Universidad Europea

TRABAJO DE FIN DE GRADO

Grado en Odontología

MESENCHYMAL STEM CELLS OF THE ORAL CAVITY. STATE OF THE ART

Madrid, Curso 2020/2021

Número identificativo **256**

Abstract

Introduction: Alexander Friedenstein described for the first time, 40 years ago, a spindle-shaped cell that did not follow the hematopoietic differentiation lineage, but had the abilities to differentiate into bone and cartilage forming cells. This cell was later named mesenchymal stem cell (MCSs) by Arnold I. Caplan 25 years ago. Ever since their discovery clinical trials and experiments have shown promising results both *in vitro* and *in vivo.*

Objectives: Summarise the current knowledge of dental MSCs in teeth and tissue regeneration in physiological conditions through an extensive review of articles, experiments, and research conducted on the matter.

Methodology: Systematic approach was conducted to collect scientific articles to answer the primary objective through credible sources. Exclusion criteria narrowed down the search to 49 unique sources that were used for this paper.

Discussion: Tooth-related structures and associated structures of the mouth, like the Bichat fat pad, have been explored as sources of MSCs. Their phenotypes have proven similar to the ones of the pancreas and bone marrow. MSCs from pulp tissue, periodontium, and Bichat fat pad has been used to regenerate lost tissue**.** Demands for easier and efficient harvesting methods shifted the interest of researchers to the mouth.

Conclusion: MSCs from different sources of the oral cavity were successfully used in pulp regeneration in pulpectomy treated canines for dogs, bone regeneration in periodontal patients, and severe atrophic mandibles. Limitations are still evident as physiological variations for individual patients will alter results and are yet to be discovered in detail by researchers. Proper protocols are still not available and need be developed for clinical use in public. Further research is therefore needed to assure a safe and stable approach to tooth-

tissue regeneration with MSCs. Despite MSCs still being in their early stages, their place in future clinical practice remains promising**.**

Resumen

Introducción: Alexander Friedenstein describió por primera vez hace 40 años un tipo celular fusiforme que no seguía el linaje de diferenciación hematopoyética, pero que tenía la capacidad de diferenciarse en células de hueso y cartílago. Este tipo celular fue posteriormente denominada célula madre mesenquimal (MSC, por sus siglas en inglés) por Arnold I. Caplan hace 25 años. Desde su descubrimiento, los ensayos clínicos y los experimentos han mostrado resultados prometedores tanto *in vitro* como *in vivo.*

Objetivos: Resumir el conocimiento actual de las MSC dentales en la regeneración de dientes y tejidos en condiciones fisiológicas a través de una extensa revisión de artículos, experimentos e investigaciones realizadas al respecto.

Metodología: Se realizó un enfoque sistemático para recopilar artículos científicos para responder al objetivo principal a través de fuentes creíbles. Los criterios de exclusión redujeron la búsqueda a las 49 citas únicas que se han utilizado para este artículo.

Discusión: Diferentes estructuras relacionadas con los dientes y asociadas a la boca, como la bolsa de grasa de Bichat, se han explorado como fuentes de MSCs. Sus fenotipos son similares a las MSCs extraídas del páncreas y la médula ósea. Se han utilizado MSC de tejido pulpar, periodontal y de la bolsa de grasa de Bichat para regenerar tejido perdido. La necesidad de métodos de recolección de MSCs más fáciles y eficientes han aumentado el interés de los investigadores hacia la boca.

Conclusión: Las MSCs provenientes de diferentes fuentes de la cavidad oral se han utilizado con éxito en la regeneración pulpar de caninos tratados con pulpectomía en perros, la regeneración ósea en pacientes periodontales y en casos de mandíbulas atróficas graves.

Las limitaciones aún son evidentes, ya que las variaciones fisiológicas en pacientes individuales alterarán los resultados obtenidos en laboratorio. Aún no se han desarrollado protocolos adecuados para el uso clínico de este tipo celular y, por lo tanto, es necesaria más investigación para garantizar un enfoque seguro y estable para la regeneración del tejido dental a través de las MSCs.

Table of Contents

Abstract 2
Resumen 4
1. INTRODUCTION 7
1.1. What is a mesenchymal stem cell and what potentials does it present? 9
1.2 Where can we find mesenchymal stem cells? 11
1.3 Mesenchymal stem cell from the oral cavity 12
1.3.1 Teeth 12
1.3.2 Gingiva 14
1.3.3 Cheek Mucosa and Floor Of The Mouth 15
1.3.4 Tongue and Hard Palate 15
1.4 Mesenchymal stem cell from associated structures of the oral cavity 18
1.4.1 Buccal Fat Pad or Bichat Fat Pad 18
1.4.2 Salivary Glands 20
1.5 Mesenchymal stem cells from teeth related structures 21
1.5.1 Dental Pulp 22
1.5.2 Exfoliated Deciduous Teeth 23
1.5.3 Apical Papilla 24
2. OBJECTIVES 25
2.1 Primary objective: 25
2.2 Secondary objectives: 25
3. METHODOLOGY 26
4. DISCUSSION 27
4.1 Mesenchymal stem cells from the Bichat fat pad 27
4.2 Mesenchymal stem cells from the major salivary glands 29
4.3 Mesenchymal stem cells from pulp and periodontium 31
5. CONCLUSION 37
6. RESPONSIBILITY 40
7. LITERATURE REVIEW 41
8. ANNEX 49

1. INTRODUCTION

About forty years ago in the mid-1960s, Alexander Friedenstein, a soviet scientist, first described a stromal cell with clonogenic potential that could give rise in culture to fibroblasts, as well as other mesodermal cells (1). They were described as spindle-shaped with proliferating abilities that formed colonies attached to plastic found in the bone marrow (2). Friedenstein discovered that the stromal cell did not adhere to the known hematopoietic cell lineage, but had the abilities to give rise to bone and cartilage forming cells instead.

Further investigations confirmed and established that these cells could indeed be isolated by plastic adherence and give rise to osteoblasts, chondrocytes, adipocytes and myoblasts. They were officially named mesenchymal stem cells (MSCs) more than 25 years ago by Arnold I. Caplan (1).

Currently, MSCs or MSC-like cells have been established from several tissues including fetal tissues, circulating blood, placenta, amniotic fluid, heart, skeletal muscle, adipose tissue, synovial tissue and pancreas (3). Therefore, MSCs represent a class of stem cells found in humans and mammals which can be isolated and differentiated in culture to induce a variety of mesodermal tissues including bone, cartilage, adipose tissue, muscle or even heart cardiomyocytes. For all these facts, MSCs are studied for their potential use in regenerative medicine. Whether MSCs from different tissues are a unique cell type or a family of related stem cells is currently a debated topic.

The oral cavity is a source of MSCs and these cells can be found in teeth, the apical papilla, alveolar bone, maxillary sinus, PDL granulation tissue, Bichat fat pad and salivary glands. Different types of MSCs can be isolated from teeth. Dental Pulp Stem Cells (DPSCs) are teeth MSCs that can be isolated from the pulp of permanent adult teeth (4). DPSCs can be

differentiated into osteoblast, chondrocytes, adipocytes and myoblasts like MSCs isolated from other tissue, representing therefore an easily accessible source to isolate them. Importantly, a small-scale phase-I clinical trial has been published regarding the possibility to use DPSCs to replace infected pulp tissue by *Nakashima and Iohara* in 2014, reinforcing the idea of using MSCs in teeth repair in the future (5).

The periodontal ligament is the source of Periodontal Ligament Stem Cells (PDLSCs) (6). These cells are still poorly defined regarding their origin, location as well as their *in vivo* differentiation capacity. Since periodontal disease is the major cause of tooth loss in western countries and the replacement of periodontal tissue is difficult, understanding the nature of PDLSCs is the focal point of several recent studies.

Since their discovery, MSCs have remained a biological enigma with huge potential. In this review, a brief description of their discovery and the current knowledge about their nature will be given. Furthermore, a description of MSCs in the oral cavity, specifically focusing on teeth-derived MSCs and MSCs from associated structures, like DPSCs and PDLSCs, will be highlighted extensively. The position of MSCs currently, and the knowledge surrounding their use in teeth and tissue repair in physiological conditions will also be discussed in depth.

1.1. What is a mesenchymal stem cell and what potentials does it present?

Mesenchymal stem cells (MSCs) received their name, MSCs, about 25 years ago. Since then, the terms MSC and marrow stromal cell have been used to describe the same type of cell interchangeably. However, it's important to highlight the fact that neither of the two terms describes this type of cell in a sufficient matter. The word mesenchymal derives from the word mesenchyme, the embryonic connective tissue, that originated from the mesoderm and that follows the hematopoietic differentiation lineage. This interchange of words is a common mistake made by many as MSCs do not follow the hematopoietic differentiation lineage but are multipotent cells that can differentiate into a variety of cells, such as chondrocytes, myocytes, adipocytes and osteoblasts. The word marrow stromal cell however, originates from stromal cells which are connective tissue cells creating the infrastructure and scaffold in which the functional cell of the specific tissue will reside and be situated. As this describes only one of the many functions of an MSCs it's therefore apparent that the word is not sufficient enough to describe and cover the many other functions

Pluripotency is defined as the capacity of a cell to give rise to all the different cell types of an adult organism. Embryonic Stem cells (ESCs) are pluripotent cells and were isolated first from mice in the 80s. It was not until the end of the 90s that it was possible to isolate human Embryonic Stem cells (hESCs). In 2004 the knowledge surrounding hESCS was further improved as the first words of the possibility to reprogram and adult cell into ESC-like cells was published. These cells were termed induced-Pluripotent Stem cells or iPSCs. *Shinya Yamanaka* was awarded the Nobel prize in 2012 for this discovery. Despite their potential, there is currently no clinical protocol based on embryonic stem cells, mainly due to their capacity to form tumors. Usage of these cells has strong ethical concerns.

MSCs however, are multipotent stromal cells. Multipotency is defined as “cells that have the capacity to self-renew by dividing and to develop into multiple specialized cell types present in a specific tissue or organ. Most adult stem cells are multipotent stem cells” (7). Multipotency describes a progenitor cell, meaning cells implicated in tissue homeostasis and tissue repair and therefore, have stem cell-like activity. MSCs have the gene activation potential to differentiate themselves into discrete cell types. Less ethical complications are therefore surrounded these cells, even though ethics will always present itself as a large influential factor in biomedicine.

1.2 Where can we find mesenchymal stem cells?

Multipotent stem cells such as mesenchymal stem cells have been found in various locations of the body. It has been found in various parts such as the umbilical cord blood, adipose tissue, cardiac cells, bone marrow, and in more specific places in the oral cavity like third molars and the periodontium. The periodontium is a well-known term used within dentistry and describes the structures around a tooth. These structures are the periodontal ligament (PDL), alveolar bone, gingiva and cementum in which most of them have been shown to be a source of MSCs.

More specifically, MSCs have been harvested and located in the dental pulp, apical papilla, maxillary sinus, Bichat fat pad and also the major salivary glands. Out of all the sources of MSCs in the oral cavity, the PDL and dental pulp have shown to be the more common places to harvest MSCs. They both present about 27%, individually, of the MSCs harvested from the oral cavity (Figure 1) (8).

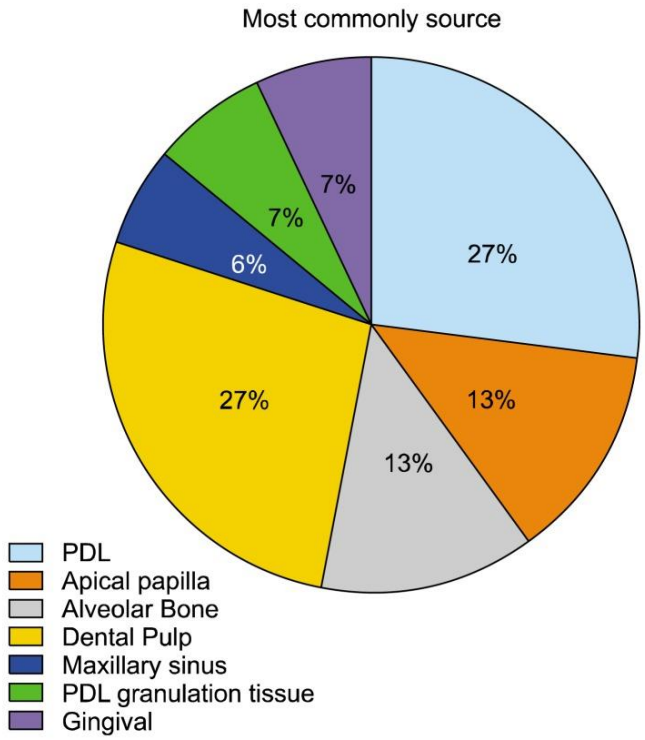

Fig.1 Table of the most common sources of harvesting mesenchymal stem cells in the oral cavity

1.3 Mesenchymal stem cell from the oral cavity

The oral cavity is defined as the area located posteriorly to the teeth. It is limited by the hard and soft palate superiorly, the floor of the mouth inferiorly, the cheeks laterally and the pharynx posteriorly. It is considered the first section of the digestive tract serving as the primary entrance point for food to the body. It presents functions such as speech and breaking down food. It also functions as an organ aiding respiration secondary to the lungs. The main structures found here are the teeth, gingiva, retromolar trigon, hard palate, cheek mucosa, tongue and the floor of the mouth. Other associated structures such as the major and minor salivary glands and Bichat fat pad are also found here. The oral cavity has traditionally been looked at as an area of expertise for dentists with teeth as their main focus. However, new demands for efficient and easy harvesting of MSCs have shifted researchers' interest to the mouth and its associated structures.

1.3.1 Teeth

The teeth are the hardest substance in the human body and are composed primarily of three layers: enamel, dentin, cementum (Figure 2). The outermost layer is called the enamel (Figure 2) and presents itself as the hardest and most mineralized layer. The underlying layer is called the dentin (Figure 2) and is composed of cylindrical tube-like structures called the dentin tubules. Changes in an environment like temperature or acidity will follow the path of the tubules and be registered by the nerves in the inner chamber of the tooth (9), this chamber is called the pulp (Figure 2) and is located in the pulp chamber. The pulp presents itself as a sack-like structure derived from neural crest cells from the ectomesenchyme. During odontogenesis, the neural crest cells proliferate and undergo condensation to form the dental papilla from which the mature pulp is later developed. The characteristics of the pulp resemble the ones of the embryonic connective tissue which is surrounded by a layer of specialized cells

and odontoblasts. The pulp is highly innervated and vascularized and is therefore the main source of pain related to dental pathologies (10). The potentials of the pulp are yet to be clearly defined, but the findings of mesenchymal stem cells have allowed us to further explore the true potential of the pulp.

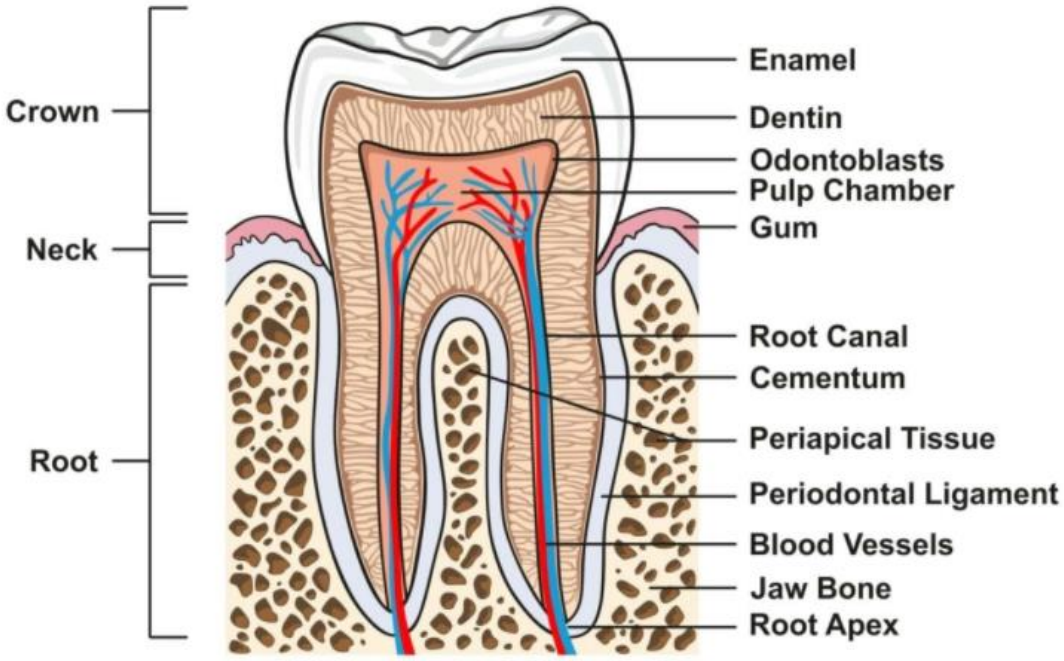

Fig.2 Dental anatomy presenting all the main anatomical landmarks

1.3.2 Gingiva

The surrounding and protective tissue found around the teeth is known as the gingiva. It is composed of stratified squamous epithelium with an underlying layer of connective tissue called the lamina propria. The two layers connect to each other through projections from the lamina propria piercing into the epithelial layer. These projections are called the connective tissue papilla and are separated by epithelial and rete ridges. The epithelial layer derives from the ectoderm whilst the underlaying lamina propria derives from the mesoderm and neural crest cells. The gingiva offers protection and a seal surrounding teeth and bone assuring resistance to mechanical trauma and microorganisms. It presents itself with great tissue regenerative potential that's been explained through several mechanisms. One of these mechanisms is the modulation of adult stem cells. The gingiva's rapid self-renewal potential has therefore been of great interest and emphasized within oral mucosa stem cell studies.

Its easy accessibility for the dentist has therefore allowed it to be one of the main sources of mesenchymal cells for tissue regeneration specifically. However, further research is needed to determine the true potential of the gingiva within the world of mesenchymal stem cell research (11)

1.3.3 Cheek Mucosa and Floor Of The Mouth

Other areas of the oral cavity that have shown great potential are the cheek mucosa and the floor of the mouth. Having mentioned both, their actual structure is not of much interest in regards to MSC's but rather the anatomical structures found within these two areas. Those of the major salivary glands located inferiorly to the floor of the mouth (submandibular gland) and the parotid gland in the cheek area, and also the buccal fat pad/Bichat fat pad located laterally to the inner cheek mucosa. The retromolar trigon does indeed follow the same principles as the anatomical position of the retromolar tragion is less relevant in itself but it being the birthplace of 3rd molars has made it rather interesting for the harvesting and accessibility of MSC's related to 3rd molars.

1.3.4 Tongue and Hard Palate

As for the tongue and hard palate; these areas in the oral cavity have not shown to be areas of great interest within the field of MSC's. However, their relevancy within the field of regenerative dentistry, specifically within tissue repair, remains relevant and highly useful for today's practitioners. The hard palate especially has shown to be the most common area, together with the maxillary tuberosity, to harvest tissue grafts within implantology and periodontology (12,13). The hard palate is composed of two bones in particular: the palatal process of the maxillary bone and the joining of the two palatine bones, more specifically the horizontal plates of both palatine bones. The underlying bony structures are layered with a protective layer of keratinized mucosa. The keratinized mucosa and underlying bone are described as the hard palate.

Experiments in mice have shown that mouse embryonic palatal mesenchymal cells do indeed present strong osteogenic potential like MSC's harvested from other structures. Their use has mainly been towards bone regeneration or as coadjuvant cells to help initiate regeneration of the palate in cases of cleft palate (14). Regeneration of palatal bone has also shown possible; however, this has been done with help from the MSC's of exfoliated deciduous teeth cell sheet and not MSC's from the hard palate itself. (15) The potential of the MSC's found in the hard palate is yet to be described in full detail in regards to their use in humans and remains still unknown when compared to MSCs from other locations.

The oral cavity has a lot of other related structures, which are not included in the definition of the oral cavity. Two of these associated structures are the Buccal fat bad or Bichat fat pad, located in the cheek area, and the major salivary glands meaning the Parotid gland, Submandibular gland and Sublingual gland.

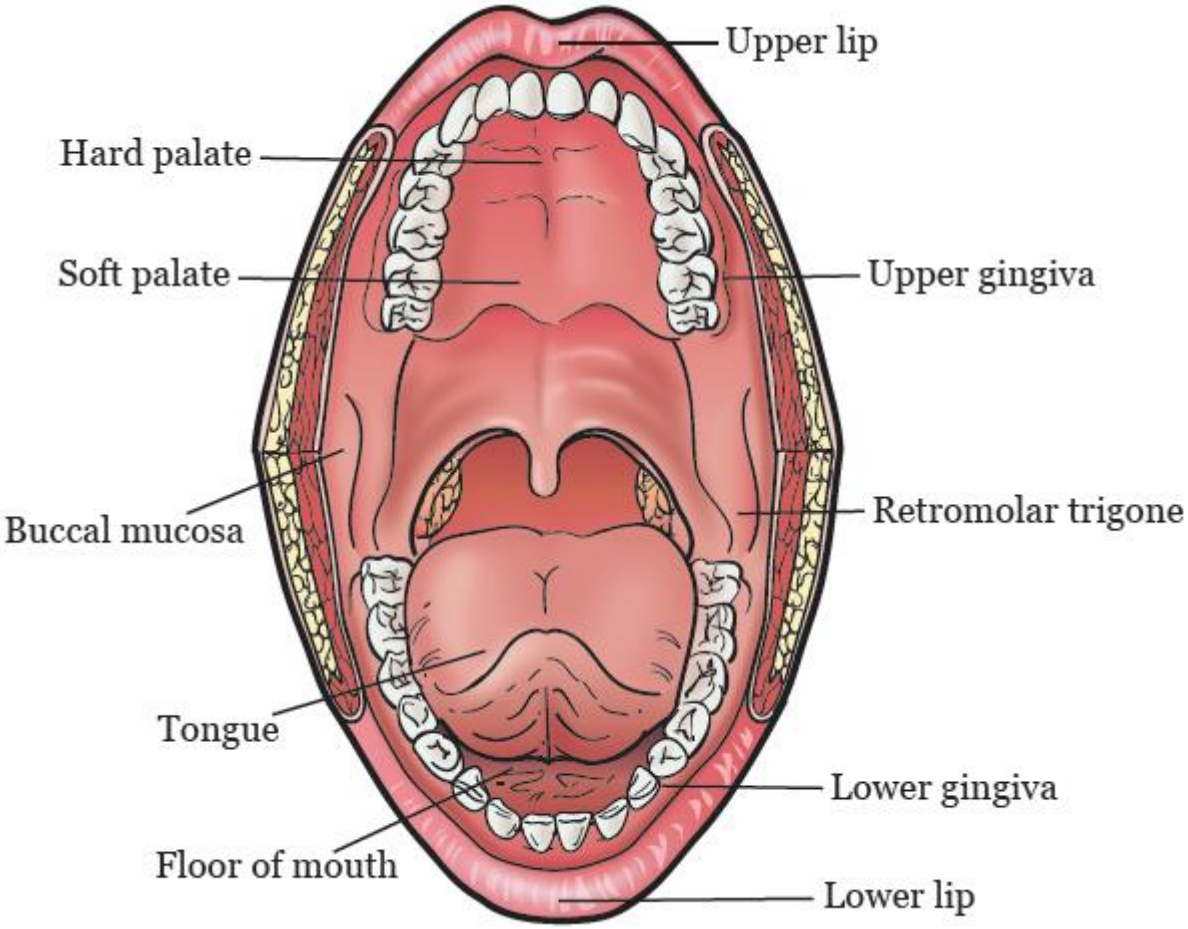

Fig.3 Anatomy of the oral cavity highlighting the anatomical landmarks

1.4 Mesenchymal stem cell from associated structures of the oral cavity

1.4.1 Buccal Fat Pad or Bichat Fat Pad

The buccal fat pad has traditionally been looked at as an associated structure to the oral cavity without much function. It is not until recent times, the last decade, that its function and potentials have been highlighted in articles and research (16). The buccal fat pad is an anatomical structure located in the cheek with adjacent structures such as the facial artery, parotid gland and its duct and buccinator muscle located close by. It is composed of three main lobes called the posterior, intermediate and anterior lobe, all encapsulated by independent membranes. The three lobes together are considered the body of the buccal fat pad extending in four directions: Pterygopalatine, Pterygoid, Temporal and Buccal extension (17). Its physiological function is mainly predominant during the early stages of life. Fat tissue differentiation has been reported to begin as early as the second trimester of gestation with its size increasing up until the 29^{th} week of gestation (18). The buccal fat pad allows for cheek prominence of adults and new-borns being the first adipose tissue that develops. The three main functions are separation of the masticatory muscle from adjacent bony structures, the protective function of the neurovascular bundle and the prevention of negative pressure in newborns during sucking (17). Its application today within the fields of maxillofacial surgery and regular oral surgery have progressed increasingly (19). Buccal fat pad flaps have been commonly used for either sinus complications or pathologies causing oroantral communication and fistulas. However, in recent times scientists have developed knowledge allowing them to explore further the regenerative properties relying on adipose-derived stem cells from the buccal fat pad (17).

Harvesting of MSCs have traditionally been derived from bone marrow aspiration. However, new studies suggest now that MSCs harvested from adipose tissue is equally or even superior in some situations. Adipose tissue does present a large cell yield of between 100 to

500 times greater than the ones of bone marrow (20). This tissue can be obtained from minimally invasive techniques and minimal discomfort for the patient. As only a small sample is required the procedures can be minimally invasive and still obtain a high number of MSCs. One of the more common locations of adipose tissue in the oral cavity is the Bichat fat pad. The cells found here present themselves with a similar phenotype to other adipose tissues from other locations of the body. Adipose tissue is composed of mainly two different fractions. One fraction being the stromal vascular fraction including the MSCs, fibroblasts and erythrocytes (20) The other fraction is mature adipocytes. Dedifferentiated fat cells from the buccal fat pad have been produced from mature adipocytes from ceiling culture technique (20)

From the two different fractions it was showed that the MSCs from the stromal vascular fraction were key to further differentiate adipose tissue, chondrocytes, osteocytes, myocytes and neurons. Cells taken from Bichat fat was in 2010 isolated and under an appropriate condition, differentiated into chondrocytes, osteoblasts or adipocytes *in vivo* (21). Other research has also been conducted showing recent use of buccal fat pad stem cells together with iliac bone block grafts to regenerate new bone formation in jaws presenting extensive atrophy. The cells harvested from the buccal fat pad and their potential to differentiate into osteoblasts have been proven. Their great potential to regenerate both bone and periodontal tissue allows us to consider the buccal fat pad as a source of mesenchymal stem cells in regenerative dentistry (20).

1.4.2 Salivary Glands

The oral cavity is composed of many different types of salivary glands. The major salivary gland composes most of the salivary production whilst the minor salivary glands are found all over the mouth with less saliva production. The three main salivary glands are the parotid gland, sublingual gland and the submaxillary gland (submandibular). These are considered to be the major glands. The minor glands are found all over the oral cavity especially concentrated on the palate, buccal wall, lips and tongue. All salivary glands contribute to the many functions of the mouth like speech, taste, deglutition, mastication and also lubrication.

Their composition is mainly based around two different cell types that produce saliva. These are the mucous and serous acinar cells and myoepithelial cells. These cells, controlled by both cholinergic and adrenergic innervation, facilitate saliva extraction and modification of salivary composition (22). Other cells found in salivary glands are stem or progenitor cells. These cells are characterized by presenting the actions of self-renewal and differentiation capacity. Their main function is therefore to replace damaged cells and give the salivary glands the possibility of self-renewal. However, these cells' potential to differentiate into cells related to dentistry is still yet to be discovered. Harvesting of such cells have been done with the aim to treat damaged or atrophic/necrotic glands and not the regeneration of other tissues.

1.5 Mesenchymal stem cells from teeth related structures

New and modern stem cell research have allowed us for the first time to explore the secrets of tissue development. Tissue regeneration is a natural process occurring due to the presence of stem cells in our body. They present the ability to self-regenerate and differentiate into new cells and therefore create new tissue. Even though tissue regeneration is a natural process, factors like age have shown to heavily slow down the regenerative potential. This means that with age, the body is less capable to repair damage from degenerative, ischemic, inflammatory and tumor based-diseases (23). In the last decades, further investigation of stem cells has shown that these cells cannot only be isolated from the more common sources like bone marrow, pancreas and connective tissue, but also from teeth, tooth-related tissues and associated structures of the mouth. The dental pulp, exfoliated deciduous teeth, PDL, dental follicle and apical papilla have all shown to be potential sources of these cells (23). Cells collected from either, but especially the pulp, have shown great potential and capacity to differentiate into many different cell types (23). One of their greater features has been described as their osteoblastic potential and dentinogenic potential through osteoblasts and odontoblasts (24). Other differential potentials from the pulp are adipogenic, chondrogenic, myogenic and neurogenic differentiation through adipocytes, chondrocytes and neural cells. (23,24)

1.5.1 Dental Pulp

Tissue engineering with the help of mesenchymal stem cells have presented itself as a potential groundbreaking discovery. The topic of dental pulp regeneration within dentistry and especially regenerative endodontics has for the first time been introduced into the world of dentistry. The dental pulp, together with the dentin, is what keeps a tooth vital. The pulp presents itself as a sack-like structure containing cells, connective tissue, vessels and nerve endings. This vascular structure is found within the tooth itself inside an area called the pulp chamber. The layer surrounding the pulp chamber is the dentin. The dentin and pulp together are considered the endodontium, or the dentin-pulp complex. They act as one complex and are in many ways interrelated depending on each other for the tooths survival. The pulp itself is highly vascularized and innervated including various cell types such as fibroblasts, odontoblasts, histocytes, macrophages, mast cells and plasma cells (25). The function of the pulp can be described easily in four ways: formation and nutrition of the dentin, and defense and innervation of the tooth (25).

The stem cells found in the pulp are multipotent with great proliferative capabilities that can be cryopreserved with immunosuppressive properties. Earlier studies have also shown that the pulp express markers such as CD13, CD29, CD44, CD59 together with many other CD markers, and STRO-1 (23). Both *in vivo* and *in vitro* studies have been conducted on the matter. Experiments conducted *in vitro* showed results were MSCs from the dental pulp formed mineralized nodules under osteoconductive conditions creating dentin-like material which was microscopically proven similar to physiological dentin (23). Furthermore, when a similar trial was conducted *in vivo*, structures similar to reparative dentin was developed on top of, the already present, physiological dentin (23)

Other studies have also been conducted describing great potentials from the MSCs harvested from the pulp. It was proven that when pulp MSCs were applied together with hydroxyapatite/ tricalcium phosphate it produced structures close to dentin. This structure, when microscopically examined, showed a newly created line of odontoblast surrounding a pulp-like tissue *in vivo* (26,27).

1.5.2 Exfoliated Deciduous Teeth

As already mentioned, human exfoliated deciduous teeth (HEDT) could also be a source of stem cells. These stem cells present similar properties to the ones of the pulp but are considered more accessible (28). HEDT are considered clinically and biologically discarded tissue due to these teeth being exfoliated naturally at age 6-12 and are described as the most promising source of mesenchymal stem cells for tissue regeneration (28).

Cells from HEDT have been shown to present a greater proliferation rate. Experiments conducted in 2010 showed that when stem cells from HEDT were transplanted onto the dorsum of immunocompromised mice, dentin-like tissue was formed. However, the stem cells were unable to recreate or regenerate the dentin-pulp complex. These results suggested that the generation of odontoblasts from HEDT is possible *in vivo* (29).

1.5.3 Apical Papilla

The apical papilla has presented itself as a new source of stem cell. It's been described as a potentially new type of cell found specifically in immature permanent teeth. The dental papilla comes from the ectomesenchyme overlaying the dental lamina during tooth development. This portion specifically will later develop, becoming the pulp, after odontoblasts encapsulates it and produce dental tissue. The apical papilla, or the apical portion, is located further down in the root apex during root development. This portion have been named the apical papilla and through several recent studies have shown great capabilities to aid and regenerate new tissue (30). MSCs surface marker STRO-1 has been found in the apical papilla and was the first proof of their existence. The apical papilla appears on the root apex of immature permanent teeth as a little sack, loosely adhered, that can be extracted easily with a pair of tweezers (30). These mesenchymal stem cells can be isolated and grown in cultures where they have shown to undergo dentinogenic differentiation with stimulants like dexamethasone with L-ascorbate-2-phosphate and inorganic phosphate (30). They have also shown further differentiation potentials like osteogenic differentiation, adipogenic and neurogenic capabilities (23,30)

The cells extracted from the apical papilla have shown special use in root formation. Cells from the apical papilla showed to present potentials in apexogenesis. Trials showed that when transplanted into an immature tooth presented with periradicular periodontitis or abscess, the cells from the apical papilla had the ability to induce the formation of the root tip. (23). Similar results were shown in an experiment where a young immature central incisor underwent root canal treatment whilst keeping the apical papilla intact (30). The formation of the root continued even though the pulp was removed. Further investigation is of course needed; however, the results highlight the potentials of the apical papilla.

2. OBJECTIVES

Mesenchymal stem cells remain a key topic in today's scientific world. Their use can be potentially without great limitations and their use in developmental and regenerative dentistry is slowly emerging as a focal point in research. I would therefore like to approach this paper answering the following objectives:

2.1 Primary objective:

- **Summarize the current knowledge of dental MSCs in teeth repair and tissue in physiological conditions.**
- **Discuss possible applications of teeth MSCs in regenerative dentistry.**

2.2 Secondary objectives:

- **Definition of MSCs**
- **Describe the different sources of MSCs inside the oral cavity**
- **Link the human anatomy of the oral cavity with the sources of oral cavity MSCs**
- **Describe dental MSCs**

3. METHODOLOGY

A systematic approach was conducted to collect and find scientific articles to answer the primary objective: to summarise current knowledge of dental MSCs in teeth and tissue repair in physiological conditions and discuss possible applications of MSC in regenerative dentistry. Trusted and well-known article databases were used such as Medline, PubMed, Wiley, ResearchGate, Elsevier and Hindawi. The online library of Universidad Europea de Madrid was also used to gain access to journals and books. With the title "Mesenchymal stem cells. State of the art", keywords such as mesenchymal stem cells, regenerative dentistry, oral cavity, tooth and tissue regeneration were used to find and collect articles as a backbone of information for this paper. Initially, a total of 83 unique references were found and identified after the removal of duplicates. After abstract screening with exclusion criteria such as articles cannot be older than 10 years and *in vivo* studies only, the article base was narrowed down to 68 articles. A further thorough article assessment revealed a total of 49 unique articles fulfilling the requirements previously set and deemed relevant to answer the primary objective and therefore used as direct references for this paper. The remaining 19 articles of the 68 were used indirectly to gain sufficient knowledge and help guide this thorough review. The primary objective will be answered by firstly explaining the basic knowledge required for this content. The definition of MSCs, where MSCs can be harvested from, the potential use of MSCs and previous history. Experiments and trials on the matter will then be discussed to summarize and enlighten the current state of MSCs in teeth and tissue repair in physiological conditions and their application in regenerative dentistry.

4. DISCUSSION

As mentioned briefly before, the oral cavity, together with some of its surrounding structures have proven to be sources of MSC's. Their use in today's biomedicine remains unclear when it comes to direct tissue regeneration. Several experiments have however been conducted showing signs of potentially groundbreaking discoveries within tissue regeneration and dentistry. This technology and the use of MSCs are still far from being common practice in most clinics or hospitals, however their discovery and potential may help guide regenerative dentistry towards a new an intuitive direction.

4.1 Mesenchymal stem cells from the Bichat fat pad

The Bichat fat pad has traditionally been looked at as a structure without much purpose and potential. Surgeons have traditionally used the Bichat fad pad for its anatomical structure, shape and convenient location as cover in sinus-related complications. However, recently researchers have started to understand more of the tissue potential the Bichat fat pad presents. A study published in 2017 explored the potential use of MSCs from the Bichat fat pad, more specifically adipose tissue-derived MSCs for bone regeneration. MSCs from adipose tissue has traditionally been harvested from underlying subcutaneous tissue. The difference between the two adipose tissues harvested from different locations have shown small differences. When compared their behavior and features have been described as quite similar with both being sensitive to osteo-differentiative stimuli (31). This was confirmed by *Farré-Guasch et. al* in 2010 when they isolated fatty tissue from the Bichat fat pad and compared it to the abdominal subcutaneous tissue. The two adipose derived stem cells from the fat pad and abdomen showed to have similar phenotype, and when stimulated under *in vivo* conditions showed to both have great capabilities to further differentiate into chondrocytes, osteoblasts and adipocytes. (21).

Further investigation was conducted in 2012 when *Shiraishi et.al* demonstrated the possibility to form and engineer bone from MSCs derived from buccal fat pad (32). Adipose-derived stem cells from the Bichat fat pad were isolated after surgical removal of the pad in patients with jaw deformity. The cells were then transplanted in pockets created on the spine of a mouse for 8 weeks. The newly formed bone-like tissue was then later analysed and compared. (32). The results showed that calcified nodules had been created in the space. The tissue created presented osteocyte-like cells, similar to the cells found in natural bone. The bone-like structure occupied between 13-40% of the space created showing variable results in regards to the amount of bone-like tissue created. Nevertheless, the findings demonstrated by Shiraishi proved that adipose-derived stem cells from the Bichat fat pad can indeed induce bone formation. Its relevancy remains still unclear as better technology and research have to be conducted to allow for reliable bone engineering in patients in day-to-day practice

More research has also been conducted in more recent times. In 2016, *Khojasteh et. al.* assessed the potential to use MSCs derived from the Bichat fat pad as coadjuvant graft material together with iliac bone graft for treatment of alveolar ridge defects. A smaller number of patients, eight in total, were selected to undergo a procedure for extensive jaw atrophy. The procedure constituted of reconstructive surgery using iliac crest bone blocks. The spaces between the bone blocks were filled with cells derived from the Bichat fat pad. The level of newly formed bone was assessed in six points along the alveolar ridge using cone beam computed tomography (CBCT) as the main tool of assessment. The results showed that the test group receiving cells derived from the Bichat fat pad presented greater new bone formation than patients in the control group not receiving it. The bone formation in the test group was 65.32% compared to the 49.21% in the controlled group translating to 3.94 ± 1.62mm to the 3.01 ± 0.89mm in the controlled group (33). This study did not investigate the use of the cells

from the Bichat fat pad alone, however it demonstrated its potential usefulness as a coadjuvant tool due to its differentiation potential. This further highlights the potentials cells derived from the Bichat fat pad may present

4.2 Mesenchymal stem cells from the major salivary glands

The major salivary glands present themselves as an easily accessible source of MSCs. Their potential has been described mainly *in vitro* in which cells have been extracted and isolated from major salivary glands like the parotid gland and submandibular gland. The potential displayed by these cells have been researched and shown great ability to differentiate along all three mesenchymal lineages (34). The cells found in the major salivary glands have been compared to the ones of the pancreas, which is already known as a source of mesenchymal stem cells within regenerative medicine. When extracted cells from the pancreas and salivary gland were compared the results showed that they both presented similar abilities to differentiate spontaneously into cells with the three embryonic layers. Their phenotypes were also described as comparable with proven differentiation potential into adipogenic, chondrogenic and osteogenic cells. The similarity in MSCs from both was explained by their common embryonic origin; the foregut (35). Discoveries like this have allowed researchers to direct their interest towards the major salivary glands and their potential.

A recent study by *Najafi et.al* investigated the possibility to regenerate functional salivary gland tissue in a rat presenting a necrotic submandibular gland. MSCs were extracted and later cultured from healthy rats and locally injected into the atrophic gland. When later examined by a histopathologist the results showed that the glands receiving MSC therapy presented a considerably lower density of the serous acini than the group of rats not receiving therapy. A further comparison of the rats receiving therapy and the healthy rats showed that histopathologically the two presented similar density of their glandular structure compared to

the high density and morphological changes found in the ones not receiving therapy. Additionally, further testing showed that the overall serous and mucin secretion increased together with the cell amount in the atrophic gland over a 2-week post-transplant period. This study proved that transplanted MSCs indeed could regenerate the necrotic duct and increase secretory granules (36).

A similar study was also conducted in 2013 by *Lim.et al* using rodents. Mice were induced salivary gland dysfunction through radiation with 15Gy. Measures of salivary flow was noted pre- and post-radiation. A local injection of MSCs was then carried out into the submandibular gland, similarly done to the study conducted by *Najafi et al.* The results showed a considerable increase in salivary flow for the mice undergoing MSCs treatment compared to the control group not receiving the injection. The increase in salivary flow was around 41% and concluded that MSCs from the salivary gland could increase and improve salivary flow in dysfunctional glands (37)

Other studies have also been conducted on the matter. *Jensen et al. 2014* conducted a study reviewing the MSC therapy for patients presenting salivary gland dysfunction, xerostomia or salivary gland dysfunction induced by radiotherapy. The more severe forms of xerostomia resulting in reduced quality are found in Sjögren syndrome and patients undergoing radiotherapy for head and neck cancer. The study highlighted the current role of MSCs in salivary gland research. Several articles were mentioned, including *Lim et al. 2013* and many others. *Jensen.et al* highlights that even though studies conducted with direct MSCs injections into the dysfunctioning gland have presented positive and promising outcomes; transferring this knowledge to humans is still far from common clinical practice. Real-life patients present larger variations in destruction related to their radiotherapeutic dosage. The damage caused is

therefore specific to the patient which would alter the results considerably compared to mice (38)

As the studies mentioned about presents themselves as great discoveries to build on further for the future; the question of whether or not MSCs from salivary glands can be used in regenerative dentistry remains unknown. The studies presented previously only conclude that MSCs from salivary glands can be effective and beneficial in salivary gland issues. Whereas this is a great discovery in itself and for regenerative medicine, their use in regenerative dentistry is still unknown and no studies have been conducted so far on the matter. MSCs from these glands seem to limit themselves to salivary gland-related issues only and thus not appropriate to use in regenerative dentistry based on today's knowledge.

4.3 Mesenchymal stem cells from pulp and periodontium

The pulp and the periodontal ligament have been two known common sources of multipotent MSCs. Many studies, both *in vivo* and *in vitro*, have been conducted on the matter. Cells from the dental pulp and periodontal ligament presents great potential and their multilineage differentiated *in vitro* and dental tissue regeneration *in vivo* have both been proven (39). Their known ability to regenerate dental tissue was discovered in the early stages of their discovery. However recent times have shown that their abilities stretch over a far greater differentiation lineage which have increased the popularity and interest around these cells (40).

Ravindran et al. conducted two studies in 2014 with the aim to regenerate dental pulp with the help of MSCs from the dental pulp, periodontal ligament and human marrow stromal cells. They established early that in order to develop and engineer a new dental pulp some vital factors had to be present. These factors are the MSCs themselves, a scaffold and a growth factor (41). The scaffold used in this study was a biomimetic pulp extracellular matrix (ECM). By

implanting MSCs from dental pulp, periodontal ligament and also human bone marrow stromal cells separately within a biomimetic pulp ECM Ravindran obtained odontogenic differentiation over a four-week period. Furthermore, vascularization through angiogenesis with help from an external vascular growth factor (VEGF) was also generated. Vascularization was achieved regardless of MSC type with help from this growth factor. With this Ravindran et al proved the possibility to develop healthy pulp tissue when implanting MSC from either dental pulp, periodontal ligament or marrow cells within a biomimetic ECM and growth factor, both *in vivo* and *in vitro* (41,42). Previous research had also been conducted on the matter with Iohara in 2011 and Ishizaka in 2012. Both proving the possibility to regenerate pulp-like tissue in canines after a pulpectomy with the help of pulp derived MSCs (43,44)

Iohara conducted in 2011 a study aiming to regenerate dental pulp in dogs after pulpectomy of their canine. This *in vivo* study was conducted with a total of sixty teeth from fifteen individual dogs. Dental pulp cells would then be isolated from their maxillary teeth previously and then transplanted later into the canines together with a scaffold and growth factor. The canine had undergone a previous pulpectomy following a standardized protocol for pulp removal pre-endodontic treatment in dogs. A control group of seven teeth without any intervention was kept as control. The results showed that when autologous pulp cells together with SDF-1were transplanted together into the root canal the apical formation was induced. After fourteen days pulp-like tissue was formed covering the root canal ranging from 5-30% of the total root canal surface. It was found that this pulp-like tissue had not only been created within the pulp chamber itself but also extending into the dentinal tubules. The tissue was fixed within the chamber due to odontoblast-like cells adhering to the dentin walls. After ninety days the pulp-like tissue had continued extending further into the cement-enamel junction, now filling a majority of the root canal. The most coronal part of the tissue created showed a

morphological build different from normal pulp. The tissue here was spindle shape whilst the middle and apical part resembled more of a natural pulp being stellate-like (43). Iohara proved through this study the possibility to regenerated pulp-like tissue with neurogenesis and vasculogeneis *in vivo*. This was the first time that complete pulp regeneration with pulp cells (CD105+) and SDF-1 had been done presenting itself as a potentially valuable clinical discovery for regenerative dentistry and endodontics especially. However, Iohara did not fail to mention that their discoveries did present some limitations. The use of autologous pulp cells is fundamental for the success of pulp regeneration as demonstrated in the study. These cells are not as widely available as normal pulp tissue as the cells are limited to patients with discarded teeth which also presents adequate pulp. Furthermore, it was mentioned that another requirement is a healthy and younger patient. Age and diabetes were mentioned as two factors limiting the pulp potential and might require a different approach (43).

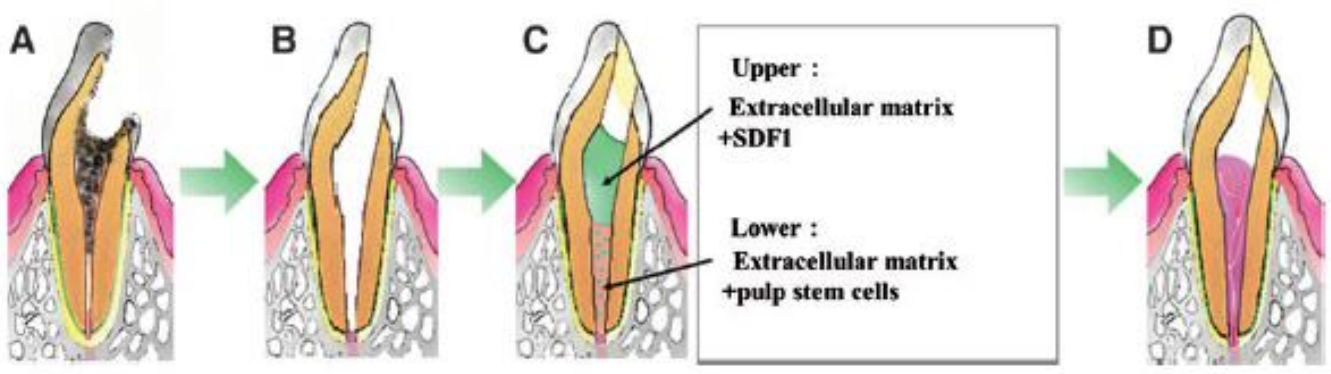

Fig 4: A: Opening of the infected tooth, **B:** Pulpectomy of infected pulp, **C:** Treatment methodology, **D:** Final results with newly generated pulp

Iohara's findings were later proven again by Ishizaka in 2012. A similar study was conducted with the same aim, however now using CD31 – cells from pulp instead of CD105+. SDF-1 was still used. The autologous cells were collected from extracted canines and later transplanted using thirty teeth from ten individual dogs. Ishizaka found that pulpal stem cells from previously extracted canines together with SDF-1 had induced and created pulp-like structure after fourteen days. This pulp-like structure had also developed vascularization and innervation. An odontoblastic layer was seen layering the pulp-like tissue allowing it to adhere to the dentin walls. Some odontoblastic structures were also discovered within the pulpal tissue itself. More odontoblastic development was found within the pulpal tissue when adipose cells were used instead of pulpal cells. This indicated that adipose MSCs might present a higher tendency to follow odontoblastic lineage than pulp MSCs (44). Ishizaka concluded that MSCs from bone marrow, adipose or pulp can indeed induce pulp regeneration *in vivo* using canines from dogs. Furthermore, it was concluded that MSCs presents comparable potential *in vivo* independent of their source (44). This confirms that pulp regeneration is a real possibility in the clinic even though further studies and trials have to be conducted before seeing pulp regeneration with help from MSCs as common day-to-day practice.

Further research has expanded the idea of using MSCs from the dental pulp to regenerate the periodontium; gingiva, PDL, alveolar bone and cementum. Treatment of periodontal disease is highly standardized and common practice for all clinics. Periodontal regeneration however is a new term introduced to the world of dentistry and has shown variable results with the help of MSCs. Periodontal regeneration should however not be confused with a periodontal repair. Periodontal repair is already a common phenomenon practiced by most implantologists and oral surgeons. The idea of using autologous grafts like bone grafts, allografts or alloplastic materials have already been introduced and is found as a common

practice amongst many practices around the world. Periodontal repairs aim to replace missing bone or ridge defects with materials from either the patient's own body, synthetic materials or bone from same or different species. Bone presents a unique ability to generate itself completely if space and the correct environment is created. The graft material provides this space and the bone will in most cases replace the grafted material and replace it with natural bone (45). This is however considered a repair of the tissue and not regeneration.

Periodontal regeneration has presented itself as a true challenge as regeneration of the periodontium in a patient with periodontal disease means regeneration of bone, ligament and cementum (46). MSCs have therefore allowed us for the first time to truly accept the challenge of periodontal regeneration due to their differential potential. Alloplastic transplantation of pulp-derived MSCs have shown to be an appropriate solution with promising results (40).

Trails on mice were conducted in 2019 by Qiao on the effects of MSCs from exfoliated human primary teeth injected in mice with periodontal disease to see the effects on the regeneration of the periodontium. SHED was obtained from an already established oral cell bank. *Qiao et.al* used a total of fifteen mice, divided in three groups with five mice in each. A control group was obtained together with a group periodontal group and SHED group. SHED was then injected locally in three different administration sites: mesial, distal and in the middle of the molars. After a two-week period, the mice were reevaluated and the results showed a rapid proliferation rate of SHED. The capacity of SHED to undergo osteogenic and adipogenic differentiation was also proven. The SHED group had developed a greater periodontal regeneration than the periodontal group. CT scans were prescribed to evaluate the bone level and the SHED group presented greater new bone formation compared to the periodontal group, however bone levels were not generated back to a physiological state.

Furthermore, it was detected that the number of osteoclasts and inflammatory factors were reduced in the SHED group allowing them to believe the possible inhibitory activity of SHED on both. Further research is however required to draw any conclusions on SHED's effect on osteoclasts and inflammatory factors. However, the overall results showed alveolar bone regeneration when SHED was injected locally in mice to treat periodontal disease (47).

The effects on bone regeneration in humans with help from MSCs have also been conducted. *Ferrarotti et al* conducted research in 2017 aimed at regenerating infrabony defects with dental MSCs. A randomized clinical trial was carried out following two groups of patients over a twelve-month period. The MSCs were isolated and collected from an extracted tooth of the patient. A surgical approach was then followed raising a flap and surgically clean the root surface before the MSCs were placed. Fifteen boney defects received MSCs within a scaffold of collagen bio complex whilst the remaining fourteen boney defects received MSCs and collagen sponge. The control group received a protocolized approach for surgical debridement of a periodontal patient without any MSCs. The results showed that the overall gain in clinical attachment loss (CAL) was 4.9 ± 1.4mm and the reduction of probing depth (PD) was 4.5±1.9 mm at twelve-month control. The results for the control group were lower at CAL gain 2.9±2.2mm and PD reduction at 3.4 ± 1.7mm at twelve-month control (48).

The limitations of the study should however not be overlooked despite positive results. Factors such as smoking habits, systemic disease with affectation on periodontal healing, pregnancy and furcation defects were excluded. This allows for the study to be conducted in a controlled environment contrary to the reality in day-to-day practice as a periodontal patient commonly present at least one of the excluded factors (49)

5. CONCLUSION

The art of dentistry has been known for thousands of years. It is a profession that is rapidly evolving year by year. As of today, dentistry is a highly protocolized profession with new and modernized treatment methods and ways of diagnosing. The current state of dentistry is modern and efficient with safe methods of approach. Modernised technology stands central and is key to achieving successful results for patients, however it is not without its limitations. Even though today's current technology has allowed dentistry to advance into new dimensions of safety, esthetics and efficiency, limitations especially within regeneration of soft and hard tissue, as well as dental tissue, remains apparent.

After four decades since the discovery of mesenchymal stem cells their presence have gained rapid traction within biomedicine. The idea of implanting a cell with almost unlimited potential to regenerate seemingly any tissue have sparked light on potentially groundbreaking discoveries in regenerative dentistry and medicine. Mesenchymal stem cells are multipotent stem cells with abilities to differentiate into chondrocytes, myocytes, adipocytes and osteoblasts. They do not follow the traditional hematopoietic differentiation lineage known for pluripotent stem cells from the bone marrow. Their potential to create new bone, cartilage, and even dental tissue have allowed mesenchymal stem cells to introduce themselves as a focal point within regenerative dentistry. Today's practice in regards to bone creation and healing, especially within periodontics and surgery, is more centered around grafts and membranes to repair the tissue rather than regenerate.

Increased knowledge about MSCs have allowed more efficient ways of harvesting them. By exploring the mouth and associated structures, researchers have found that some of the anatomy here, like the Bichat fat pad, dental pulp and periodontal ligament, presents equal potential to MSCs harvested from other sources like the marrow or pancreas. Discoveries such as this might deem to stand central in further MSCs research. Easy access to sources of MSCs through minimally invasive techniques will allow the cells to be more widely available, and permit harvesting prior to future studies to be more comfortable for the patient and economically beneficial for the researchers.

After reviewing several articles undergoing trials and experiments the current role of MSCs in regenerative dentistry stands unassertive. Their potentials have been explored greatly inside the laboratory and the findings throughout the years have allowed scientists to further the knowledge of MSCs ability to regenerate tissue from *in vitro* to *in vivo*. Studies conducted have proven positive results *in vivo* within topics like bone regeneration in periodontal patients and complete pulp regeneration in dogs. Positive results such as these permit the idea that MSCs could be a viable method of tissue regeneration within dentistry in the future.

However, despite several studies showing great results *in vivo*. It is not to be forgotten the many challenges and limitations the future might bring. The majority of studies conducted today are conducted in relativity-controlled patients or samples allowing maximum potential from the cells. In some instances, the environment is even modified to improve results. It is not to be dismissed that patients in day-to-day practice do not present these benefits in normal physiological conditions. Patients will in most cases present interfering factors such as systemic diseases, medications, habits like drinking or smoking or in most cases; a combination of all three. The human factor will always be there and alter results. The unassertiveness surrounding

MSCs today is largely based on their fault to be used in a wide range of patients and physiological conditions.

Today, the current status of MSCs research stands at a crossroad. Their potentials have been proven both in the lab, and in real living animals or humans, but are yet to be introduced into the general market and practices. Further research has to be conducted to generate necessary protocols, assure safety and efficacy, and at the same time assure affordability for the practitioner. MSCs find themselves at an early stage of life and usability, however there's no doubt that MSCs do indeed have a role in the future of regenerative dentistry.

6. RESPONSIBILITY

This extensive overview of the current knowledge of MSCs and the position it holds in today's scientific world calls for analysis of several articles, experiments and research conducted on the matter. Research allows a professional field such as dentistry to expand and developed. New technology has allowed dentistry as a profession to evolve and expand into greater dimensions of treatment, efficiency and patient comfort. Limitations are however still present and especially in the field of periodontics, endodontics and surgery. MSCs present themselves with a unique ability to regenerate lost tissue. Repairs of lost tissue is commonly practiced in many clinics and hospitals around the world, however complete regeneration has never been possible before. The researchers and dental professionals of today have a responsibility to further expand and drive the profession further. As of today, the world of MSCs research stands at an early but exciting stage of its life. Introducing MSCs fully into regenerative dentistry can potentially revolutionize the field. Exploring their potentials further and developing methods to integrate them into the dental profession is therefore necessary as it will bring huge benefits to patients and quality of life.

7. LITERATURE REVIEW

1. Gomez-Salazar M, Gonzalez-Galofre ZN, Casamitjana J, Crisan M, James AW, Péault B. Five Decades Later, Are Mesenchymal Stem Cells Still Relevant? Front Bioeng Biotechnol. 2020;8(February).

2. Sharpe PT. Dental mesenchymal stem cells. Dev. 2016;143(13):2273–80

3. Berebichez-Fridman R, Montero-Olvera P. Sources and Clinical Applications of Mesenchymal Stem Cells: State-of-the-art review. Sultan Qaboos University Medical Journal [SQUMJ]. 2018;18(3):264.

4. Li B, Chen X, Jin Y. Tooth and Dental Pulp Regeneration. A Roadmap to Non-Hematopoietic Stem Cell-based Therapeutics. 2019;:367-392.

5. Nakashima M, Iohara K. Mobilized Dental Pulp Stem Cells for Pulp Regeneration: Initiation of Clinical Trial. Journal of Endodontics. 2014;40(4):S26-S32.

6. Abdel Meguid E, Ke Y, Ji J, El-Hashash A. Stem cells applications in bone and tooth repair and regeneration: New insights, tools, and hopes. Journal of Cellular Physiology. 2017;233(3):1825-1835.

7. Kassmer S, Langenbacher A, Tomaso A, Shendy N, Raghu D, Roy S et al. Multipotent stem cells - Latest research and news | Nature [Internet]. Nature.com. 2020 [cited 6 December 2020]. Available from: https://www.nature.com/subjects/multipotent-stem-cells

8. Avinash K, Malaippan S, Dooraiswamy JN. Methods of isolation and characterization of stem cells from different regions of oral cavity using markers: A systematic review. Int J Stem Cells. 2017;10(1):12–20.

9. Hoffman, M., 2021. The Teeth (Human Anatomy): Diagram, Names, Number, and Conditions. [online] WebMD. Available at: <https://www.webmd.com/oral-health/picture-of-the-teeth#1> [Accessed 2 February 2021].

10. Yu, C. and Abbott, P., 2007. An overview of the dental pulp: its functions and responses to injury. Australian Dental Journal, 52, pp.S4-S6.

11. Zhao, J., Volponi, A., Caetano, A. and Sharpe, P., 2020. Mesenchymal Stem Cells in Teeth. Encyclopedia of Bone Biology, pp.109-118.

12. Ioannou, A., Kotsakis, G., McHale, M., Lareau, D., Hinrichs, J. and Romanos, G., 2015. Soft Tissue Surgical Procedures for Optimizing Anterior Implant Esthetics. International Journal of Dentistry, 2015, pp.1-9.

13. Said, K., Abu Khalid, A. and Farook, F., 2020. Anatomic factors influencing dimensions of soft tissue graft from the hard palate. A clinical study. Clinical and Experimental Dental Research, 6(4), pp.462-469.

14. Shi, L., Li, B., Zhang, B., Zhen, C., Zhou, J. and Tang, S., 2019. Mouse embryonic palatal mesenchymal cells maintain stemness through the PTEN-Akt-mTOR autophagic pathway. Stem Cell Research & Therapy, 10(1).

15. Lee, J., Kim, H., Park, J., Lee, D., Zhang, S., Green, D., Okano, T., Hong, J. and Jung, H., 2019. Developing palatal bone using human mesenchymal stem cell and stem cells from exfoliated deciduous teeth cell sheets. Journal of Tissue Engineering and Regenerative Medicine, 13(2), pp.319-327.

16. Loukas, M., Kapos, T., Louis, R., Wartman, C., Jones, A. and Hallner, B., 2006. Gross anatomical, CT and MRI analyses of the buccal fat pad with special emphasis on volumetric variations. Surgical and Radiologic Anatomy, 28(3), pp.254-260.

17. Hassani A, Shahmirzadi S, Saadat S. Applications of the Buccal Fat Pad in Oral and Maxillofacial Surgery. A Textbook of Advanced Oral and Maxillofacial Surgery Volume 3. 2016;.

18. Poissonnet, C., Burdi, A. and Bookstein, F., 1983. Growth and development of human adipose tissue during early gestation. Early Human Development, 8(1), pp.1-11.

19. Singh, J., Prasad, K., Lalitha, R. and Ranganath, K., 2010. Buccal pad of fat and its applications in oral and maxillofacial surgery: a review of published literature (February) 2004 to (July) 2009. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 110(6), pp.698-705.

20. Salehi-Nik N, Rezai Rad M, Kheiri L, Nazeman P, Nadjmi N, Khojasteh A. Buccal Fat Pad as a Potential Source of Stem Cells for Bone Regeneration: A Literature Review. Stem Cells Int. 2017;2017.

21. Farré-Guasch E, Martí-Pagès C, Hernández-Alfaro F, Klein-Nulend J, Casals N. Buccal Fat Pad, an Oral Access Source of Human Adipose Stem Cells with Potential for Osteochondral Tissue Engineering: An In Vitro Study. Tissue Engineering Part C: Methods. 2010;16(5):1083-1094.

22. Pringle S, Van Os R, Coppes RP. Concise review: Adult salivary gland stem cells and a potential therapy for xerostomia. Stem Cells. 2013;31(4):613–9.

23. Rodríguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramírez MC, Blanquer M, et al. Mesenchymal stem cells derived from dental tissues. Int Endod J. 2011;44(9):800–6.

24. Huang GTJ, Gronthos S, Shi S. Critical reviews in oral biology & medicine: Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in Regenerative Medicine. J Dent Res. 2009;88(9):792–806.

25. Ghannam MG, Alameddine H, Bordoni B. Anatomy, Head and Neck, Pulp (Tooth) [Updated 2020 Aug 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537112/

26. Gronthos S, Mankani M, Brahim J, Robey P, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and invivo. Proceedings of the National Academy of Sciences. 2000;97(25):13625-13630.

27. Batouli S, Miura M, Brahim J, Tsutsui T, Fisher L, Gronthos S et al. Comparison of Stem-cell-mediated Osteogenesis and Dentinogenesis. Journal of Dental Research. 2003;82(12):976-981.26.

28. Nakajima K, Kunimatsu R, Ando K, Hiraki T, Rikitake K, Tsuka Y et al. Success rates in isolating mesenchymal stem cells from permanent and deciduous teeth. Scientific Reports. 2019;9(1).

29. Sakai V, Zhang Z, Dong Z, Neiva K, Machado M, Shi S et al. SHED Differentiate into Functional Odontoblasts and Endothelium. Journal of Dental Research. 2010;89(8):791-796.

30. Huang G, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and BioRoot Engineering. Journal of Endodontics. 2008;34(6):645-651.

31. Broccaioli E, Niada S, Rasperini G, Ferreira L, Arrigoni E, Yenagi V et al. Mesenchymal Stem Cells from Bichat's Fat Pad:In VitroComparison with Adipose-Derived Stem Cells from Subcutaneous Tissue. BioResearch Open Access. 2013;2(2):107-117.

32. Shiraishi T, Sumita Y, Wakamastu Y, Nagai K, Asahina I. Formation of Engineered Bone with Adipose Stromal Cells from Buccal Fat Pad. Journal of Dental Research. 2012;91(6):592-597.

33. Khojasteh A, Sadeghi N. Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study. International Journal of Oral and Maxillofacial Surgery. 2016;45(7):864-871.

34. Okumura K, Shinohara M, Endo F. Capability of Tissue Stem Cells to Organize into Salivary Rudiments. Stem Cells International. 2012;2012:1-11.

35. Gorjup E, Danner S, Rotter N, Habermann J, Brassat U, Brummendorf T et al. Glandular tissue from human pancreas and salivary gland yields similar stem cell populations. European Journal of Cell Biology. 2009;88(7):409-421.

36. Najafi S, Nosrati H, Faraji Z, Mohamadnia A, Shirian S, Mortazavi S et al. Reconstruction of necrotic submandibular salivary gland using mesenchymal stem cells. Heliyon. 2020;6(10):e05162.

37. Lim J, Yi T, Choi J, Jang Y, Lee S, Kim H et al. Intraglandular transplantation of bone marrow-derived clonal mesenchymal stem cells for amelioration of post-irradiation salivary gland damage. Oral Oncology. 2013;49(2):136-143.

38. Jensen D, Oliveri R, Trojahn Kølle S, Fischer-Nielsen A, Specht L, Bardow A et al. Mesenchymal stem cell therapy for salivary gland dysfunction and xerostomia: a systematic review of preclinical studies. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2014;117(3):335-342.e1.

39. Lei M, Li K, Li B, Gao L, Chen F, Jin Y. Mesenchymal stem cell characteristics of dental pulp and periodontal ligament stem cells after in vivo transplantation. Biomaterials. 2014;35(24):6332-6343.

40. Ledesma-Martínez E, Mendoza-Núñez V, Santiago-Osorio E. Mesenchymal Stem Cells Derived from Dental Pulp: A Review. Stem Cells International. 2016;2016:1-12.

41. Ravindran S, Huang C, George A. Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs. Frontiers in Physiology. 2014;4

42. Ravindran S, Zhang Y, Huang C, George A. Odontogenic Induction of Dental Stem Cells by Extracellular Matrix-Inspired Three-Dimensional Scaffold. Tissue Engineering Part A. 2014;20(1-2):92-102.

43. Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M et al. Complete Pulp Regeneration After Pulpectomy by Transplantation of CD105+ Stem Cells with Stromal Cell-Derived Factor-1. Tissue Engineering Part A. 2011;17(15-16):1911-1920.

44. Ishizaka R, Iohara K, Murakami M, Fukuta O, Nakashima M. Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials. 2012;33(7):2109-2118.

45. Kumar P, Fathima G, Vinitha B. Bone grafts in dentistry. Journal of Pharmacy and Bioallied Sciences. 2013;5(5):125.

46. Hernández-Monjaraz B, Santiago-Osorio E, Ledesma-Martínez E, Aguiñiga-Sánchez I, Sosa-Hernández N, Mendoza-Núñez V. Dental Pulp Mesenchymal Stem Cells as a Treatment for Periodontal Disease in Older Adults. Stem Cells International. 2020;2020:1-12.

47. Y. Q. Qiao, L. S. Zhu, S. J. Cui, T. Zhang, R. L. Yang, and Y. H. Zhou, “Local administration of stem cells from human exfoliated primary teeth attenuate experimental periodontitis in mice,” The Chinese Journal of Dental Research, vol. 22, no. 3, pp. 157–163, 2019.

48. Ferrarotti F, Romano F, Gamba M, Quirico A, Giraudi M, Audagna M et al. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial. Journal of Clinical Periodontology. 2018;45(7):841-850.

49. AlJehani Y. Risk Factors of Periodontal Disease: Review of the Literature. International Journal of Dentistry. 2014;2014:1-9

50. Bianco P, Robey PG, Simmons PJ. Mesenchymal Stem Cells: Revisiting History, Concepts, and Assays. Cell Stem Cell. 2008;2(4):313–9.

51. Zheng C, Chen J, Liu S, Jin Y. Stem cell-based bone and dental regeneration: a view of microenvironmental modulation. Int J Oral Sci. 2019;11(3):1–16.

52. Broccaioli E, Niada S, Rasperini G, Ferreira LM, Arrigoni E, Yenagi V, et al. Mesenchymal stem cells from Bichat's fat pad: In vitro comparison with adipose-derived stem cells from subcutaneous tissue. Biores Open Access. 2013;2(2):107–17.

53. Sanz AR, Carrión FS, Chaparro AP. Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering. Periodontol 2000. 2015;67(1):251–67

54. Väänänen HK. Mesenchymal stem cells. Ann Med. 2005;37(7):469–79.

55. Caplan AI. Mesenchymal stem cells: Time to change the name! Stem Cells Transl Med. 2017;6(6):1445–51.

56. Narang S, Sehgal N. Stem cells: A potential regenerative future in dentistry. Indian J Hum Genet. 2012;18(2):150–4.

57. Yang JW, Shin YY, Seo Y, Kim HS. Therapeutic functions of stem cells from oral cavity: An update. Int J Mol Sci. 2020;21(12):1–24.

58. Bansal R, Jain A. Current overview on dental stem cells applications in regenerative dentistry. J Nat Sci Biol Med. 2015;6(1):29–34.

59. Murray IR, Péault B. Q&A: Mesenchymal stem cells - where do they come from and is it important? BMC Biol [Internet]. 2015;13(1):4–9. Available from: http://dx.doi.org/10.1186/s12915-015-0212-7

60. Shi S, Miura M, Seo BM, Robey PG, Bartold PM, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofacial Res. 2005;8(3):191–9.

61. Metcalf D. Concise Review: Hematopoietic Stem Cells and Tissue Stem Cells: Current Concepts and Unanswered Questions. Stem Cells. 2007;25(10):2390-2395.

62. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo B, Zhang C et al. Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine. PLoS ONE. 2006;1(1):e79.

63. Pittenger M, Discher D, Péault B, Phinney D, Hare J, Caplan A. Mesenchymal stem cell perspective: cell biology to clinical progress. npj Regenerative Medicine. 2019;4(1).

64. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J et al. Concise Reviews: Characteristics and Potential Applications of Human Dental Tissue-Derived Mesenchymal Stem Cells. STEM CELLS. 2015;33(3):627-638.

65. Junior A, Pinheiro C, Tanikawa D, Ferreira J, Amano M, Bueno D. Mesenchymal Stem Cells from Human Exfoliated Deciduous Teeth and the Orbicularis Oris Muscle: How Do They Behave When Exposed to a Proinflammatory Stimulus?. Stem Cells International. 2020;2020:1-15.

66. Nishino Y, Yamada Y, Ebisawa K, Nakamura S, Okabe K, Umemura E et al. Stem cells from human exfoliated deciduous teeth (SHED) enhance wound healing and the possibility of novel cell therapy. Cytotherapy. 2011;13(5):598-605.

67. Giuliani, A., Manescu, A., Langer, M., Rustichelli, F., Desiderio, V., Paino, F., De Rosa, A., Laino, L., d'Aquino, R., Tirino, V. and Papaccio, G., 2013. Three Years After Transplants in Human Mandibles, Histological and In-Line Holotomography Revealed That Stem Cells Regenerated a Compact Rather Than a Spongy Bone: Biological and Clinical Implications. STEM CELLS Translational Medicine, 2(4), pp.316-324.

68. Hernández-Monjaraz, B., Santiago-Osorio, E., Ledesma-Martínez, E., Aguiñiga-Sánchez, I., Sosa-Hernández, N. and Mendoza-Núñez, V., 2020. Dental Pulp Mesenchymal Stem Cells as a Treatment for Periodontal Disease in Older Adults. Stem Cells International, 2020, pp.1-12.

Fig 1: Avinash K, Malaippan S, Dooraiswamy JN. Methods of isolation and characterization of stem cells from different regions of oral cavity using markers: A systematic review. Int J Stem Cells. 2017;10(1):12–20.

Fig. 2: Forest Hill Family Dental. 2021. The Structure Of Your Teeth | Forest Hill Family Dental. [online] Available at: <https://foresthillfamilydental.com/813-2/> [Accessed 7 February 2021].

Fig 3: Memorial Sloan Kettering Cancer Center. 2021. Early Stage Oral Cavity Cancer. [online] Available at: <https://www.mskcc.org/cancer-care/patient-education/early-stage-oral-cavity> [Accessed 7 February 2021].

Fig.4: Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M et al. Complete Pulp Regeneration After Pulpectomy by Transplantation of CD105+ Stem Cells with Stromal Cell-Derived Factor-1. Tissue Engineering Part A. 2011;17(15-16):1911-1920.

8. ANNEX

frontiers in Bioengineering and Biotechnology

REVIEW
published: 28 February 2020
doi: 10.3389/fbioe.2020.00148

Five Decades Later, Are Mesenchymal Stem Cells Still Relevant?

Mario Gomez-Salazar[†], Zaniah N. Gonzalez-Galofre[†], Joan Casamitjana[1], Mihaela Crisan[1], Aaron W. James[2,3] and Bruno Péault[1,2]*

[1] MRC Centre for Regenerative Medicine and Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, United Kingdom, [2] Orthopaedic Hospital Research Center and Broad Stem Cell Research Center, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States, [3] Department of Pathology, Johns Hopkins University, Baltimore, MD, United States

Mesenchymal stem cells are culture-derived mesodermal progenitors isolatable from all vascularized tissues. In spite of multiple fundamental, pre-clinical and clinical studies, the native identity and role in tissue repair of MSCs have long remained elusive, with MSC selection *in vitro* from total cell suspensions essentially unchanged as a mere primary culture for half a century. Recent investigations have helped understand the tissue origin of these progenitor cells, and uncover alternative effects of MSCs on tissue healing *via* growth factor secretion and interaction with the immune system. In this review, we describe current trends in MSC biology and discuss how these may improve the use of these therapeutic cells in tissue engineering and regenerative medicine.

Keywords: tissue engineering, mesenchymal stem cell, pericyte, cell therapy, adventitia

OPEN ACCESS

Edited by:
Ranieri Cancedda, Independent Researcher, Genova, Italy

Reviewed by:
Ivan Martin, University of Basel, Switzerland
Mary Murphy, National University of Ireland Galway, Ireland

***Correspondence:**
Bruno Péault
bpeault@mednet.ucla.edu
[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Tissue Engineering and Regenerative Medicine, a section of the journal Frontiers in Bioengineering and Biotechnology

Received: *16 August 2019*
Accepted: *13 February 2020*
Published: *28 February 2020*

Citation:
Gomez-Salazar M, Gonzalez-Galofre ZN, Casamitjana J, Crisan M, James AW and Péault B (2020) Five Decades Later, Are Mesenchymal Stem Cells Still Relevant? Front. Bioeng. Biotechnol. 8:148. doi: 10.3389/fbioe.2020.00148

INTRODUCTION

Three main classes of stem cells can be used, in theory, for tissue regeneration and engineering. Organ resident, lineage specific stem cells should be ideal candidates but are rare, difficult to identify and purify and usually impossible to "expand" in culture. Conversely, culture adapted pluripotent stem cells may exhibit ultimate therapeutic potential, should their engraftment, differentiation and cycling be accurately controlled (reviewed in Robinton and Daley, 2012). In a third category fall multipotent cells endowed with mesodermal differentiation potential that proliferate in extended cultures of dissociated pre- and post-natal vascularized tissues, the prototype of which is the mesenchymal stem cell. MSCs have been used in around 1000 clinical trials (see ClinicalTrials.gov) in multiple indications as diverse as musculo-skeletal defects, disorders of the immune system including auto-immune diseases, and myocardial infarcts. In spite of this popularity, the MSC remains a biologic enigma, since retrospective derivation in culture has long concealed the true native identity of this cell, the role of which in tissue regeneration is also incompletely understood. Initially defined as a true stem cell driving cell-for-cell replacement, the MSC is now recognized primarily as a growth factor secretor and immunomodulatory agent (Sacchetti et al., 2007; Caplan, 2017). These combined functions drive tissue healing and rejuvenation, although their respective contributions to tissue repair remain unknown.

We have herein collated classic and recent results on mesenchymal stem cell phenotype, potentials and innate identity, and speculated about the future of MSCs in cell therapies and tissue engineering.

© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 2273-2280 doi:10.1242/dev.134189

REVIEW

Dental mesenchymal stem cells

Paul T. Sharpe*

ABSTRACT

Mammalian teeth harbour mesenchymal stem cells (MSCs), which contribute to tooth growth and repair. These dental MSCs possess many *in vitro* features of bone marrow-derived MSCs, including clonogenicity, expression of certain markers, and following stimulation, differentiation into cells that have the characteristics of osteoblasts, chondrocytes and adipocytes. Teeth and their support tissues provide not only an easily accessible source of MSCs but also a tractable model system to study their function and properties *in vivo*. In addition, the accessibility of teeth together with their clinical relevance provides a valuable opportunity to test stem cell-based treatments for dental disorders. This Review outlines some recent discoveries in dental MSC function and behaviour and discusses how these and other advances are paving the way for the development of new biologically based dental therapies.

KEY WORDS: Dental, Incisor, MSCs, Pulp, Teeth

Introduction

Teeth are composed of two layers of mineral – an outer layer of enamel and an inner layer of dentine that encloses the soft inner fibroblast pulp tissue (Fig. 1). Multiple different stem cell populations have been described in teeth and their supporting structures, many of which share *in vitro* properties with bone marrow-derived mesenchymal stem cells (MSCs; see Box 1). For this reason, these stem cell populations are collectively referred to as dental MSCs, although not all dental MSCs are equal in terms of their phenotypic and functional properties (reviewed in Volponi and Sharpe, 2013).

Dental MSCs play an important role in tooth homeostasis and repair. In the dental pulp, these cells remain active throughout life and generate odontoblasts, which function to repair damaged dentine. In addition, dental MSCs located in the periodontal ligament also play a role in repair, and may also be involved in homeostatic turnover of this tissue. But not all tooth tissue can be repaired or replaced; the cells that form enamel, the epithelial-derived ameloblasts, are lost when teeth erupt and thus damage to enamel cannot be naturally repaired. Current clinical treatments to repair tooth damage involve the use of inorganic materials and clinical regeneration of periodontal ligament tissue is currently very difficult. An understanding of the properties of the different resident dental MSC populations can thus inform the development of novel, biologically based dental therapies.

Our understanding of dental MSCs – what characterizes them and how they might be used in the clinic – has grown in recent years, as several discoveries have shed light on various aspects of dental MSC function and behaviour. This Review brings together these advances and provides an overview of some of the key findings in identification and heterogeneity of dental MSCs. The role of dental MSCs in endogenous tooth repair is also discussed, as well as how these cells can be used to treat several different dental disorders: from restoration of tooth pulp to mineral formation. Finally, some future directions regarding dental MSC research and its application in the clinic provides an interesting perspective on how to move this field forward in order to realise the potential that these cells hold for regenerative medicine.

Department of Craniofacial Development and Stem Cell Biology, Dental Institute, Kings College London, London SE1 9RT, UK.

*Author for correspondence (paul.sharpe@kcl.ac.uk)

P.T.S., 0000-0003-2116-9561

Dental MSCs: what are they and where do they come from?

Most of what is known, and indeed the 'dogma' regarding adult MSCs has come from the bone marrow. In many respects the tooth pulp can be considered to be similar to bone marrow. Both are highly vascularized, innervated 'soft' tissues that are surrounded by mineral. In both bone marrow and dental pulp, the MSCs are capable of differentiating into cells that generate the mineral. In bone marrow, this function is performed by the osteoblasts, whereas in teeth it is performed by the odontoblasts, which are derived from the dental pulp stem cells (DPSCs). Importantly, however, DPSCs have more restricted differentiation than bone marrow cells *in vivo*. Dental pulp can therefore provide a simple model system to study mesenchymal stem cells that is easily accessible and has a defined structure.

The dental MSCs that are the topic of this review are neural crest-derived (ecto)mesenchymal cells that are located in the pulp of deciduous and permanent adult teeth and the periodontal ligament (Gronthos et al., 2000, 2002; Miura et al., 2003; Seo et al., 2004; Balic et al., 2010; Koyama et al., 2009; Waddington et al., 2009; Wang et al., 2012). Each dental MSC population is named according to its tissue of origin, e.g. stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs) and so on (Fig. 2). In mice, the relative contributions of mesenchymal stem cells to growth and/or repair can be distinguished by studying incisors, which grow continuously throughout life (Fig. 3A) and by studying molars, which do not grow at all in adult mice.

Identifying dental MSCs

When tooth pulp is removed and cultured, a population of cells can be rapidly established that shows all the characteristics attributed to MSCs such as clonogenicity, expression of defining markers such as CD90 (Thy1 – Mouse Genome Informatics), CD73 (Nt5e), CD105 (Eng) markers and multi-lineage differentiation following appropriate stimulation (Gronthos et al., 2000, 2002; Miura et al., 2003). Labelling of pericytes using *Ng2-Cre* (*Ng2* is also known as *Cspg4*) and reporter lines shows that cells with all the characteristics of odontoblasts are derived from pericytes following experimental damage to the pulp (Feng et al., 2011). Thus, with the limited genetic lineage tracing that has been carried out to date, it appears that DPSCs can be derived from pericytes. However, pericytes are

Sultan Qaboos University Med J, August 2018, Vol. 18, Iss. 3, pp. e264–277, Epub. 19 Dec 18
Submitted 20 Feb 18
Revision Req. 18 Mar 18; Revision Recd. 16 Apr 18
Accepted 10 May 18
DOI: 10.18295/SQUMJ.2018.18.03.002

REVIEW

Sources and Clinical Applications of Mesenchymal Stem Cells

State-of-the-art review

*Roberto Berebichez-Fridman[1,2] and Pablo R. Montero-Olvera[2]

مصادر الخلايا الجذعية الوسيطة واستخداماتها السريرية

مراجعة حديثة

روبرتو بيريبتشيز–فريدمان و بابلو رودريغو مونتيرو–أولفيرا

ABSTRACT: First discovered by Friedenstein in 1976, mesenchymal stem cells (MSCs) are adult stem cells found throughout the body that share a fixed set of characteristics. Discovered initially in the bone marrow, this cell source is considered the gold standard for clinical research, although various other sources—including adipose tissue, dental pulp, mobilised peripheral blood and birth-derived tissues—have since been identified. Although similar, MSCs derived from different sources possess distinct characteristics, advantages and disadvantages, including their differentiation potential and proliferation capacity, which influence their applicability. Hence, they may be used for specific clinical applications in the fields of regenerative medicine and tissue engineering. This review article summarises current knowledge regarding the various sources, characteristics and therapeutic applications of MSCs.

Keywords: Mesenchymal Stem Cells; Adult Stem Cells; Regenerative Medicine; Cell Differentiation; Tissue Engineering.

الملخص: الخلايا الجذعية الوسيطة التي تم اكتشافها لأول مرة بواسطة فريدنشتاين في عام 1976 هي الخلايا الجذعية البالغة الموجودة في جميع أنحاء الجسم والتي تشترك في مجموعة ثابتة من الخصائص. تم اكتشاف هذه الخلايا في البداية في نخاع العظام، ويعتبر هذا المصدر الخلوي هو المعيار الذهبي للبحوث السريرية، على الرغم من أنه تم منذ ذلك الحين تحديد مصادر أخرى متنوعة–بما في ذلك الأنسجة الدهنية، ولب الأسنان، وخلايا الدم الطرفية المحركة والأنسجة المشتقة من مشيمة الولادة. وعلى الرغم من كونها متشابهة، فإن الخلايا الجذعية الوسيطة المستمدة من تلك المصادر مختلفة تمتلك خصائص مميزة ومزايا وعيوب، بما في ذلك إمكاناتها في التمايز وقدرتها على الانتشار، والتي تؤثر على قابليتها للتطبيق. ومن ثم، يمكن استخدامها في تطبيقات سريرية محددة في مجالات الطب التجديدي وهندسة الأنسجة. تلخص هذه المقالة المعرفة الحالية فيما يتعلق بمختلف المصادر والخصائص والتطبيقات العلاجية للخلايا الجذعية الوسيطة.

الكلمات المفتاحية: الخلايا الجذعية الوسيطة؛ الخلايا الجذعية للبالغين؛ الطب التجديدي؛ تمايز الخلايا؛ هندسة الانسجة.

Stem cells can be distinguished based on their differentiation potential and source within the human body. Embryonic stem cells are totipotent, because they can form both embryonic and extra-embryonic structures.[1] Furthermore, embryonic stem cells can proliferate indefinitely under specific culture conditions and retain the ability to differentiate into cell types of the three embryonic germ layers.[1,2] In contrast, adult stem cells are undifferentiated multipotent stem cells obtained from adult individuals and differentiate into the cell types that constitute their respective source tissues; accordingly, cells originating from neuronal tissue can differentiate into neurons, oligodendrocytes or astrocytes. This characteristic plasticity is an attribute of mesenchymal stem cells (MSCs), which are unspecialised cells with the ability to self-renew.[3,4]

Human MSCs are plastic-adherent cells that differentiate into cells that originate from the ectoderm and endoderm.[3–5] Moreover, they can abandon their unspecialised or undifferentiated states and transform into other mesenchymal lineages. Thus, they can regenerate bone, cartilage and fat and even become endothelial cells, muscle cells or neurons under physiological and experimental conditions.[3,4] While evidence suggests that MSCs are present in almost all human tissues, they were first isolated from mononuclear cells derived from bone marrow (BM).[3,6]

As MSCs are responsible for tissue repair, growth, wound healing and cell substitution resulting from physiological or pathological causes, they have various therapeutic applications such as in the treatment of central nervous system afflictions like spinal cord lesions.[4]

[1]*Department of Orthopaedic Surgery, American British Cowdray Medical Center, Mexico City, Mexico;* [2]*Tissue Engineering, Cell Therapy & Regenerative Medicine Unit, National Institute of Rehabilitation, Mexico City, Mexico*
**Corresponding Author's e-mail: berebichezmd@gmail.com*

Chapter 15

Tooth and Dental Pulp Regeneration

Bei Li[1], **Xi Chen**[1,2], **Yan Jin**[1]

[1]*State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi Key Laboratory of Oral Diseases, Center for Tissue Engineering, Fourth Military Medical University, Xi'an, China;* [2]*Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, China*

1. INTRODUCTION

Recently, advances in stem cell biology and tissue engineering have combined to accelerate the development of new cell-based regenerative therapies. Teeth are ectodermal organs that are derived, during embryonic development, from sequential reciprocal interactions between oral epithelial cells and cranial neural crest–derived mesenchymal cells. Teeth are unique complex organs that contain both hard (dentin and enamel) and soft (pulp and periodontium) tissues; thus, full restoration requires the regeneration of both types of tissues. Stem cell–based therapies are emerging as viable alternatives for accomplishing this regenerative process. Healthy teeth harbor stem cells throughout life and are likely to play a key role in maintaining tooth tissue. However, if robust stem cells are either not present or present in insufficient numbers (e.g., due to inflammation or tissue trauma), repair will not take place [1,2]. If the normal reparative/regenerative process fails, ex vivo expanded stem cells may be a good source of viable cells for replenishing the lost cells and/or repairing/recreating the stem cell niche and facilitate tissue regeneration [3].

Preclinical in vitro and in vivo studies have shown that cell-based dental pulp and periodontal regeneration therapies produce reliable and effective results in the management of tooth regeneration. In addition, several clinical trials have already begun or are in the planning stages that use ex vivo expanded dental stem cells to regenerate pulp or periodontal tissue in human patients [4]. In the case of cell-based therapies, stem cells or progenitor cells must be expanded in vitro to obtain sufficient number of cells to achieve a therapeutic effect. However, since stem cells are biologic entities that are extremely sensitive to their environment, their function/activity may change

A Roadmap to Nonhematopoietic Stem Cell-Based Therapeutics. https://doi.org/10.1016/B978-0-12-811920-4.00015-X

Mobilized Dental Pulp Stem Cells for Pulp Regeneration: Initiation of Clinical Trial

Misako Nakashima, PhD, and Koichiro Iohara, PhD

Abstract

Stem cell therapy is a potential strategy to regenerate the dentin-pulp complex, enabling the conservation and restoration of functional teeth. We assessed the efficacy and safety of pulp stem cell transplantation as a prelude before the initiation of clinical trials. Granulocyte-colony stimulating factor (G-CSF) induces subsets of dental pulp stem cells to form mobilized dental pulp stem cells (MDPSCs). Good manufacturing practice is a prerequisite for the isolation and expansion of MDPSCs that are enriched in stem cells, expressing a high level of trophic factors with properties of high proliferation, migration, and antiapoptotic effects and endowed with regenerative potential. The quality of clinical-grade MDPSCs was assured by the absence of abnormalities/aberrations in karyotype and the lack of tumor formation after transplantation in immunodeficient mice. Autologous transplantation of MDPSCs with G-CSF in pulpectomized teeth in dogs augmented the regeneration of pulp tissue. The combinatorial trophic effects of MDPSCs and G-CSF on cell migration, antiapoptosis, immunosuppression, and neurite outgrowth were also confirmed *in vitro*. Furthermore, MDPSCs from the aged donors were as potent as the young donors. It is noteworthy that there were no significant age-related changes in biological properties such as stability, regenerative potential, and expression of the senescence markers in MDPSCs. On the other hand, autologous transplantation of MDPSCs with G-CSF induced less regenerated pulp tissue in the aged dogs compared with the young dogs. In conclusion, the preclinical safety, feasibility, and efficacy of pulp regeneration by MDPSCs and G-CSF were established. Therefore, the standardization and establishment of regulatory guidelines for stem cell therapy in clinical endodontics is now a reality. *(J Endod 2014;40:S26–S32)*

Key Words

Angiogenesis, CD105, dentinogenesis, mobilized dental pulp stem cells, neurogenesis, preclinical trial, pulpectomy, pulp regeneration, preclinical trial, stem cell isolation method

In an aging society, the elderly face challenges in the maintenance of health including dental health, and the dental profession has emphasized the importance of the preservation of teeth for optimal quality of life. Dental pulp has multiple functions in the homeostasis of teeth, and maintenance of the function of pulp tissue is critical for the longevity of teeth. The ideal approaches for endodontic treatment are conservation of healthy tooth structure, prevention of microleakage from pulp cavity, and maintenance of the properties and mechanical strength of the tooth structure. Stem cell therapy with pulp stem/progenitor cells is a useful strategy to regenerate the dentin-pulp complex (1). Innervation and vasculature of dental pulp are intimately associated in pulp homeostasis, and both angiogenesis/vasculogenesis and neurogenesis are essential for pulp regeneration. We have previously shown complete pulp regeneration harnessing $CD105^+$ dental pulp stem cells (DPSCs) and pulp $CD31^-$ side population (SP) cells with stromal cell–derived factor 1 (SDF-1) in a canine pulpectomy model (2–4). The high regenerative potential of SP cells including angiogenesis/vasculogenesis and neurogenesis/reinnervation after transplantation in models of hind limb (2, 5, 6) and brain ischemia (6–8) and ectopic tooth root transplantation was shown (6). Thus, certain DPSC subsets might be useful for cell-based therapy (9–13). For clinical use, good manufacturing practice (GMP) is a prerequisite for the isolation of DPSC subsets. However, the safety of these $CD105^+$ cells and $CD31^-$ SP cells isolated by flow cytometry has not been established because there are still no GMP-grade flow cytometers. Another isolation method using GMP-grade immunomagnetic beads is not suitable for human DPSC subsets because a large number of the primary pulp cells is needed. The costs will also be prohibitive if CD105 magnetic beads are specially made to order for pulp stem cell isolation. Thus, there are no methods in place for flow cytometer or immunomagnetic beads to isolate GMP-grade DPSC subsets for clinical use.

In this investigation, we devised a method to isolate GMP-grade DPSC subsets using optimized G-CSF–induced mobilization (14) that is cost-effective and leading to safe and efficacious isolation from a small number of pulp cells. Here we present the regenerative potential of mobilized dental pulp stem cells (MDPSCs) in comparison with DPSCs in a hind limb ischemic model and an ectopic tooth root transplantation model. Potential clinical applications of MDPSCs were assessed by the safety and efficacy of pulp stem cell transplantation as a prelude for the

From the Department of Dental Regenerative Medicine, Center of Advanced Medicine for Dental and Oral Diseases, National Center for Geriatrics and Gerontology, Research Institute, Obu, Aichi, Japan.

This paper is based on a presentation from the International Association for Dental Research (IADR) Pulp Biology and Regeneration Group Satellite Meeting, which was held March 24–26, 2013 in San Francisco, California.

Address requests for reprints to Dr Misako Nakashima, Department of Oral Disease Research, National Center for Geriatrics and Gerontology, Research Institute, Obu, Aichi 474-8522, Japan. E-mail address: misako@ncgg.go.jp

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.01.020

Received: 27 March 2017 | Accepted: 28 March 2017

DOI: 10.1002/jcp.25940

MINI-REVIEW

WILEY Journal of Cellular Physiology

Stem cells applications in bone and tooth repair and regeneration: New insights, tools, and hopes

Eiman Abdel Meguid[1] | Yuehai Ke[2] | Junfeng Ji[3] | Ahmed H.K. El-Hashash[2,3,4,5]

[1] Centre for Biomedical Sciences Education, School of Medicine, Dentistry and Biomedical Sciences Queen's University, Belfast, Ireland, UK

[2] Molecular Medicine Research Centre, School of Basic Medical, Zhejiang University, Hangzhou, Zhejiang, China

[3] Dr. Li Dak Sum & Yip Yio Chin Centre of Stem Cell and Regenerative Medicine School of Medicine, Zhejiang University

[4] University of Edinburgh-Zhejiang University Institute (UoE- ZJU Institute), Edinburgh, UK

[5] Edinburgh Medical School, University of Edinburgh, Edinburgh, UK

Correspondence
Prof. Ahmed H.K. El-Hashash, PhD, (Office # 429, c/o Ms. Ajda Yang), The University of Edinburgh, Edinburgh Medical School, Zhejiang International Campus, (ZJU), Arts and Science Building, Haining, Zhejiang 314400, China.
Email: hashash05@yahoo.co.uk

Funding information
Key scientific and technological innovation group of Zhejiang Province, Grant number: 2013TD11

The exploration of stem and progenitor cells holds promise for advancing our understanding of the biology of tissue repair and regeneration mechanisms after injury. This will also help in the future use of stem cell therapy for the development of regenerative medicine approaches for the treatment of different tissue-species defects or disorders such as bone, cartilages, and tooth defects or disorders. Bone is a specialized connective tissue, with mineralized extracellular components that provide bones with both strength and rigidity, and thus enable bones to function in body mechanical supports and necessary locomotion process. New insights have been added to the use of different types of stem cells in bone and tooth defects over the last few years. In this concise review, we briefly describe bone structure as well as summarize recent research progress and accumulated information regarding the osteogenic differentiation of stem cells, as well as stem cell contributions to bone repair/regeneration, bone defects or disorders, and both restoration and regeneration of bones and cartilages. We also discuss advances in the osteogenic differentiation and bone regeneration of dental and periodontal stem cells as well as in stem cell contributions to dentine regeneration and tooth engineering.

KEYWORDS
bone, regeneration and engineering, stem cells, tissue repair, tooth

1 | INTRODUCTION

Stem cells can continuously produce unaltered daughters and could generate cells with different and more restricted properties. Stem cells can divide either symmetrically (allowing the increase of stem cell number) or asymmetrically. Asymmetric divisions keep the number of stem cells unaltered and are responsible for the generation of cells with different properties. These cells can either multiply (progenitors or transit amplifying cells) or be committed to terminal differentiation (Berika, Elgayyar, & El-Hashash, 2014; Elshahawy, Ibrahim, & El-Hashash, 2016; El-Hashash, 2015; Morrison & Kimble, 2006).

Progenitors and transit amplifying cells have a limited lifespan and, therefore, can only reconstitute a tissue for a short period of time when transplanted. In contrast, stem cells are self-renewing and thus can generate any tissue for a lifetime. This is a key property for a successful therapy. The capacity to expand stem cells in culture is an indispensable step for regenerative medicine, and a considerable effort has been made to evaluate the consequences of the cultivation on stem cell behavior. Scientists rely on indirect properties to identify stem cells such as the expression of a repertoire of surface proteins, slow cell cycle, clonogenicity, or an undifferentiated state. The evaluation of self-renewal is the ultimate way to show "stemness," which relies on the isolation and transplantation of a putative stem cell (clonal analysis) followed by its serial transplantation and long-term reconstitution of a tissue (He et al., 2009).

Recently, stem cells have been used extensively in many medical disciplines for the repair and/or regeneration of defective tissues and organs (e.g., bone, ligament, heart). New therapeutic approaches are largely inspired and based on our knowledge of embryonic development. The goal of regenerative medicine is to stepwise re-create in vitro all the mechanisms and processes that nature uses during initiation and morphogenesis of a given organ. In this context, stem cell research offers an amazing and seductive potential for body homeostasis, repair, regeneration, and pathology. The possibility of manipulating stem cells in situ using specific signaling molecules or by expanding them ex vivo is an exciting outcome of basic research. Hence, regenerative medicine has become a

pISSN 2005-3606 • eISSN 2005-5447
International Journal of Stem Cells Vol. 10, No. 1, 2017 https://doi.org/10.15283/ijsc17010

REVIEW ARTICLE

Methods of Isolation and Characterization of Stem Cells from Different Regions of Oral Cavity Using Markers: A Systematic Review

Kavarthapu Avinash, Sankari Malaippan, Jayakumar Nadathur Dooraiswamy

Department of Periodontology, Saveetha Dental College, Chennai, India

Background: Periodontitis is a destructive inflammatory disorder of the periodontium caused by the destruction of periodontal tissues namely the PDL, cementum, alveolar bone, and gingiva. Once these tissues are lost, the foremost goal of periodontal therapy is to regenerate the diseased tissues if possible to their original form, architecture, and function. Various regenerative procedures were employed and still a gap was found in achieving the goal. As stem cells are characterized by their ability to self-renew and differentiate to produce specialized cells, there could be a possibility of using them for regenerative therapy. Recently, dental tissues such as the PDL, the dental pulp and the tooth follicle have been recognized as readily available sources of adult stem cells.
Aim: The aim was to identify the various sources and methodologies in isolation of stem cells from human oral cavity and its differentiation into various lineages using markers.
Materials and Methods: The electronic databases PUBMED, GOOGLE SCHOLAR, SCIENCE DIRECT, COCHRANE LIBRARY along with a complimentary manual search of all periodontics journal till the year 2016. Thirteen articles were selected on the basis of the inclusion criteria. Isolation of stem cells from oral cavity through various methods has been evaluated and similarly characterization to different lineages were tabulated as variables of interest. They included human in-vitro and ex-vivo studies.
Results: The results showed that PDLSC's and pulpal stem cells are the most common source from where stem cells were isolated. Each source has used different methodology in isolating the stem cells and it was found that STRO-1 was the commonly used marker in all the studies mentioned.
Conclusions: The studies showed that there is no standard protocol existed in isolating the stem cells from different sources of oral cavity. Moreover, there was no standard marker or methodology used in characterization.

Keywords: Oral stem cell, Dental stem cell, Periodontal ligament stem cell, Gingival stem cell, Pulpal stem cell

Background

Stem cell research is the most fascinating area of interest today. The discovery of stem cells dates back in 1950`s when various experiments with bone marrow established their identity and their powerful role in regeneration of lost tissues. Earlier studies on human development had demonstrated that cells of embryo were able to produce every cell type in the body. Ernst Heinrich Philipp August Haeckel was the first person who described a fertilized ovule which would evolve into an organism (1). The team of scientists from the university of Madison were the first

Accepted for publication April 10, 2017, Published online May 30, 2017
Correspondence to **Kavarthapu Avinash**
Department of Periodontology, Saveetha Dental College, Ponamalle High Road, Chennai 600077, Tamilnadu, India
Tel: +919840379213, Fax: +918592234577
E-mail: avin_sourav@hotmail.com

Australian Dental Journal Supplement 2007;52:(1 Suppl):S4-S16

An overview of the dental pulp: its functions and responses to injury

C Yu,* PV Abbott*

Abstract

The dental pulp is a unique tissue and its importance in the long-term prognosis of the tooth is often ignored by clinicians. It is unique in that it resides in a rigid chamber which provides strong mechanical support and protection from the microbial rich oral environment. If this rigid shell loses its structural integrity, the pulp is under the threat of the adverse stimuli from the mouth, such as caries, cracks, fractures and open restoration margins, all of which provide pathways for micro-organisms and their toxins to enter the pulp. The pulp initially responds to irritation by becoming inflamed and, if left untreated, this will progress to pulp necrosis and infection. The inflammation will also spread to the surrounding alveolar bone and cause periapical pathosis. The magnitude of pulp-related problems should not be underestimated since their most serious consequence is oral sepsis, which can be life threatening, and hence correct diagnosis and management are essential. Clinicians must have a thorough understanding of the physiological and pathological features of the dental pulp as well as the biological consequences of treatment interventions.

Key words: Dental pulp, pulp disease, inflammation, necrosis.

Abbreviations and acronyms: CGRP = calcitonin gene-related peptides; IL = interleukins; PBF = pulp blood flow; SP = substance P; TTXr = tetrodotoxin-resistant; TTXs = tetrodotoxin-sensitive.

INTRODUCTION

The dental pulp resides in a rigid chamber comprising dentine, enamel and cementum, which provide strong mechanical support and protection from the microbial rich oral environment. However, if this rigid shell loses its structural integrity, the pulp is under the threat of the adverse stimuli from the mouth. Caries, cracks, fractures and open restoration margins provide pathways for micro-organisms and their toxins to enter the pulp. The response of the pulp to irritation is inflammation and, if unattended, this will eventually progress to pulp necrosis. Inflammation may spread to the surrounding alveolar bone and cause periapical pathosis. The magnitude of pulp-related problems should not be underestimated. The most serious consequence of pulp disease is oral sepsis, which can be life threatening.[1,2] If the infection spreads from the maxillary teeth, it may cause purulent sinusitis, meningitis, brain abscess, orbital cellulitis and cavernous sinus thrombosis, whereas infection from the mandibular teeth may cause Ludwig's angina, parapharyngeal abscess, mediastinitis, pericarditis, emphysema and jugular thrombophlebitis. Moreover, the number of teeth that are extracted result in mutilated dentitions, malnutrition and possible emotional problems.

Toothache is a common complaint in the dental clinic, and yet diagnosis of pulp disease is often difficult due to the seemingly unclear symptoms and the inaccessibility of the pulp for clinical tests. This is further complicated by referred toothache originating from the tissues other than the pulp. Improper diagnosis can lead to improper treatment, causing distress to the patient and embarrassment to the practitioner. An understanding of the histophysiology of the healthy pulp and the possible underlying pathological processes in the diseased pulp, careful assessment of the pain history, and appropriate clinical examination and diagnostic tests, should aid the dental practitioner in reaching an accurate diagnosis and a positive treatment outcome.

The dental pulp and its nature

The dental pulp derives from neural crest cells (the ectomesenchyme). Proliferation and condensation of these cells lead to formation of the dental papilla from which the mature pulp is derived. The mature pulp bears a strong resemblance to the embryonic connective tissue, with a layer of highly specialized cells, the odontoblasts, along its periphery.[3] The physical confinement of the dental pulp, its high incidence of sensory nerve innervation and the rich microcirculatory components make the dental pulp a unique tissue. Knowledge of the normal pulp function, its components and their interaction is necessary to provide a framework for understanding the changes that occur in diseased pulps.

Functions of the dental pulp

A fundamental question that needs to be addressed is whether the dental pulp is necessary in a fully formed

*School of Dentistry, The University of Western Australia.

Hindawi Publishing Corporation
International Journal of Dentistry
Volume 2015, Article ID 740764, 9 pages
http://dx.doi.org/10.1155/2015/740764

Review Article

Soft Tissue Surgical Procedures for Optimizing Anterior Implant Esthetics

Andreas L. Ioannou,[1] Georgios A. Kotsakis,[1] Michelle G. McHale,[1] Donald E. Lareau,[1,2] James E. Hinrichs,[1] and Georgios E. Romanos[3]

[1] *Advanced Education Program in Periodontology, University of Minnesota, 515 Delaware Street SE, Minneapolis, MN 55455, USA*
[2] *Private Practice, Edina, MN 55435, USA*
[3] *Department of Periodontology, School of Dental Medicine, Stony Brook, NY 11794, USA*

Correspondence should be addressed to Georgios A. Kotsakis; geo.kotsakis@gmail.com

Received 15 August 2014; Revised 2 November 2014; Accepted 4 November 2014

Academic Editor: Francesco Carinci

Implant dentistry has been established as a predictable treatment with excellent clinical success to replace missing or nonrestorable teeth. A successful esthetic implant reconstruction is predicated on two fundamental components: the reproduction of the natural tooth characteristics on the implant crown and the establishment of soft tissue housing that will simulate a healthy periodontium. In order for an implant to optimally rehabilitate esthetics, the peri-implant soft tissues must be preserved and/or augmented by means of periodontal surgical procedures. Clinicians who practice implant dentistry should strive to achieve an esthetically successful outcome beyond just osseointegration. Knowledge of a variety of available techniques and proper treatment planning enables the clinician to meet the ever-increasing esthetic demands as requested by patients. The purpose of this paper is to enhance the implant surgeon's rationale and techniques beyond that of simply placing a functional restoration in an edentulous site to a level whereby an implant-supported restoration is placed in reconstructed soft tissue, so the site is indiscernible from a natural tooth.

1. Introduction

Implant dentistry has been definitively established as a predictable treatment modality for replacing missing or nonrestorable teeth which yields excellent clinical success rates. During the last decade, the focus of implant research has shifted from the functional stability of the implant to its esthetic integration in the smile. The esthetics of implant restorations is dictated by two fundamental components: the reproduction of the natural tooth characteristics on the implant crown and the establishment of a soft tissue housing that will intimately embrace the crown. Therefore, the success of implant rehabilitation in the esthetic zone relies heavily on the preservation or the augmentation of peri-implant soft tissue by means of periodontal surgical procedures.

The aim of this paper is to enhance the implant surgeon's armamentarium with rationale and techniques that extend beyond the placement of a functional restoration in an edentulous site to the restoration of soft tissue harmony so that the implant-supported restoration is indiscernible from a natural tooth. This is especially important in areas of esthetic concern but not negligible in posterior sites where the added benefits of enhanced tissue contours cannot be overlooked.

2. Indications

It may not be an overstatement that every surgical implant procedure in the esthetic region constitutes an indication for soft tissue grafting. The inevitable alteration of the alveolar ridge dimensions that follows a tooth extraction often results in the placement of the implant in a site that has undergone a reduction in soft and hard tissue volume in comparison to its neighboring dentate sites [1–3]. This discrepancy is even more pronounced in single-implant sites where a concavity forms between the edentulous site and the root prominences of the neighboring dentition. Subepithelial connective tissue grafts (SCTG) or free gingival grafts (FGG) can be employed

Received: 12 December 2019 | Revised: 8 April 2020 | Accepted: 8 April 2020

DOI: 10.1002/cre2.298

ORIGINAL ARTICLE

Clinical and Experimental Dental Research WILEY

Anatomic factors influencing dimensions of soft tissue graft from the hard palate. A clinical study

Khalid N. Said[1,2,3] | Areej S. Abu Khalid[4] | Fathima F. Farook[1,3]

[1]College of Dentistry, King Saud Bin Abdul Aziz University for Health Sciences, Riyadh, Saudi Arabia

[2]Department of Dental Services, Ministry of National Guard-Health Affairs, Riyadh, Saudi Arabia

[3]King Abdullah International Medical Research Center, Riyadh, Saudi Arabia

[4]Department of Dental and Oral Health, Prince Sultan Military College of Health Sciences, Dhahran, Saudi Arabia

Correspondence
Fathima F. Farook, College of Dentistry, King Saud Bin Abdul Aziz University for Health Sciences, PO Box 3660, Riyadh, Saudi Arabia.
Email: fazrinaf@ksau-hs.edu.sa

Abstract

Objectives: The aim of the present study was to measure the thickness of the palatal mucosa in a Jordanian (Middle Eastern) population as well as identify possible factors that may influence the thickness of palatal mucosa.

Material and Methods: Sixty period on tally healthy subjects (29 males and 31 females) were selected. Fifteen measurement points were defined on the palate. The mucosal thickness in the hard palate was determined by "bone sounding" with a Hu-Friedy® round periodontal probe.

Results: The overall mean thickness of the palatal masticatory mucosa was 3.23 ± 0.47 mm. The mean thickness increased from the gingival margin to a more apical position irrespective of the tooth measured or side of the mouth in the following sequence: canine, second molar, first premolar, second premolar and lastly, the first molar. No significant difference between gender, smoking status, gingival phenotype andsides of the mouth with the thickness of palatal masticatory mucosa was determined. A significant difference between palatal shape and palatal gingival thickness was found.

Conclusions: The most appropriate site for graft harvesting is the canine-premolar area 8-13 mm from the mid-palatal aspect of each respective tooth in a Jordanian population. Except for the palatal shape, the side of the mouth, smoking, gender or gingival phenotype does not affect the graft harvest.

Clinical Relevance:

Scientific Rationale for Study: Knowledge on the thickness of the masticatory mucosa is crucialin making decisions for surgical treatment modality and may affect surgical outcome. We measured the thickness of the palatal mucosa in a Jordanian population and identified possible influencing factors.

Principal Findings: The thickness varied according to the teeth and the canine to premolar region was found to be the appropriate donor site.

Practical Implications: This information on safe zone for graft harvest can guide the periodontist to make appropriate incisions and choose the appropriate location to obtain a graft of adequate thickness and dimensions.

KEYWORDS

masticatory mucosa, palatal thickness, transgingival probing

Shi et al. Stem Cell Research & Therapy (2019) 10:217
https://doi.org/10.1186/s13287-019-1340-8

Stem Cell Research & Therapy

RESEARCH Open Access

Mouse embryonic palatal mesenchymal cells maintain stemness through the PTEN-Akt-mTOR autophagic pathway

Lungang Shi[1], Binchen Li[2], Binna Zhang[3], Congyuan Zhen[2], Jianda Zhou[4] and Shijie Tang[1*]

Abstract

Background: Both genetic and environmental factors are implicated in the pathogenesis of cleft palate. However, the molecular and cellular mechanisms that regulate the development of palatal shelves, which are composed of mesenchymal cells, have not yet been fully elucidated. This study aimed to determine the stemness and multilineage differentiation potential of mouse embryonic palatal mesenchyme (MEPM) cells in palatal shelves and to explore the underlying regulatory mechanism associated with cleft palate formation.

Methods: Palatal shelves excised from mice models were cultured in vitro to ascertain whether MEPM are stem cells through immunofluorescence and flow cytometry. The osteogenic, adipogenic, and chondrogenic differentiation potential of MEPM cells were also determined to characterize MEPM stemness. In addition, the role of the PTEN-Akt-mTOR autophagic pathway was investigated using quantitative RT-PCR, Western blotting, and transmission electron microscopy.

Results: MEPM cells in culture exhibited cell surface marker expression profiles similar to that of mouse bone marrow stem cells and exhibited positive staining for vimentin (mesodermal marker), nestin (ectodermal marker), PDGFRα, Efnb1, Osr2, and Meox2 (MEPM cells markers). In addition, exposure to PDGFA stimulated chemotaxis of MEPM cells. MEPM cells exhibited stronger potential for osteogenic differentiation as compared to that for adipogenic and chondrogenic differentiation. Undifferentiated MEPM cells displayed a high concentration of autophagosomes, which disappeared after differentiation (at passage four), indicating the involvement of PTEN-Akt-mTOR signaling.

Conclusions: Our findings suggest that MEPM cells are ectomesenchymal stem cells with a strong osteogenic differentiation potential and that maintenance of their stemness via PTEN/AKT/mTOR autophagic signaling prevents cleft palate development.

Keywords: Autophagy, Stemness, Mouse embryonic palatal mesenchyme cells, PTEN-Akt-mTOR signaling pathway

Background

The palate plays a vital role in shaping the embryonic facial primordia. Palate development in humans includes the primary palate formation (a small part of the adult hard palate) and secondary palate formation (hard and soft parts of the palate) [1]. Cleft palate is caused by abnormal formation of the secondary palate during embryonic development [2, 3]. It is one of the most common congenital birth defects (global incidence rate: 1 in 700 people). During normal palate development, the palatal shelves grow down vertically on both sides of the tongue till embryonic day 13.5 (E13.5); subsequently, these begin to elevate above the tongue at E14 and start growing horizontally towards each other. The initial contact of the palatal shelves initiates the formation of the medial edge epithelial (MEE) seam on E14.5; disintegration of the seam enables palatal fusion by E15.5 [4]. Since normal palate primarily comprises of mesenchymal cells surrounded by a thin layer of epithelial cells [2, 5–7], loss of viability of mouse embryonic palatal mesenchyme

* Correspondence: SJtang3@stu.edu.cn
[1]Department of Plastic Surgery and Burn Center, the Second Affiliated Hospital of Shantou University Medical College, North Dongxia Road, Shantou 515041, Guangdong, China
Full list of author information is available at the end of the article

Received: 6 July 2017 | Revised: 11 December 2018 | Accepted: 9 January 2019

DOI: 10.1002/term.2811

RESEARCH ARTICLE

WILEY

Developing palatal bone using human mesenchymal stem cell and stem cells from exfoliated deciduous teeth cell sheets

Jong-Min Lee[1*] | Hyun-Yi Kim[1*] | Jin-Sung Park[1] | Dong-Joon Lee[1] | Sushan Zhang[1] | David William Green[1] | Teruo Okano[2] | Jeong-Ho Hong[3] | Han-Sung Jung[1]

[1] Division in Anatomy and Developmental Biology, Department of Oral Biology, Oral Science Research Center, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea

[2] Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

[3] Department of Life Sciences, School of Life Sciences and Biotechnology, Korea University, Seoul, Korea

Correspondence
Han-Sung Jung, Division in Anatomy and Developmental Biology, Department of Oral Biology, Oral Science Research Center, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea.
Email: hsj8076@gmail.com

Funding information
National Research Foundation of Korea, Grant/Award Numbers: NRF-2016R1A5A2008630, NRF-2016R1C1B2013725 and NRF-2017M3A9E4048172; Ministry of Health and Welfare, Grant/Award Number: HI14C3266

Abstract

Cleft palate is one of the most common craniofacial defects in newborn babies. The characteristics of this genetic disease produce soft and hard tissue defects on the lip and maxilla, which cause not only aesthetic but also functional problems with speech, eating, and breathing. Bone grafts using autologous cancellous bone have been a standard treatment to repair the hard tissue defect in cleft palates. However, such grafts do not fully integrate into host bone and undergo resorption. To overcome engraftment problems, it is common to engineer new tissues with a combination of multipotent cells and biomaterial frameworks. Here, we manufactured cell sheets for bone repair of cleft palates derived from two osteogenic cell sources, human mesenchymal stem cells (hMSCs) and stem cells from human exfoliated deciduous teeth (SHEDs). Cell sheets made from hMSCs and SHEDs gave rise to in vitro calcification, which indicated the osteogenic potential of these cells. The cell sheets of hMSCs and SHEDs expressed the bone-specific osteogenic markers, osterix, osteocalcin, and osteopontin, following insertion into ex vivo-cultured embryonic palatal shelves and in ovo culture. In conclusion, we showed that osteogenic stem cell sheets have mineralization potential and might represent a new alternative to autologous bone transplantation in the reconstruction of cleft palates.

KEYWORDS

cell sheet, cleft palate, hMSC, palatal bone, regenerative medicine, SHED

1 | INTRODUCTION

Cleft lip and palate (CLP) is one of the most common human birth defects caused by various reasons (Lee et al., 2008). It begins early in utero during embryonic development and affects the base of the nose and the upper jaw bones. Asian and Native American populations show the highest affliction rates for CLP, as high as one in 500. The prevalence of CLP is one in 500 and one in 1,000 in European- and African-derived populations, respectively. These results indicate that different populations have different genetic susceptibilities to CLP (Beaty et al., 2010; Christensen & Mitchell, 1996; Mossey, Little, Munger, Dixon, & Shaw, 2009).

In mice, palatal shelves are localized on both sides of the tongue at E13.5. At E14.5, two palatal shelves are elevated on the tongue and make contact at the medial edge epithelium. After contact has been made between the palatal shelves, the medial edge epithelium forms a midline epithelial seam. At this stage, the midline epithelial seam disappears through an epithelial mesenchymal transition and apoptosis. Consequently, the palatal shelves become entirely fused. During this complex developmental event, failures in gene expression can occur, leading to insufficient tissue production and no closure between the plates, giving rise to a cleft palate (Bush & Jiang, 2012).

*Jong-Min Lee and Hyun-Yi Kim contributed equally to this work.

Surg Radiol Anat (2006) 28: 254–260
DOI 10.1007/s00276-006-0092-1

ORIGINAL ARTICLE

Marios Loukas · Theodoros Kapos · Robert G. Louis Jr
Christopher Wartman · Ashley Jones
Barry Hallner

Gross anatomical, CT and MRI analyses of the buccal fat pad with special emphasis on volumetric variations

Received: 31 May 2005 / Accepted: 15 December 2005 / Published online: 10 March 2006

Abstract The use of the buccal fat pad (BFP) has increased in popularity in recent years because of its reliability, ease of harvest, and low complication rate during oral and maxillofacial procedures. The aim of this study was to evaluate the volumetric variations of the BFP with a CT and MRI, as well as the thickness, weight and volume with conventional methods. We have examined the BFP from 80 formalin fixed adult cadavers (mean age 59) derived from both males (45) and females (35). In addition, we also examined 20 cadaveric BFPs using MR and CT imaging. Digital image analysis software was used to measure the volumetric distribution and to characterize the morphology of BFP. The BFP can be divided into three lobes (anterior, intermediate, and posterior) and has four extensions (buccal, pterygoid, pterygopalatine, and temporal). The BFP is fixed by six ligaments, to the maxilla, posterior zygoma, inner and outer rim of infraorbital fissure, temporalis tendon, and buccinator membrane.The mean volume in males was 10.2 ml and ranged 7.8–11.2 ml, while in females the mean volume was 8.9 ml and ranged 7.2–10.8 ml. Additionally, the mean thickness was 6 mm, with a mean weight of 9.7 g. These facts may be important when considering the use of the BFP in reconstruction, particularly whether the correct volume has been removed from each side in aesthetic, oral, or maxillofacial procedures.

Keywords Buccal fat pad · Maxillary reconstruction · Oral reconstruction · Facial pseudoherniation · Traumatic facial pseudolipoma

M. Loukas (✉)
Department of Anatomical Sciences, St George University, School of Medicine, True Blue Campus, St George's, Grenada
E-mail: edsg2000@yahoo.com
Tel.: +1-473-4444175

M. Loukas
Department of Education and Development, Harvard Medical School, Boston, MA, USA

T. Kapos
Department of Advanced Prosthodontics, Harvard School of Dental Medicine, Boston, MA, USA

R. G. Louis Jr · C. Wartman · A. Jones · B. Hallner
Department of Anatomy, American University of the Caribbean, Sint Maarten, Netherlands Antilles

Introduction

Although descriptions of the buccal fat pad (BFP) are typically very brief and lacking in detail in anatomical textbooks [1], they have recently received increased attention in the clinical literature. This stems largely from documentation of the use of BFP in oral and maxillofacial reconstruction [2–8]. Bichat [9] first considered the BFP, in 1802, as a well-circumscribed mass of fat (according to Khan, BFP is surrounded by a well-defined capsule giving the appearance of a well-circumscribed mass) without functional importance [10]. In modern medicine, however, clinicians realize that the BFP has several functional and therapeutic significances. In addition to its importance in filling deep tissue spaces and serving as a gliding pad during masticatory and facial muscle contraction, the BFP also acts to cushion important structures from the extrusion of muscle contraction or outer force impulsion [11]. Further exploration also indicates the BFP's role as an important structure in plastic and reconstructive procedures [12].

Several surgical procedures involve the use of the BFP as a graft or pedicled flap, including correction of a cleft palate, closure of chronic buccal fistulas and softening bone graft contours in infraorbital and maxillary deformities [5, 13, 14].

In addition, BFP herniations are very common, especially in infants and children. These herniations usually arise due to a tear in a mucosal surface or in the buccinator muscle and may proceed to push the BFP into the oral cavity [14]. The BFP can also herniate into the maxillary sinus in which case it can potentially be

Chapter 18

Applications of the Buccal Fat Pad in Oral and Maxillofacial Surgery

Ali Hassani, Solaleh Shahmirzadi and Sarang Saadat

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63133

Abstract

The buccal fat pad (BFP) has become more and more popular in oral and maxillofacial surgery. Originally, it was described as an anatomic structure without any obvious function; it was even considered to be a surgical nuisance. Nowadays, the most reported application of the BFP is the closure of oroantral communications. In this chapter, different aspects of the BFP such as its applications, anatomy, physiology, and complications are explained.

Keywords: buccal fat pad, oral reconstruction, oroantral communication, oroantral fistula, cleft palate, surgical defects

1. Introduction

Although descriptions of the buccal fat pad (BFP) are typically very brief and lacking in detail in anatomical textbooks, they have recently received increased attention in the clinical literature [1]. After the first clinical use of the BFP by Egyedi in 1977, its use has increased rapidly during these years. The BFP has become more and more popular for closing oronasal and oroantral communications (OACs) and as a versatile pedicle graft for closing postsurgical maxillary defects [2].

THE JOURNAL OF
PEDIATRICS

JULY 1988 Volume 113 Number 1, Part 1

MEDICAL PROGRESS

Growth and development of adipose tissue

Claude M. Poissonnet, MD, PhD, Marquisa LaVelle, PhD, and Alphonse R. Burdi, PhD

From the Department of Anatomy and Cell Biology and the Center for Human Growth and Development, University of Michigan, Ann Arbor, and the Department of Anthropology, Cornell University, Ithaca, New York

The study of growth and development of adipose tissue can give important clues to understanding the pathogenesis of obesity, one of the major public health and nutrition problems of our so-called developed societies. In 1981 the American Committee on Nutrition drew attention to "the need for studies of the pathogenesis of obesity in early life with an emphasis on the ontogeny of the fat organ."[1] Since then, little progress has been made in this area. There are still many difficulties in the study of adipose tissue in the human fetus. Thus the knowledge of fat tissue biology in humans is mostly indirect and leads to the problem of the relevance of animal data to man.

We focus on the cellular processes and the metabolic influences that occur during the prenatal and postnatal periods under normal and abnormal conditions. Especially important are the effects on adipose tissue growth of metabolic and dysmorphogenic disturbances such as maternal type I diabetes, intrauterine growth retardation, connective tissue dysplasia, and tumors. Our purpose is to summarize the major advances, with emphasis on recent experimental findings.

Supported in part by the U.S. Department of Health and Human Services grant No. DE 03610, from the National Institute of Dental Research.

Correspondence: Claude M. Poissonnet, MD, PhD, 107 rue Saint-Lazare, 75009 Paris, France.

Reprint requests: Alphonse R. Burdi, PhD, Department of Anatomy and Cell Biology, 5815 Medical Science Building II, The University of Michigan, Ann Arbor, MI 48109.

PRENATAL ADIPOSE TISSUE GROWTH

Adipogenesis. Relatively few studies[2-5] on human prenatal adipogenesis are available. Recently, five morphogenic phases have been identified[6]; (1) undifferentiated tissue (i.e., loose connective tissue), (2) angiogenesis (i.e., proliferation of primitive vessels associated with mesenchymal condensation), (3) mesenchymal lobules (i.e., mesenchymal cells differentiating into stellate preadipocytes within a vascular matrix), (4) primitive fat lobules (i.e., fine fat vacuoles in cell cytoplasm of mesenchymal lobules), and (5) definitive fat lobules (i.e., fat lobules well separated from each other by dense septa of perilobular mesenchymal tissue).

ACTH	Adrenocorticotropic hormone
FFA	Free fatty acids
LPL	Lipoprotein lipase

Attention has also been given to developmental timing. In the human fetus, fat tissue first arises in the facial buccal pad area. Before the fourteenth week of gestation, future fat consists of loose connective tissue composed of an amorphous ground substance and fibers (stage 1). The first indicator of adipogenesis is the aggregation of a dense mass of mesenchymal cells. Condensation of mesenchyme,

Buccal pad of fat and its applications in oral and maxillofacial surgery: a review of published literature (February) 2004 to (July) 2009

Jasmeet Singh,[a] Kavitha Prasad, MDS,[b] R. M. Lalitha, MDS,[c] and K. Ranganath, MDS,[d] Bangalore, India
DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY, M. S. RAMAIAH DENTAL COLLEGE

This review of the literature was performed to study the frequency and preference of usage of the buccal fat pad (BFP) in oral and maxillofacial reconstruction and to determine its potential versatility in various clinical applications. A computerized literature search using Medline, the JGate@Helinet database, and the Google internet search engine was performed for all relevant articles with specific keywords from February 2004 to July 2009. Focus was on the use of BFP regarding size, location, and types of defects and success and failure rates for various applications. It was found that BFP has been used most commonly for closure of oroantral communications/fistula, followed by reconstruction of maxillary defects; with closure of primary clefts, coverage of mucosal defects, etc. being other uses. Studies suggested that owing to favorable anatomic location, high vascularity, ease of handling, and low failure rate, the BFP has become the flap of choice for reconstruction of various oral defects. The size limitation of the BFP must be known to permit successful outcome. The results have been encouraging for clinicians to make use of potential benefits of the BFP in closure of defects in the oral and maxillofacial region. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:698-705)**

The buccal fat pad (BFP) has become more and more popular for closing oronasal and oroantral communications and as a versatile pedicled graft for closing post-surgical maxillary defects.[1] Originally described as an anatomic structure without any obvious function, it was for a long period even considered to be a surgical nuisance.[2-4] However, during the past 3 decades, the BFP has become a well established tool in oral and maxillofacial surgery for the closure of oroantral communications (OAC)[5] and reconstruction of small to medium-sized acquired or congenital soft tissue and bone defects in the oral cavity.[6]

The aim of the present article was to review the database available in the past 5 years regarding buccal fat pad (BFP), including its anatomy, clinical usage, success, and complications and to ascertain the reason for its preference over other modalities in various applications in oral surgery.

MATERIALS AND METHODS

A computerized literature search was performed using Medline, J-Gate@Helinet, and the Google search engine for articles published from February 2004 to July 2009 on BFP and its application. Key words used were buccal pad of fat and its application, buccal fat pad, Bichat's, oroantral communication/fistula. "And" was used as a Boolean operator to combine and narrow the search. The search produced 33 articles, which included case reports, case series, retrospective case series, randomized controlled trials, comparative studies, technical notes, and abstracts. Abstracts were not included in the review. Case series were found to be most informative regarding correlation between various uses and complications. Case reports were included for completion of discussion.

The following clinical uses of the BFP in the literature were observed: closure of OAC/orantral fistula (OAF) (270 cases), closure of postexcision defects (140 cases), as a covering for mucosal defects, closure of primary clefts, midline secondary clefts, in TMJ reconstruction, postincisional fibrotomy coverage in oral submucous fibrosis, elongation of soft palate, vocal cord augmentation, and root coverage.

BUCCAL PAD OF FAT

Terminology

The BFP was first described by Heister (1732), who believed this structure to be glandular in nature and termed it the "glandula molaris."[7,8] Bichat is credited with recognizing the true nature of the BFP. Therefore, it is commonly referred to as the boule de Bichat or bolle graisseusse in French; it is called wangenfettp-

[a]Postgraduate student.
[b]Professor.
[c]Professor and head.
[d]Professor.
Received for publication Feb 1, 2010; returned for revision Mar 12, 2010; accepted for publication Mar 14, 2010.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2010.03.017

Hindawi
Stem Cells International
Volume 2017, Article ID 8354640, 13 pages
https://doi.org/10.1155/2017/8354640

Review Article

Buccal Fat Pad as a Potential Source of Stem Cells for Bone Regeneration: A Literature Review

Nasim Salehi-Nik,[1] Maryam Rezai Rad,[2] Lida Kheiri,[3] Pantea Nazeman,[2] Nasser Nadjmi,[4] and Arash Khojasteh[1,5,6]

[1]*Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2]*Dental Research Center, Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[3]*School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[4]*University of Antwerp, The Team for Cleft and Craniofacial Anomalies, Oral and Maxillofacial Surgery, University of Antwerp, Antwerp, Belgium*
[5]*Medical Nano-Technology & Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[6]*Faculty of Medicine, University of Antwerp, Antwerp, Belgium*

Correspondence should be addressed to Arash Khojasteh; arashkhojasteh@yahoo.com

Received 3 December 2016; Revised 17 April 2017; Accepted 16 May 2017; Published 5 July 2017

Academic Editor: Heinrich Sauer

Adipose tissues hold great promise in bone tissue engineering since they are available in large quantities as a waste material. The buccal fat pad (BFP) is a specialized adipose tissue that is easy to harvest and contains a rich blood supply, and its harvesting causes low complications for patients. This review focuses on the characteristics and osteogenic capability of stem cells derived from BFP as a valuable cell source for bone tissue engineering. An electronic search was performed on all in vitro and in vivo studies that used stem cells from BFP for the purpose of bone tissue engineering from 2010 until 2016. This review was organized according to the PRISMA statement. Adipose-derived stem cells derived from BFP (BFPSCs) were compared with adipose tissues from other parts of the body (AdSCs). Moreover, the osteogenic capability of dedifferentiated fat cells (DFAT) derived from BFP (BFP-DFAT) has been reported in comparison with BFPSCs. BFP is an easily accessible source of stem cells that can be obtained via the oral cavity without injury to the external body surface. Comparing BFPSCs with AdSCs indicated similar cell yield, morphology, and multilineage differentiation. However, BFPSCs proliferate faster and are more prone to producing colonies than AdSCs.

1. Introduction

Mesenchymal stem cells (MSCs) derived from bone marrow aspirates have been frequently used as a cell source in bone tissue engineering [1]. However, several problems are associated with the clinical application of bone marrow stem cells (BMSCs) [1]. The harvesting procedure is associated with pain and discomfort for patients, and their differentiation capability is dependent on the donor age [2].

Adipose tissues have been introduced as a promising source of MSCs that can be obtained with minimal discomfort for patients, since subcutaneous adipose tissues are usually discarded after aesthetic surgical procedures. In addition, several studies have shown that the cell yield from adipose tissues is 100 to 500 times greater than that from bone marrow aspirates [3–5]. Therefore, minimally invasive procedures can be used to obtain a high number of MSCs with similar multilineage capabilities [6–8]. However, not all patients undergo liposuction, and fat distribution is dependent on body weight.

TISSUE ENGINEERING: Part C
Volume 16, Number 5, 2010

DOI: 10.1089/ten.tec.2009.0487

Buccal Fat Pad, an Oral Access Source of Human Adipose Stem Cells with Potential for Osteochondral Tissue Engineering: An *In Vitro* Study

Elisabet Farré-Guasch, D.D.S., M.D.,[1,2] Carles Martí-Pagès, M.D., FEBOMS, Ph.D.,[1,3]
Federico Hernández-Alfaro, D.D.S., M.D., F.E.B.O.M.S., Ph.D.,[1,4]
Jenneke Klein-Nulend, Ph.D.,[5] and Núria Casals, Ph.D.[2,6]

Stem cells offer an interesting tool for tissue engineering, but the clinical applications are limited by donor-site morbidity and low cell number upon harvest. Recent studies have identified an abundant source of stem cells in subcutaneous adipose tissue. Adipose stem cells (ASCs) present in adipose tissue are able to differentiate to several lineages and express multiple growth factors, which makes them suitable for clinical application. Buccal fat pad (BFP), an adipose-encapsulated mass found in the oral cavity, could represent an easy access source for dentists and oral surgeons. The stromal vascular fraction obtained from fresh BFP-derived adipose tissue and passaged ASCs were analyzed to detect and quantify the percentage of ASCs in this tissue. Here we show that BFP contains a population of stem cells that share a similar phenotype with ASCs from abdominal subcutaneous fat tissue, and are also able to differentiate into the chondrogenic, adipogenic, and osteogenic lineage. These results define BFP as a new, rich, and accessible source of ASCs for tissue engineering purposes.

Introduction

TISSUE ENGINEERING is an emerging field that allows regeneration with restitution of lost tissues, combining the principles of bioengineering, cell transplantation, and biomaterial engineering. There is increasing biological knowledge regarding human development that will likely allow new future therapies to satisfy the clinical patient's needs.

In the late 1960s Friedenstein *et al.* demonstrated that the mesenchymal stroma from human bone marrow contains a population of cells that proliferate when cultured on plastic and differentiate to cell lineages derived from the mesoderm, such as chondrocytes and osteoblasts.[1] Later, these precursor spindle-shaped cells are referred to as mesenchymal stem cells (MSCs),[2] and shown to differentiate to several lineages *in vitro*[3] and *in vivo*,[4–6] making these cells promising candidates for mesodermal defect repair. However, the clinical use of MSCs provides several problems, such as pain associated with the harvest procedure, complexity of the technique, and low cell number upon purification, especially in old donors.[7] This makes an *ex vivo* expansion step necessary to obtain therapeutic cell doses, which is time consuming and expensive, and contains the risk of cell contamination and cell loss.

Adipose tissue represents a promising source of MSCs available in large quantities, which does not require the use of general anesthesia and results in minimal patient discomfort. Further, adipose tissue yields higher numbers of MSCs than bone marrow, which could avoid cell expansion.[8] The nomenclature of MSCs from adipose tissue varies widely, but the consensus reached at the Second Annual International Fat Applied Technology Society Meeting (2004, Pittsburgh, PA) was to use the term "adipose stem cells" (ASCs). ASCs are able to differentiate to multiple mesenchymal tissue cell types, such as osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes, and endothelial cells, and there is growing evidence suggesting that they can also give rise to cells from other lineages, such as ectoderm and endoderm.[9–12] In addition, adipose tissue secretes a variety of angiogenic and antiapoptotic growth factors that makes fat a promising source for reconstructive surgery.[13] ASCs have

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry and [2]Department of Basic Sciences, Faculty of Medicine and Health Sciences, International University of Catalonia, Barcelona, Spain.
[3]Hospital Clínic de Barcelona, Barcelona, Spain.
[4]Institute of Maxillofacial Surgery and Implantology, Teknon Medical Center, Barcelona, Spain.
[5]Department of Oral Cell Biology, Research Institute MOVE, ACTA-University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands.
[6]CIBER Institute of Physiopathology of Obesity and Nutrition (CB06/03), Instituto de Salud Carlos III, Madrid, Spain.

STEM CELLS
REGENERATIVE MEDICINE

Concise Review: Adult Salivary Gland Stem Cells and a Potential Therapy for Xerostomia

SARAH PRINGLE,[a,b] RONALD VAN OS,[c] ROBERT P. COPPES[a,b]

[a]Department of Cell Biology; [b]Department of Radiation Oncology; and [c]European Research Into Biology of Ageing, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Key Words. Hyposalivation • Regeneration • Stem/progenitor cells • Cellular therapy

ABSTRACT

The ability to speak, swallow, masticate, taste food, and maintain a healthy oral cavity is heavily reliant on the presence of saliva, the hugely important effect of which on our everyday lives is often unappreciated. Hyposalivation, frequently experienced by people receiving radiation therapy for head and neck cancers, results in a plethora of symptoms whose combined effect can drastically reduce quality of life. Although artificial lubricants and drugs stimulating residual function are available to ameliorate the consequences of hyposalivation, their effects are at best transient. Such management techniques do not address the source of the problem: a lack of functional saliva-producing acinar cells, resulting from radiation-induced stem cell sterilization. Post-radiotherapy stimulation of cell proliferation only results in improved saliva secretion when part of the tissue has been spared or when the dose to the salivary gland (SG) remains below a certain level. Therefore, stem cell replacement therapy may be a good option to treat radiation-induced hyposalivation. Substantial progress has been made lately in the understanding of cell turnover in the SG, and the recent identification of stem and progenitor cell populations in the SG provides a basis for studies toward development of a stem cell-based therapy for xerostomia. Here, we review the current state of knowledge of SG stem cells and their potential for use in a cell-based therapy that may provide a more durable cure for hyposalivation. STEM CELLS *2013;31:613–619*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

More than 40,000 new patients in the U.S.A. are expected to be diagnosed with head and neck cancer in 2012 [1]. The majority of these patients will be treated with radiotherapy (RT) alone, or in combination with chemotherapy and/or surgery, with a consequent 5-year-survival rate of approximately 50% for non-metastatic locally advanced disease [2]. While significantly improving the patient's chances of survival, RT treatment often results in unavoidable co-irradiation of normal tissues surrounding the tumor, such as the salivary glands (SGs). Although protocols have been developed to minimize early and late loss of gland function following RT, 40% of head and neck cancer patients receiving the most modern intensity modulated RT will still experience moderate or severe xerostomia [3–7].

Induced by radiation, SG dysfunction and consequential hyposalivation causes many post-treatment complications, including hampered speech, dental problems, difficulties with swallowing and food mastication, impaired taste, and nocturnal oral discomfort. Hyposalivation and the resultant symptoms are together termed xerostomia ("dry mouth syndrome"), can lead to a dramatic loss in quality of life for the patient, and remains extremely difficult to manage [3, 4, 8, 9]. This review describes recent progress in our comprehension of radiation-induced hyposalivation, the characterization of rodent and human SG stem cells, and advances in design of an adult stem cell-based therapy for long-term treatment of hyposalivation in post-RT patients.

A CELLULAR BASIS FOR RADIATION-INDUCED LONG-TERM HYPOSALIVATION

The SGs of mice, rats, and humans are composed basically of two saliva-producing cells types, namely mucous and serous acinar cells, myoepithelial cells, which facilitate saliva expulsion and a ductal cell system which modifies saliva composition and through which saliva is secreted into the oral cavity (Fig. 1). Intertwined cholinergic and adrenergic nerve fibers stimulate saliva production and also indirectly affect SG secretion through innervation of the blood vessels that supply the glands. The whole consortium of cells is kept in close physical proximity to each other by supporting stromal tissue [10, 11] (Fig. 1). The impact of RT on function of SGs is bifaceted. Saliva-producing acinar cells are largely postmitotic in nature, and according

Author contributions: S.P.: conception and design, collection and/or assembly of data, data analysis and interpretation, and manuscript writing; R.v.O.: conception and design and manuscript writing; R.P.C.: conception and design, manuscript writing, and final approval of manuscript.

Correspondence: Robert P. Coppes, Ph.D., Department of Cell Biology, University of Groningen—University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. Telephone: +050-3632709; Fax: +050-3632913; e-mail: r.p.coppes@umcg.nl Received October 17, 2012; accepted for publication December 17, 2012; first published online in STEM CELLS *EXPRESS* January 17, 2013. 1066-5099/2013/$30.00/0 doi: 10.1002/stem.1327

International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01877.x

Mesenchymal stem cells derived from dental tissues

F. J. Rodríguez-Lozano[1], C. Bueno[2], C. L. Insausti[1], L. Meseguer[1], M. C. Ramírez[2], M. Blanquer[1], N. Marín[1], S. Martínez[2] & J. M. Moraleda[1]

[1]Cell Therapy Unit, Hospital Universitary Virgen de la Arrixaca, Faculty of Medicine, University of Murcia, Murcia; and [2]Institute of Neurosciences, University Miguel Hernández, Alicante, Spain

Abstract

Rodríguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramírez MC, Blanquer M, Marín N, Martínez S, Moraleda JM. Mesenchymal stem cells derived from dental tissues. *International Endodontic Journal*, **44**, 800–806, 2011.

Regeneration of tissues occurs naturally due to the existence of stem cells with the capacity to self-regenerate and differentiate; however, regenerative capacity decreases with age, and in many cases, regeneration is not sufficient to repair the damage produced by degenerative, ischaemic, inflammatory, or tumour-based diseases. In the last decade, advances have been made in the understanding of stem cells, the genes that control the alternative fates of quiescence and differentiation, and the niches that provide specific signals that modulate cell fate decisions. Embryonic stem-cell research is shedding light on the secrets of development. Adult stem cells (AS cells) are available from several sources. Bone marrow and connective tissue have been used in preliminary clinical trials for regenerative therapy. Recently, several types of AS cells have been isolated from teeth, including dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, dental follicle progenitor stem cells and stem cells from apical papilla. Preliminary data suggest that these cells have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes and neural cells. If confirmed, these data would support the use of these cells, which are easily obtained from extracted teeth, in dental therapies, including in regenerative endodontics, providing a new therapeutic modality.

Keywords: dental pulp stem cells, dental stem cells, mesenchymal stem cells, periodontal ligament stem cells, stem cells.

Received 18 July 2010; accepted 28 February 2011

Introduction

Cell therapy and tissue engineering have become a promising approach for dental pulp repair, especially in regenerative endodontics, where the regeneration of the dental-pulp complex with stem cells has been investigated (Ishimatsu *et al.* 2009, Kadar *et al.* 2009). However, the potential for pulp-tissue regeneration from implanted stem cells is yet to be tested in extensive clinical trials to evaluate their efficacy and safety (Murray *et al.* 2007). The use of stem cells in endodontic treatments such as apexification requires autogenous stem cells that revascularize dental pulp tissue, provide appropriate signalling molecule(s) as well as an ideal scaffold that will promote controlled cell growth and differentiation (Hargreaves *et al.* 2008). Although the challenges of introducing endodontic tissue engineering therapies are substantial, the potential benefits to patients and the profession are ground-breaking (Hans & Shetty 2009).

Stem cells are defined as clonogenic, self-renewing progenitor cells that can generate one or more specialized cell types (Moraleda *et al.* 2006). Based on their origin, there are two main types of stem cells: embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). ES cells are stem cells derived from the inner cell mass of an early,

Correspondence: Prof Jose M. Moraleda Jiménez, Servicio de Hematología y Hemoterapia, Unidad de Trasplante Hematopoyético y Terapia Celular, Hospital Universitario Virgen de la Arrixaca, Ctra. Madrid-Cartagena, El Palmar, 30120 Murcia, Spain (Tel.: 968 369532; fax: 968 369088; e-mail: jmoraled@um.es).

CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

G.T.-J. Huang[1]*, S. Gronthos[2], and S. Shi[3]

[1]University of Maryland, College of Dental Surgery, Dental School, Department of Endodontics, Prosthodontics and Operative Dentistry, 650 West Baltimore St., Baltimore, MD 21201, USA; [2]Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Adelaide 5000, South Australia, Australia; and [3]Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, CA 90033, USA; *corresponding author, ghuang@umaryland.edu

J Dent Res 88(9):792-806, 2009

Mesenchymal Stem Cells Derived from Dental Tissues *vs.* Those from Other Sources: Their Biology and Role in Regenerative Medicine

ABSTRACT

To date, 5 different human dental stem/progenitor cells have been isolated and characterized: dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and dental follicle progenitor cells (DFPCs). These post-natal populations have mesenchymal-stem-cell-like (MSC) qualities, including the capacity for self-renewal and multilineage differentiation potential. MSCs derived from bone marrow (BMMSCs) are capable of giving rise to various lineages of cells, such as osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells. The dental-tissue-derived stem cells are isolated from specialized tissue with potent capacities to differentiate into odontogenic cells. However, they also have the ability to give rise to other cell lineages similar to, but different in potency from, that of BMMSCs. This article will review the isolation and characterization of the properties of different dental MSC-like populations in comparison with those of other MSCs, such as BMMSCs. Important issues in stem cell biology, such as stem cell niche, homing, and immunoregulation, will also be discussed.

KEY WORDS: MSCs, DPSCs, SHED, SCAP, PDLSCs, DFPCs, stem cell niche, apical papilla, stem cell homing, tissue regeneration.

DOI: 10.1177/0022034509340867

Received April 23, 2008; Last revision February 3, 2009; Accepted February 13, 2009

INTRODUCTION

Stem cell biology has become an important field for the understanding of tissue regeneration and implementation of regenerative medicine. Since the discovery and characterization of multipotent mesenchymal stem cells (MSCs) from bone marrow (BM), MSC-like populations from other tissues have now been characterized based on the 'gold standard' criteria established for BMMSCs (Friedenstein *et al.*, 1976; Caplan, 1991; Prockop, 1997; Pittenger *et al.*, 1999; Gronthos *et al.*, 2003). Of those, MSC-like populations from adipose tissues and umbilical cord blood have been shown to be promising alternative multipotent MSC sources (Mareschi *et al.*, 2001; Zuk *et al.*, 2001). These MSCs are capable of giving rise to at least 3 cell lineages: osteogenic, chondrogenic, and adipogenic. Other lineages, such as myogenic, neurogenic, and tenogenic, may also be derived from BMMSCs. The search for MSC-like cells in specific tissues has led to the discovery of a variety of stem cells in every organ and tissue in the body in the past decades (reviewed by Baksh *et al.*, 2004; Porada *et al.*, 2006; Kolf *et al.*, 2007). Dental-tissue-derived MSC-like populations are among many other stem cells residing in specialized tissues that have been isolated and characterized. The first type of dental stem cell was isolated from the human pulp tissue and termed 'post-natal dental pulp stem cells' (DPSCs) (Gronthos *et al.*, 2000). Subsequently, 3 more types of dental-MSC-like populations were isolated and characterized: stem cells from exfoliated deciduous teeth (SHED) (Miura *et al.*, 2003), periodontal ligament stem cells (PDLSCs) (Seo *et al.*, 2004), and stem cells from apical papilla (SCAP) (Sonoyama *et al.*, 2006, 2008). Recent studies have identified a fifth dental-tissue-derived progenitor cell population, referred to as 'dental follicle precursor cells' (DFPCs) (Morsczeck *et al.*, 2005). However, the precise relationship among these different stem cell populations remains unclear.

During the characterization of these newly identified dental stem cells, certain aspects of their properties have been compared with those of BMMSCs. Dental stem cells display multidifferentiation potential, with the capacity to give rise to at least 3 distinct cell lineages: osteo/odontogenic, adipogenic, and neurogenic. Differences have been noted between the dental stem cell populations and BMMSCs, where dental stem cells appear to be more committed to odontogenic rather than osteogenic development. To date, dental-tissue-derived stem/progenitor cells have been used for tissue-engineering studies in large animals to assess their potential in pre-clinical

Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*

S. Gronthos, M. Mankani, J. Brahim, P. Gehron Robey, and S. Shi*

Craniofacial and Skeletal Diseases Branch, National Institute on Dental Research, National Institutes of Health, Bethesda, MD 20892

Edited by Darwin J. Prockop, Tulane University, New Orleans, LA, and approved September 26, 2000 (received for review July 5, 2000)

Dentinal repair in the postnatal organism occurs through the activity of specialized cells, odontoblasts, that are thought to be maintained by an as yet undefined precursor population associated with pulp tissue. In this study, we isolated a clonogenic, rapidly proliferative population of cells from adult human dental pulp. These DPSCs were then compared with human bone marrow stromal cells (BMSCs), known precursors of osteoblasts. Although they share a similar immunophenotype *in vitro*, functional studies showed that DPSCs produced only sporadic, but densely calcified nodules, and did not form adipocytes, whereas BMSCs routinely calcified throughout the adherent cell layer with clusters of lipid-laden adipocytes. When DPSCs were transplanted into immunocompromised mice, they generated a dentin-like structure lined with human odontoblast-like cells that surrounded a pulp-like interstitial tissue. In contrast, BMSCs formed lamellar bone containing osteocytes and surface-lining osteoblasts, surrounding a fibrous vascular tissue with active hematopoiesis and adipocytes. This study isolates postnatal human DPSCs that have the ability to form a dentin/pulp-like complex.

odontoblast | dentin | *in vivo* transplantation

During tooth formation, interactions between epithelial and dental papilla cells promote tooth morphogenesis by stimulating a subpopulation of mesenchymal cells to differentiate into odontoblasts, which in turn form primary dentin. Morphologically, odontoblasts are columnar polarized cells with eccentric nuclei and long cellular processes aligned at the outer edges of dentin (1). After tooth eruption, reparative dentin is formed by odontoblasts in response to general mechanical erosion or disruption, and through dentinal degradation caused by bacteria (2). These odontoblasts are thought to arise from the proliferation and differentiation of a precursor population, residing somewhere within the pulp tissue (3). Despite extensive knowledge of tooth development, and of the various specialized tooth-associated cell types, little is known about the characteristics and properties of their respective precursor cell populations in the postnatal organism.

To date, the identification and isolation of an odontogenic progenitor population from adult dental pulp tissue has never been done. It is known that in certain conditions, cultures of pulp cells derived from early developing dental root tissue and pulp tissue can develop an odontoblast-like appearance with the capacity to form mineralized nodules *in vitro* (4), a trait normally attributed to cultures of bone or bone marrow cells (5, 6). More is known about the characteristics of multipotent bone marrow stromal cells (BMSCs) and their potential to develop into osteoblasts, chondrocytes, adipocytes, myelosupportive fibrous-stroma, and perhaps even muscle and neural tissues (7–12). They are characterized by their high proliferative capacity *ex vivo*, whereas maintaining their ability to differentiate into multiple stromal cell lineages. The tissue-specific differentiation of BMSCs seems to be dependent on their state of differentiation and commitment, and the microenvironment in which they are located. By analogy, we speculated that adult dental pulp tissue might also contain a population of multipotential stem cells.

In the present study, clonogenic and highly proliferative cells were derived from enzymatically disaggregated adult human dental pulp, which we have termed DPSCs, and compared with BMSCs, cells with known stem cell character (13). We have previously shown that human bone is generated after xenogeneic transplantation of BMSCs with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier vehicle (9). We therefore explored the possibility that isolated *ex vivo*-expanded human DPSCs would also be capable of regenerating a dentin/pulp-like structure *in vivo* under similar conditions.

Materials and Methods

Subjects and Cell Culture. Normal human impacted third molars were collected from adults (19–29 years of age) at the Dental Clinic of the National Institute of Dental and Craniofacial Research under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. Tooth surfaces were cleaned and cut around the cementum-enamel junction by using sterilized dental fissure burs to reveal the pulp chamber. The pulp tissue was gently separated from the crown and root and then digested in a solution of 3 mg/ml collagenase type I (Worthington Biochem, Freehold, NJ) and 4 mg/ml dispase (Boehringer Mannheim) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-μm strainer (Falcon). Bone marrow cells, processed from marrow aspirates of normal human adult volunteers (20–35 years of age), were purchased from Poietic Technologies (Gaithersburg, MD) and then washed in growth medium. Single-cell suspensions (0.01 to 1×10^5/well) of dental pulp and bone marrow were seeded into 6-well plates (Costar) with alpha modification of Eagle's medium (GIBCO/BRL) supplemented with 20% FCS (Equitech-Bio, Kerrville, TX)/100 μM L-ascorbic acid 2-phosphate (Wako Pure Chemicals, Osaka)/2 mM L-glutamine/100 units/ml penicillin/100 μg/ml streptomycin (Biofluids, Rockville, MD), and then incubated at 37°C in 5% CO_2. To assess colony-forming efficiency, day 14 cultures were fixed with 4% formalin, and then stained with 0.1% toluidine blue. Aggregates of ≥50 cells were scored as colonies. Conditions for the induction of calcified bone matrix deposition *in vitro* were as reported (6). The proliferation rate of subconfluent cultures (first passage) of DPSCs and BMSCs was assessed by bromodeoxyuridine (BrdUrd) incorporation for 24 h by using a Zymed BrdUrd staining kit (Vector Laboratories).

Immunohistochemistry. Primary DPSCs and BMSCs were subcultured into 8-chamber slides (2×10^4 cells/well) (Nunc). The

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: DPSC, dental pulp stem cell; BMSC, bone marrow stromal cell; HA/TCP, hydroxyapatite/tricalcium phosphate; BrdUrd, bromodeoxyuridine; DSPP, dentin sialophosphoprotein; CFU-F, colony-forming unit-fibroblast.

*To whom reprint requests should be addressed. E-mail: sshi@dir.nidr.nih.gov.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.240309797. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.240309797

RESEARCH REPORTS
Biological

S. Batouli[1‡], M. Miura[1‡], J. Brahim[2], T.W. Tsutsui[1], L.W. Fisher[1], S. Gronthos[3], P. Gehron Robey[1], and S. Shi[1*]

[1]Craniofacial and Skeletal Diseases Branch, [2]Clinical Research Core, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Building 30, Room 222, NIDCR/NIH, 30 Convent Drive MSC-4320, Bethesda, MD 20892; [3]Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Frome Road Adelaide 5000, South Australia, Australia; ‡authors contributing equally to this work; *corresponding author, sshi@dir.nidcr.nih.gov

J Dent Res 82(12):976-981, 2003

Comparison of Stem-cell-mediated Osteogenesis and Dentinogenesis

ABSTRACT

The difference between stem-cell-mediated bone and dentin regeneration is not yet well-understood. Here we use an *in vivo* stem cell transplantation system to investigate differential regulation mechanisms of bone marrow stromal stem cells (BMSSCs) and dental pulp stem cells (DPSCs). Elevated expression of basic fibroblast growth factor (bFGF) and matrix metalloproteinase 9 (MMP-9, gelatinase B) was found to be associated with the formation of hematopoietic marrow in BMSSC transplants, but not in the connective tissue of DPSC transplants. The expression of dentin sialoprotein (DSP) specifically marked dentin synthesis in DPSC transplants. Moreover, DPSCs were found to be able to generate reparative dentin-like tissue on the surface of human dentin *in vivo*. This study provided direct evidence to suggest that osteogenesis and dentinogenesis mediated by BMSSCs and DPSCs, respectively, may be regulated by distinct mechanisms, leading to the different organization of the mineralized and non-mineralized tissues.

KEY WORDS: bone marrow stromal stem cell, dental pulp stem cell, osteoblast, odontoblast, transplantation.

Received May 14, 2003; Last revision September 18, 2003; Accepted September 23, 2003.

INTRODUCTION

Adult bone marrow stromal stem cells (BMSSCs) and adult dental pulp stem cells (DPSCs) are multipotent stem cells capable of differentiating into various cell types, including, but not limited to, osteoblasts/odontoblasts, adipocytes, and neural cells (Prockop, 1997; Azizi *et al.*, 1998; Gronthos *et al.*, 2000, 2002; Bianco *et al.*, 2001). Previous studies also demonstrated that BMSSCs are able to differentiate into chondrocytes and muscle cells (Prockop, 1997; Ferrari *et al.*, 1998; Johnstone *et al.*, 1998). Although bone and dentin are similar in their matrix protein composition, their organ structures are totally different. One of the most striking characteristics is that BMSSCs and DPSCs can generate a bone/marrow organ structure and a dentin/pulp complex, respectively (Krebsbach *et al.*, 1997; Gronthos *et al.*, 2000). However, the detailed mechanisms involved in the initiation and maintenance of the bone/marrow organ and dentin/pulp complex have yet to be determined. In this study, we demonstrate that bFGF and MMP-9, two important angiogenic factors, are temporally expressed in the connective tissue compartment of BMSSC transplants prior to marrow formation. In contrast, dentin sialoprotein (DSP), a highly specific dentin protein, is highly expressed during dentinogenesis in the DPSC transplants. Furthermore, we found that DPSCs were capable of forming reparative dentin-like tissue on the surface of human dentin *in vivo*. This study suggests that BMSSCs and DPSCs use distinct regulatory mechanisms to control their *in vivo* tissue regeneration.

MATERIALS & METHODS

Subjects and Cell Culture

Bone marrow mononuclear cells were purchased from Poietic Technologies (Gaithersburg, MD, USA). Human impacted third molars were collected from adults (19-29 yrs of age) at the Dental Clinic of the National Institute of Dental & Craniofacial Research under a protocol approved by the NIH Office of Human Subjects Research. Human DPSCs were isolated and cultured as previously described (Gronthos *et al.*, 2000, 2002). Briefly, the pulp tissue was separated from the crown and root and then digested in a solution of 3 mg/mL collagenase type I (Worthington Biochem, Freehold, NJ, USA) and 4 mg/mL dispase (Boehringer Mannheim GmbH, Mannheim, Germany) for 1 hr at 37°C. Single-cell suspensions (from 0.01 to 1 x 10^5/well) of bone marrow and dental pulp were cultured in six-well plates (Costar, Cambridge, MA, USA) with alpha modification of Eagle's Medium (GIBCO BRL, Grand Island, NY, USA) supplemented with 15% fetal calf serum (Equitech-Bio Inc., Kerrville, TX, USA), 100 μM L-ascorbic acid 2-phosphate (WAKO, Tokyo, Japan), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Biofluids Inc., Rockville, MD, USA), then incubated at 37°C in 5% CO_2.

Transplantation

Approximately 4.0 x 10^6 of *ex vivo* expanded BMSSCs and DPSCs were mixed with 40 mg of hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder

SCIENTIFIC REPORTS
natureresearch

OPEN

Success rates in isolating mesenchymal stem cells from permanent and deciduous teeth

Kengo Nakajima, Ryo Kunimatsu*, Kazuyo Ando, Tomoka Hiraki, Kodai Rikitake, Yuji Tsuka, Takaharu Abe & Kotaro Tanimoto

Stem cells from human exfoliated deciduous teeth (SHED) and human dental pulp stem cells (hDPSCs) have emerged as attractive cell sources for bone regeneration. However, the specific teeth and the conditions most suitable for stem cell isolation remain unclear. Therefore, the success rate of SHED and hDPSCs isolation, the patient age and remaining root length in deciduous teeth were evaluated. Successful isolation was defined as when the cell culture was maintained up to the third passage without any contamination or other issues. Remaining tooth length was calculated using the root-to-crown ratio from patient X-rays and compared to the norm value from the literature. The overall successful isolation rate of SHED and hDPSCs was 82% and 70%. The average patient ages at extraction of the deciduous teeth and permanent teeth were 11 years and 9 months, and 22 years and 10 months respectively. In the successful SHED group, the average remaining root length of the anterior deciduous teeth was 71.4%, and that of the deciduous molars was 61.4%. Successful isolation appears to be associated with patient age, length of the remaining root, and also mechanical stress and other factors. Tooth selection criteria need to be identified to improve the success rate.

Mesenchymal stem cells (MSCs) have emerged as a promising tool for tissue regeneration. Since the isolation of bone marrow MSCs (BMMSCs), MSC-like cells have been continuously discovered from various tissues. Human dental pulp stem cells (hDPSCs) were first isolated in 2000[1], followed by isolation of stem cells from human exfoliated deciduous teeth (SHED) in 2003[2]. Subsequently, stem cells from the periodontal ligament and apical papilla were isolated and characterised[3,4]. SHED are derived from the pulp of deciduous teeth, which are clinically and biologically discarded tissues. Thus, among these MSC sources of the dental tissue, SHED represent the most accessible and promising cell source for tissue regeneration.

We previously reported successful bone regeneration using autogenous BMMSCs in a dog model of artificial alveolar cleft[5,6]. However, since bone marrow collection is an invasive procedure for the patient, we have focused on the bone regeneration potential of SHED and hDPSCs both *in vitro* and *in vivo*. Indeed, the *in vivo* transplantation of human SHED and hDPSCs for bone regeneration has been reported, similar to human BMMSCs (hBMMSCs)[7]. *In vitro*, SHED and hDPSCs show high proliferation activity, and have similar differentiation ability to osteoblasts as that of hBMMSCs[8]. Moreover, SHED have been applied for regeneration of mineralised tissue[9–13]. Thus, SHED and hDPSCs could be ideal tools for bone regeneration. However, the tooth source that is most suitable for the isolation of MSCs and ultimate bone regeneration remains to be determined. Accordingly, in this preliminary study, we evaluated the success rate of SHED and hDPSCs isolation from deciduous and permanent teeth and related this rate to the condition of the teeth and general patient characteristics.

Methods

Cell isolation and culture. Human dental pulp tissue from both permanent and deciduous teeth was obtained from clinically healthy patients who required extraction for orthodontic treatment at Hiroshima University Hospital. The collection of tissues and isolation of SHED and hDPSCs from patients were approved by the preliminary review board of the Epidemiological Research Committee of Hiroshima University (approval number: E-20-1). hDPSCs were isolated and cultured as previously described[1,2]. In brief, the extracted permanent teeth were split using bone forceps at the cementum-enamel junction after the periodontal tissue was removed.

Department of Orthodontics and Craniofacial Developmental Biology, Division of Dental Sciences, Biomedical Sciences Major, Hiroshima University Graduate School of Biomedical & Health Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8553, Japan. *email: ryoukunimatu@hiroshima-u.ac.jp

RESEARCH REPORTS
Biological

V.T. Sakai[1,2], Z. Zhang[2], Z. Dong[2], K.G. Neiva[2], M.A.A.M. Machado[1], S. Shi[3], C.F. Santos[4], and J.E. Nör[2,5,6]*

[1]Department of Pediatric Dentistry, Orthodontics and Community Health, Bauru School of Dentistry, University of São Paulo, Brazil; [2]Angiogenesis Research Laboratory, Department of Cariology, Restorative Sciences and Endodontics, University of Michigan School of Dentistry, 1011 N. University, Rm. 2309, Ann Arbor, MI 48109-1078, USA; [3]Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California; Los Angeles, CA, USA; [4]Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, São Paulo, SP, Brazil; [5]Department of Biomedical Engineering, University of Michigan College of Engineering; and [6]Department of Otolaryngology, University of Michigan School of Medicine; *corresponding author, jenor@umich.edu

J Dent Res 89(8):791-796, 2010

SHED Differentiate into Functional Odontoblasts and Endothelium

ABSTRACT

Studies on mechanisms underlying the differentiation of dental pulp stem cells are critical for the understanding of the biology of odontogenesis and for dental tissue engineering. Here, we tested the hypothesis that stem cells from exfoliated deciduous teeth (SHED) differentiate into functional odontoblasts and endothelial cells. SHED were seeded in tooth slice/scaffolds and implanted subcutaneously into immunodeficient mice. SHED differentiated into functional odontoblasts that generated tubular dentin, as determined by tetracycline staining and confocal microscopy. These cells also differentiated into vascular endothelial cells, as determined by beta-galactosidase staining of LacZ-tagged SHED. *In vitro*, vascular endothelial growth factor (VEGF) induced SHED to express VEGFR2, CD31, and VE-Cadherin (markers of endothelium) and to organize into capillary-like sprouts. VEGF induced ERK and AKT phosphorylation (indicative of differentiation), while inhibiting phosphorylation of STAT3 (indicative of 'stemness'). Collectively, this work demonstrates that SHED can differentiate into angiogenic endothelial cells and odontoblasts capable of generating tubular dentin.

KEY WORDS: endodontics, stem cells, tissue engineering, odontogenesis, angiogenesis.

DOI: 10.1177/0022034510368647

Received September 10, 2009; Last revision February 23, 2010; Accepted February 26, 2010

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

INTRODUCTION

Dental pulp tissue engineering is an expanding field that aims for the replacement of an irreversibly inflamed or necrotic pulp by a healthy and functionally competent tissue that is capable of forming new dentin. Such a strategy is particularly attractive for the treatment of necrotic immature permanent teeth, since it has the potential of allowing for the completion of vertical and lateral root development (Nör, 2006). Successful engineering of any connective tissue requires the quick and effective induction of angiogenesis. The primary function of vasculature in the engineered constructs is to ensure the efficient delivery of nutrients and oxygen to cells invading from the host tissue and cells transplanted in the scaffolds (Jabbarzadeh *et al.*, 2008). The discovery of a cell type that is capable of differentiating into functional vascular endothelial cells would certainly be beneficial for dental pulp tissue engineering. In addition, better understanding of the differentiation potential of dental pulp stem cells is critical for the field of tooth development. Particularly, it is not clear if the blood vessels of the dental papilla are recruited from the neighboring mesenchyme, or if local stem cell populations are capable of forming *de novo* vascular structures (vasculogenesis). Here, we used a combination of *in vitro* and *in vivo* approaches to evaluate whether SHED are capable of differentiating into functional odontoblasts and angiogenic endothelium.

MATERIALS & METHODS

Tooth Slice/Scaffolds

SHED (Miura *et al.*, 2003) were cultured in alpha-MEM (Invitrogen, Grand Island, NY, USA) supplemented with 10% FBS and penicillin/streptomycin. Extracted non-carious human third molars were collected in the Department of Oral Surgery at the University of Michigan with the donors' informed consent and Institutional Review Board approval. Tooth slices (1 mm thick) were prepared, the pulp tissue was removed, and poly-L-lactic acid (PLLA) (Boehringer Ingelheim, Germany) scaffolds were cast within the pulp chamber (Appendix Fig. 1), as described previously (Cordeiro *et al.*, 2008). Specimens were treated with 10% EDTA for 1 min and randomly assigned to:

Published in final edited form as:
J Endod. 2008 June ; 34(6): 645–651. doi:10.1016/j.joen.2008.03.001.

The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and BioRoot Engineering

George T.-J. Huang, DDS, MSD, DSc[*], **Wataru Sonoyama, DDS, PhD**[§], **Yi Liu, DDS, PhD**[‡], **He Liu, DDS, PhD**[‖], **Songlin Wang, DDS, PhD**[‡], and **Songtao Shi, DDS, PhD**[†]

[*]*University of Maryland, College of Dental Surgery, Dental School, Department of Endodontics, Prosthodontics and Operative Dentistry, Baltimore, Maryland* [†]*Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, California* [‡]*Salivary Gland Disease Center and the Molecular Laboratory for Gene Therapy, Capital Medical University School of Stomatology, Beijing, China* [§]*Department of Oral and Maxillofacial Rehabilitation, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan* [‖]*Peking University School of Stomatology, Beijing, China.*

Abstract

Some clinical case reports have shown that immature permanent teeth with periradicular periodontitis or abscess can undergo apexogenesis after conservative endodontic treatment. A call for a paradigm shift and new protocol for the clinical management of these cases has been brought to attention. Concomitantly, a new population of mesenchymal stem cells residing in the apical papilla of permanent immature teeth recently has been discovered and was termed stem cells from the apical papilla (SCAP). These stem cells appear to be the source of odontoblasts that are responsible for the formation of root dentin. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to completion. This article reviews current findings on the isolation and characterization of these stem cells. The potential role of these stem cells in the following respects will be discussed: (1) their contribution in continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and (2) their potential utilization for pulp/dentin regeneration and bioroot engineering.

Keywords

Apexogenesis; apical papilla; bioroot engineering; dental pulp stem cells; immature teeth; periodontal ligament stem cells; pulp regeneration; stem cells from human exfoliated deciduous teeth; stem cells from the apical papilla

A number of recent clinical case reports have revealed the possibilities that many teeth that traditionally would receive apexification may be treated for apexogenesis. A call for a paradigm shift and new protocol for the clinical management of these cases has been made by the authors (1-3). A recent scientific finding, which may explain in part why apexogenesis can occur in these infected immature permanent teeth, is the discovery and isolation of a new population of mesenchymal stem cells (MSCs) residing in the apical papilla of incompletely developed teeth (4,5). These cells are termed stem cells from the apical papilla (SCAP), and they differentiate

Address requests for reprints to Dr George T.-J. Huang, University of Maryland, College of Dental Surgery, Dental School, Department of Endodontics, Prosthodontics and Operative Dentistry, 650 West Baltimore St, Baltimore, MD 21201. E-mail address: ghuang@umaryland.edu..

BioResearch Open Access
Volume 2, Number 2, April 2013

DOI: 10.1089/biores.2012.0291

Mesenchymal Stem Cells from Bichat's Fat Pad: *In Vitro* Comparison with Adipose-Derived Stem Cells from Subcutaneous Tissue

Eugenio Broccaioli,[1,*] Stefania Niada,[1,2,*] Giulio Rasperini,[1,3] Lorena Maria Ferreira,[1] Elena Arrigoni,[1] Vijay Yenagi,[1] and Anna Teresa Brini[1,2]

Abstract

Adipose-derived stem/stromal cells (ASCs) are progenitor cells used in bone tissue engineering and regenerative medicine. Since Bichat's fat pad is easily accessible for dentists and maxillo-facial surgeons, we compared the features of ASCs from Bichat's fat pad (BFP-ASCs) with human ASCs from subcutaneous adipose tissue (SC-ASCs). BFP-ASCs isolated from a small amount of tissue were characterized for their stemness and multidifferentiative ability. They showed an important clonogenic ability and the typical mesenchymal stem cell immunophenotype. Moreover, when properly induced, osteogenic and adipogenic differentiation markers, such as alkaline phosphatase activity, collagen deposition and lipid vacuoles formation, were promptly observed. Growth of both BFP-ASCs and SC-ASCs in the presence of human serum and their adhesion to natural and synthetic scaffolds were also assessed. Both types of ASCs adapted rapidly to human autologous or heterologous sera, increasing their proliferation rate compared to standard culture condition, and all the cells adhered finely to bone, periodontal ligament, collagen membrane, and polyglycol acid filaments that are present in the oral cavity or are commonly used in oral surgery. At last, we showed that amelogenin seems to be an early osteoinductive factor for BFP-ASCs, but not SC-ASCs, *in vitro*. We conclude that Bichat's fat pad contains BFP-ASCs with stemness features that are able to differentiate and adhere to biological supports and synthetic materials. They are also able to proliferate in the presence of human serum. For all these reasons we propose BFP-ASCs for future therapies of periodontal defects and bone regeneration.

Key words: amelogenin; biomaterials; buccal fat pad; mesenchymal stem/stromal cells; oral bone regeneration

Introduction

Mesenchymal stem/stromal cells (MSCs) represent important suitable candidates in regenerative medicine applications for the treatment of tissues damaged by trauma or pathological diseases. They have been isolated from bone marrow, adipose tissue, tendon, periodontal ligament, synovial membranes, trabecular bone, skin, periostium, and muscle.[1] Even though bone marrow represents the more used source of stem cells (BMSCs) in the clinical field, adipose tissue is a valid alternative source of MSCs. It is easily accessible in large quantities with a minimal invasive harvesting procedure and allow a high number of adipose-derived mesenchymal stem/stromal cells (ASCs) to be obtained.[2,3] ASCs show a multilineage differentiation capacity similar to that of BMSCs.[4–6] The growth factor secretome of MSCs was characterized by Wang et al.,[7] and the secretory activity of MSCs favors a regenerative microenvironment at sites of tissue injury.[8]

Adipose tissue withdrawn during plastic surgery is a discarded tissue, and the usual anatomical regions from which this tissue is collected are the abdomen, breast, buttock, knee, and thigh. In this study, we have characterized human ASCs isolated from the buccal fat pad, usually called Bichat's fat pad (BFP), one of the encapsulated fat masses in the cheek. It is a deep fat pad located on either side of the face between the buccinator muscle and several more superficial muscles, including the masseter, the zygomaticus major,

[1]Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy.
[2]Galeazzi Orthopedic Institute Research Hospital (IRCCS), Milan, Italy.
[3]Ca Granda Polyclinic Foundation Research Hospital (IRCCS), Milan, Italy.
*These authors contributed equally.

RESEARCH REPORTS
Biomaterials & Bioengineering

T. Shiraishi[1], Y. Sumita[1]*, Y. Wakamastu[1], K. Nagai[2], and I. Asahina[1]

[1]Department of Regenerative Oral Surgery, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan; and [2]Blood Transfusion Service, Nagasaki University Hospital, Nagasaki, Japan; *corresponding author, y-sumita@nagasaki-u.ac.jp

J Dent Res 91(6):592-597, 2012

Formation of Engineered Bone with Adipose Stromal Cells from Buccal Fat Pad

ABSTRACT

A robust method for inducing bone formation from adipose-derived stromal cells (ADSCs) has not been established. Moreover, the efficacy of strong osteogenic inducers including BMP-2 for ADSC-mediated bone engineering remains controversial. Meanwhile, the buccal fat pad (BFP), which is found in the oral cavity as an adipose-encapsulated mass, has been shown to have potential as a new accessible source of ADSCs for oral surgeons. However, to date, there have been no reports that define the practical usefulness of ADSCs from BFP (B-ADSCs) for bone engineering. Here, we report an efficient method of generating bone from B-ADSCs using rhBMP-2. The analyses show that B-ADSCs can differentiate *in vitro* toward the osteoblastic lineage by the addition of rhBMP-2 to culture medium, regardless of the presence of osteoinductive reagents (OSR), as demonstrated by measurements of ALP activity, *in vitro* calcification, and osteogenic gene expression. Interestingly, adipogenic genes were clearly detectable only in cultures with rhBMP-2 and OSR. However, *in vivo* bone formation was most substantial when B-ADSCs cultured in this condition were transplanted. Thus, B-ADSCs reliably formed engineered bone when pre-treated with rhBMP-2 for inducing mature osteoblastic differentiation. This study supports the potential translation for B-ADSC use in the clinical treatment of bone defects.

KEY WORDS: buccal fat pad, adipose tissue, mesenchymal stem cells, cultured condition, BMP-2, bone regeneration.

DOI: 10.1177/0022034512445633

Received November 23, 2011; Last revision March 21, 2012; Accepted March 21, 2012

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

INTRODUCTION

To date, bone-marrow-derived stromal cells (BMSCs) have been frequently used as a cell source in studies on bone engineering (Lecanda *et al.*, 1997; Uchida *et al.*, 2009; Agata *et al.*, 2010). However, most BMSCs are heterogeneous cell populations, and BMSCs from bone-marrow aspirate contain relatively few uncommitted mesenchymal stem cells (MSCs; Sacchetti *et al.*, 2007). Furthermore, BMSCs lose some capacity to differentiate into osteoblasts during culture expansion and passage (Agata *et al.*, 2010). An ideal source of cells for culture and subsequent clinical use must (1) provide a substantial number of MSCs at initial harvest, and (2) be from an easily accessible donor site with minimal morbidity.

Adipose tissue contains uncommitted stem cells that are similar to bone-marrow MSCs. Moreover, adipose-derived stromal cells (ADSCs), like BMSCs, are heterogeneous populations, but they reportedly have on the order of 100- to 500-fold more MSCs than do BMSCs after initial harvest (Fraser *et al.*, 2006). Therefore, many recent studies have assessed the potential of ADSCs for bone engineering (Levi *et al.*, 2010; Ying *et al.*, 2012). ADSCs have shown their ability to differentiate into osteogenic lineage *in vitro* (Zuk *et al.*, 2002; Lin *et al.*, 2008; Quarto *et al.*, 2008). However, their actual inducibility of *in vivo* bone formation is still under debate. Some previous studies reported that ADSCs may have an inferior potential for osteogenesis compared with BMSCs (Im *et al.*, 2005; Yoshimura *et al.*, 2007). For adipose tissue to become a potent source of MSCs for clinical use, efficient methods to apply ADSCs for bone engineering must be investigated further. Recently, Farrè-Guasch *et al.* showed that the buccal fat pad (BFP) contains a population of stem cells that share a similar phenotype with ADSCs from abdominal subcutaneous adipose tissue (Farrè-Guasch *et al.*, 2010). Under appropriate conditions, ADSCs from BFP (B-ADSCs) can differentiate to chondrocytes, osteoblasts, or adipocytes *in vitro*. Therefore, BFP might be a potential cell source for bone engineering in oral and maxillofacial areas, because it is easy to harvest and provides a reliable volume of tissue for oral surgeons.

It is well-known that the recombinant human bone morphogenetic protein-2 (rhBMP-2) enhances the effects of osteoinductive reagents (OSR) on osteogenic differentiation of BMSCs (Lecanda *et al.*, 1997). However, it has been suggested that rhBMP-2 may not influence the osteogenic differentiation of ADSCs (Chou *et al.*, 2011; Zuk *et al.*, 2011). Moreover, though rhBMP-2 has been shown to significantly enhance bone regeneration directly, direct implantation of high doses is known to induce substantial swelling that may cause airway obstruction when applied to oral and cervical areas (Jung *et al.*, 2003; Patel *et al.*, 2006). An efficient delivery method for the clinical use of rhBMP-2 also remains to be developed.

Int. J. Oral Maxillofac. Surg. 2016; xxx: xxx–xxx
http://dx.doi.org/10.1016/j.ijom.2016.01.003, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Pre-Implant Surgery

Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study

A. Khojasteh[1,2], N. Sadeghi[1]
[1]Dental Research Centre, Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]School of Medicine, University of Antwerp, Antwerp, Belgium

A. Khojasteh, N. Sadeghi: Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study. Int. J. Oral Maxillofac. Surg. 2016; xxx: xxx–xxx.

Abstract. Stem cell therapy for the treatment of bone defects is an alternative or adjunct to autologous bone grafting. This study assessed the efficacy of buccal fat pad-derived stem cells (BFPSCs) with iliac bone block grafting for the treatment of extensive human alveolar ridge defects. Eight patients with extensive jaw atrophy were selected for this study. The jaws were reconstructed with non-vascularized anterior iliac crest bone blocks. Gaps between the blocks were filled with freeze-dried bone granules and covered with a collagen membrane. In the test group ($n = 4$), these granules were seeded with BFPSCs. Cone beam computed tomography scans were used to assess the amount of new bone formed at six sites in each patient. Trephine biopsies of 2-mm were also taken from the graft site during implant placement for histomorphometric analysis. The mean bone width change at the graft site was greater in the test group than in the control group (3.94 ± 1.62 mm vs. 3.01 ± 0.89 mm). New bone formation was 65.32% in the test group versus 49.21% in the control group. The application of BFPSCs in conjunction with iliac bone block grafts may increase the amount of new bone formation and decrease secondary bone resorption in extensively atrophic jaws.

Key words: mesenchymal stem cell; tissue engineering; regenerative medicine; bone defect; bone graft.

Accepted for publication 7 January 2016

Hindawi Publishing Corporation
Stem Cells International
Volume 2012, Article ID 502136, 11 pages
doi:10.1155/2012/502136

Review Article

Capability of Tissue Stem Cells to Organize into Salivary Rudiments

Kenji Okumura,[1] Masanori Shinohara,[2] and Fumio Endo[1]

[1] *Department of Pediatrics, Kumamoto University School of Medicine, Honjo 1-1-1, Kumamoto 860-8556, Japan*
[2] *Department of Oral and Maxillofacial Surgery, Kumamoto University, Kumamoto, Japan*

Correspondence should be addressed to Fumio Endo, fendo@kumamoto-u.ac.jp

Received 8 September 2011; Accepted 5 January 2012

Academic Editor: Zev Gartner

Branching morphogenesis (BrM), an essential step for salivary gland development, requires epithelial-mesenchymal interactions. BrM is impaired when the surrounding mesenchyme is detached from the salivary epithelium during the pseudoglandular stage. It is believed that the salivary mesenchyme is indispensable for BrM, however, an extracellular matrix gel with exogenous EGF can be used as a substitute for the mesenchyme during BrM in the developing salivary epithelium. Stem/progenitor cells isolated from salivary glands in humans and rodents can be classified as mesenchymal stem cell-like, bone-marrow-derived, duct cell-like, and embryonic epithelium-like cells. Salivary-gland-derived progenitor (SGP) cells isolated from duct-ligated rats, mice, and swine submandibular glands share similar characteristics, including intracellular laminin and α6β1-integrin expression, similar to the embryonic salivary epithelia during the pseudoglandular stage. Progenitor cells also isolated from human salivary glands (human SGP cells) having the same characteristics differentiate into hepatocyte-like cells when transplanted into the liver. Similar to the dissociated embryonic salivary epithelium, human SGP cells aggregate to self-organize into branching organ-like structures on Matrigel plus exogenous EGF. These results suggest the possibility that tissue stem cells organize rudiment-like structures, and the embryonic cells that organize into whole tissues during development are preserved even in adult tissues.

1. Introduction

Salivary glands are small digestive organs that have a wide variety of functions and vary greatly in the dominant cell type in acini as well as cytodifferentiation of the acinar cells depending on the major glands. Salivary glands synthesize and secrete a large variety of polypeptides including growth factors that have systemic effects. Both epidermal growth factor (EGF) and nerve growth factor (NGF) isolation from mouse salivary gland are especially well known since researchers studying the topic were awarded a Nobel prize [1]. The EGF system regulates not only gastrointestinal mucosal constancy in adults, but salivary gland development during embryonic periods.

In this paper, we first discuss the histological and developmental biological aspects of the salivary gland, which are helpful for understanding their stem/progenitor cell characteristics. Branching morphogenesis (BrM) is a developmental process for epithelial cell-forming branching tubules that are present in various exocrine organs such as the lungs as well as mammary, prostate, and lacrimal glands. BrM is well characterized in the salivary glands, and these glands have contributed as a good experimental model in developmental biology for over 50 years. BrM is a result of epithelial-mesenchymal interactions and is regulated by extracellular matrix (ECM) composition and growth factors. Many ECM proteins including collagens, laminins, proteoglycans, and fibronectin play important roles in salivary gland morphogenesis [2]. Among these proteins, laminins are essential components of the basement membrane (BM), and ECM receptor integrins expressed on salivary glands epithelia play important roles in BrM. Perturbations in laminins and integrin interactions induce abnormal BrM. Interestingly, the BrM process does not necessarily demand mesenchymal cells, and BM-like substratum plus exogenous EGF induce BrM in mouse salivary rudiment *in vitro* [3].

Available online at www.sciencedirect.com
ScienceDirect
European Journal of Cell Biology 88 (2009) 409–421
European Journal of Cell Biology
www.elsevier.de/ejcb

Glandular tissue from human pancreas and salivary gland yields similar stem cell populations

Erwin Gorjup[a,1], Sandra Danner[b,1], Nicole Rotter[c], Jens Habermann[d], Ute Brassat[e], Tim H. Brummendorf[e], Sascha Wien[a], Andreas Meyerhans[f], Barbara Wollenberg[c], Charli Kruse[b], Hagen von Briesen[a,*]

[a]*Department of Cell Biology and Applied Virology, Fraunhofer Institute for Biomedical Engineering, Ensheimer Straße 48, D-66386 St. Ingbert, Germany*
[b]*Fraunhofer Institute for Marine Biotechnology, Lübeck, Germany*
[c]*Department of Otorhinolaryngology, University Hospital of Schleswig-Holstein, Lübeck, Germany*
[d]*Department of Surgery, University Hospital of Schleswig-Holstein, Lübeck, Germany*
[e]*University Cancer Center Hamburg (UCCH), University Hospital Hamburg-Eppendorf, Hamburg, Germany*
[f]*Institute of Virology, University of the Saarland, Homburg/Saar, Germany*

Received 16 November 2007; received in revised form 23 January 2009; accepted 25 February 2009

Abstract

Stem cells derived from pancreatic tissue are well characterized and exhibit a broad plasticity as they can differentiate beyond lineage boundaries into many cell types. The aim of this study was the comparative characterization of pancreatic stem cells with one other derivate of the embryonic foregut, namely salivary glands, for the existence of similar stem cell populations. The expression of stem cell markers as well as lineage-specific markers was detected by reverse transcription polymerase chain reaction, flow cytometry and immuncytochemical staining. The isolated cells from salivary glands and pancreas grew adherently in vitro and could be maintained for up to 55 and 46 population doublings, respectively. Cells from both tissues showed a comparable phenotype. They expressed different embryonic and adult stem cell markers and had the ability to differentiate spontaneously into cells representing the three embryonic germ layers. Additionally, the directed differentiation of glandular stem cells into the mesodermal lineage was achieved, yielding adipogenic, osteogenic and chondrogenic cells from salivary gland stem cells as well as osteogenic and chondrogenic cells from pancreatic stem cells. Here, we compared two stem cell populations from different glandular tissues which showed similar phenotypes and analogous properties. During embryonic development the two exocrine glands originate from the foregut, which might be the explanation for these intriguing resemblances.

Keywords: Adult stem cells; Progenitor cells; Exocrine glands; Pancreas; Salivary gland; Differentiation

Introduction

Stem cells with their high potential for self renewal and their capability to emerge different cell types of the

*Corresponding author. Tel.: +49 6894 980 286; fax: +49 6894 980 400.
E-mail address: hagen.briesen@ibmt.fraunhofer.de (H. von Briesen).
[1]These authors contributed equally to this study.

0171-9335/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2009.02.187

Heliyon 6 (2020) e05162

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Reconstruction of necrotic submandibular salivary gland using mesenchymal stem cells

Shamsoulmolouk Najafi [a,b,c], Haleh Nosrati [d], Zahra Faraji [e], Abdolreza Mohamadnia [f,g], Sadegh Shirian [h,i,j], Seyed Mostafa Mortazavi [k], Naghmeh Bahrami [l,m,*]

[a] Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Dept. of Oral & Maxillofacial Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[c] Dept of Oral & Maxillofacial Medicine, School of Dentistry, International Campus, Tehran University of Medical Sciences, Tehran, Iran
[d] Tehran University of Medical Sciences, Tehran, Iran
[e] Tehran University of Medical Science, Tehran, Iran
[f] Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and lung Diseases (NRITLD), ShahidBeheshti University of Medical Sciences, Tehran, Iran
[g] Department of Biotechnology, School of Advanced Technologies in Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran
[h] Department of Pathology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[i] Shiraz Molecular Pathology Research Center, Dr Daneshbod Lab, Shiraz, Iran
[j] Shefa Neuroscience Research Center, Tehran, Iran
[k] Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Alborz University of Medical Science, Karaj, Iran
[l] Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[m] Craniomaxillofacial Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Biochemistry
Cancer research
Cell biology
Molecular biology
Regenerative medicine
Stem cell research
Dry mouth
Salivary glands reconstruction
Stem cell transplantation

ABSTRACT

Background: The efficacy of mesenchymal stem cells (MSCs) to treat the necrotic tissue of salivary glands (SGs) has yet investigated.

Objective: This study was conducted to investigate the potential capacity of MSCs to restore the function and regenerate the necrotic submandiular gland in the rat animal model.

Methods: Twenty-one Sprague–Dawley rats were provided from a breeding colony and randomly divided into three groups including the positive control or induced SG atrophy without treatment, the treatment group or induced SG atrophy with MSCs isolated transplantation and the negative control group consists of healthy rats. The atrophic and necrotic submandiular gland was induced using intraoral duct ligation of the main duct of submandiular gland for one month. The isolated stem cells were confirmed using flow cytometry for CD90 and CD 105. The isolated MSCs were cultured and injected to submandiular gland and the potential efficacy of MSCs to treat the atrophic submandibular glands was evaluated using histopathology on two weeks post-transplantation. To detect the acinar cell protein secretory granules, Alcian Blue and periodic acid shift (PAS) staining were done. For the demonstration of mitotic index or proliferation rate of the SG epithelia tissue, Ki-67 and Smbg proteins expression were evaluated using immunohistochemistry.

Results: The locally injected MSCs could regenerate the overall histological structure of the necrotic submandibular gland tissue within 2 weeks of post-transplantation. Alcian Blue and PAS staining indicated that the mean amount of serous and mucin secretions in the treatment group was significantly increased compared to the positive control groups. We have also found that the treatment group significantly express higher Ki-67 protein, as a diagnostic marker for cell mitosis and proliferation rate, and lower Smbg protein, as a diagnostic marker, for damage to the submandibular gland than that of control group.

Conclusion: This study demonstrates the therapeutic benefits of MSCs isolated from the SG in treating atrophic and necrotic SGs in a rat model. MSCs may be potential candidates for cell-based therapies targeting hypofunction of SG induced by a range of diseases or because of surgery and radiotherapy of head and neck cancers.

* Corresponding author.
E-mail address: n-bahrami@sina.tums.ac.ir (N. Bahrami).

https://doi.org/10.1016/j.heliyon.2020.e05162
Received 15 August 2020; Received in revised form 21 September 2020; Accepted 1 October 2020

Oral Oncology 49 (2013) 136–143

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Intraglandular transplantation of bone marrow-derived clonal mesenchymal stem cells for amelioration of post-irradiation salivary gland damage

Jae-Yol Lim [a,b,e], TacGhee Yi [b,c,e], Jeong-Seok Choi [a,b], Yun Ho Jang [a,b], Songyi Lee [a,b], Hun Jung Kim [d], Sun U. Song [b,c,e], Young-Mo Kim [a,b,*,e]

[a] Department of Otorhinolaryngology – Head and Neck Surgery, Inha University School of Medicine, Incheon, Republic of Korea
[b] Translational Research Center, Inha University School of Medicine, Incheon, Republic of Korea
[c] Inha Research Institute for Medical Sciences, Inha University School of Medicine, Incheon, Republic of Korea
[d] Department of Radiation Oncology, Inha University School of Medicine, Incheon, Republic of Korea

ARTICLE INFO

Article history:
Received 20 June 2012
Received in revised form 15 August 2012
Accepted 17 August 2012
Available online 13 September 2012

Keywords:
Salivary glands
Head and neck neoplasms
Radiotherapy
Xerostomia
Mesenchymal stem cells

SUMMARY

Objectives: External irradiation in head and neck cancers may induce irreversible hyposalivation and consequent xerostomia, stemming from radiation damage to salivary glands (SGs). As cell-based therapy has been reported to be able to repair or restore damaged SG tissues, we attempted to determine whether bone marrow-derived clonal mesenchymal stem cells (BM-cMSCs) can ameliorate irradiation-induced salivary gland damage via a murine model.

Methods: External irradiation at a dose of 15 Gy was delivered to the neck fields of C57BL/6 mice. We directly administered either homologous mouse BM-cMSCs labeled with PKH26 (treatment group) or PBS (control group) into SGs 24 h after irradiation. Salivary flow rate (SFR) and lag time of salivation were measured at 12 weeks after transplantation. At 4 and 12 weeks post-transplantation, we performed morphological, histological, and immunofluorescent examinations. Transdifferentiation of administered BM-cMSCs into salivary epithelial cells was observed by confocal microscopy.

Results: SFR was significantly increased in BM-cMSCs-transplanted mice compared with PBS-injected mice at 12 weeks after transplantation. Administration of BM-cMSCs preserved the microscopic morphologies of SGs, with more functional acini in BM-cMSC-transplanted SGs than in PBS-injected SGs. Immunofluorescent staining revealed less apoptotic cells and increased microvessel density in BM-cMSC-transplanted SGs compared with PBS-injected SGs. PKH-26 labeled BM-cMSCs were detected in transplanted SGs at 4 weeks after transplantation and *in vivo* transdifferentiation of BM-cMSCs into acinar cells was also observed.

Conclusion: This study suggests that BM-cMSCs can ameliorate salivary damage following irradiation and can be used as a source of cell-based therapy for restoration of irradiation-induced salivary hypofunction.

Introduction

Salivary hypofunction and consequent xerostomia are common and significant complications of irradiation treatment in head and neck cancers. Nearly 64% of long-term survivors of head and neck cancers after radiation therapy experience moderate to severe xerostomia.[1] Irradiation-induced salivary hypofunction usually occurs shortly after radiotherapy: salivary flow decreases 50–60% in the first week and finally declines to approximately 20% at 7 weeks after conventional radiotherapy.[2] This significant change is typically irreversible and a life-long problem.[3]

Salivary hypofunction may contribute to a variety of oral sequelae, as well as general health problems. Patients with xerostomia commonly experience swallowing difficulty, loss of taste, difficulty in speech, oral candidiasis, and dental caries. In the end, these complications may lead to nutritional insufficiency and weight loss.[4–6] Moreover, irradiation-induced salivary hypofunction not only affects quality of life of patient, but also interrupts the course of radiotherapy, posing tumor control.[7]

While there have been many of advances in SG-sparing radiation delivery techniques, it is still impossible to successfully prevent irradiation-induced salivary hypofunction in all patients, indicating a need for new biologic insights for which to prevent or restore the obstinate complications of radiotherapy. Recently, an increasing

* Corresponding author at: Department of Otorhinolaryngology – Head and Neck Surgery, Inha University School of Medicine, 3-Ga Shinheung-dong, Jung-Gu, Incheon 400-711, Republic of Korea. Tel.: +82 32 890 3472; fax: +82 32 890 3580.
E-mail address: ymk416@inha.ac.kr (Y.-M. Kim).
* These authors contributed equally to this manuscript.

1368-8375/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.oraloncology.2012.08.010

Mesenchymal stem cell therapy for salivary gland dysfunction and xerostomia: a systematic review of preclinical studies

David Hebbelstrup Jensen, MD,[a] Roberto Stefan Oliveri, MD, PhD,[b] Stig-Frederik Trojahn Kølle, MD,[c] Anne Fischer-Nielsen, MD, DMSc,[b] Lena Specht, MD, DMSc,[d] Allan Bardow, DDS, PhD,[e] and Christian Buchwald, MD, DMSc[a]

University of Copenhagen and Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark

The most severe forms of xerostomia and salivary gland dysfunction, as well as a severely reduced quality of life, are seen in Sjögren syndrome (SS) and after radiotherapy for head and neck cancer. For both conditions, no effective regenerative therapies yet exist. Thus, the aim of this article was to assess, through systematic review, the potential benefit of mesenchymal stem cell (MSC) therapy in radiation-induced and SS-related salivary gland dysfunction and xerostomia. We searched PubMed/MEDLINE, Embase, Web of Science, the Cochrane Database of Systematic Reviews, the World Health Organization Clinical Trials Registry Platform, and Google Scholar. We identified 6 separate study comparisons eligible for inclusion. Owing to the limited number of studies, we conclude that more randomized, adequately powered clinical trials are needed to validate the potential beneficial effect of MSCs on salivary gland dysfunction and xerostomia. Nonetheless, the preliminary studies identified in the present review were encouraging for further research. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:335-342)

OVERVIEW

Xerostomia, or dry mouth syndrome, is the subjective feeling of dry mouth, which may or may not be associated with a reduced secretion of saliva. The feeling of oral dryness is generally not observed until the salivary flow is reduced by more than 50%.[1] Numerous conditions can lead to a reduced secretion of saliva, and hence xerostomia; it occurs most commonly as a side effect of many drugs. Among the more severe conditions, which also have the greatest effect on the secretion rate and normally a chronic perspective, are Sjögren syndrome (SS) and effects of radiotherapy for head and neck cancers (radiation-induced xerostomia [RIX]). Both RIX and SS are characterized by a progressive loss of acinar cells in the salivary glands and thus a progressive decline in saliva production. Patients with xerostomia have a severely reduced quality of life, and currently no treatments are aimed at regenerating the acinar cells and thus restoring normal salivary function. However, recent studies have suggested that treatment with mesenchymal stem cells (MSCs) might be a promising regenerative treatment option for increasing saliva flow rates (SFRs) and thus relieving xerostomia. The aim of this study was to assess the potential benefit of MSC therapy in RIX and SS-related salivary gland dysfunction and xerostomia, based on a systematic review of the published literature on the subject.

Although the etiologies of RIX and SS-related salivary gland dysfunction are very different, the effects of both are comparable in terms of their chronic and progressive nature and their severe effect on SFRs, which nearly always leads to pathologically reduced salivary flow (i.e., hyposalivation, International Classification of Diseases [ICD]-10 K11.7) and xerostomia (i.e., ICD-10 R68.2). Without any comparison in mind, both conditions are dealt with in tandem in the present review, because MSC therapy could become a treatment option for both. Thus, at this preclinical stage of MSC therapy for RIX and SS-related salivary gland dysfunction, all positive findings should be regarded as encouraging for future treatment strategies.

Statement of Clinical Relevance

The treatment of xerostomia after radiotherapy and Sjögren syndrome is unsatisfactory. Mesenchymal stem cell therapy has previously been evaluated in numerous clinical trials with positive results. However, a critical appraisal of this intervention for xerostomia has not been conducted.

David H. Jensen is supported by a grant from the Candys Foundation, a nonprofit foundation. This study did not receive other grants from any funding agency in the public, commercial, or not-for-profit sectors.

[a]Department of Otorhinolaryngology, Head & Neck Surgery and Audiology, Copenhagen University Hospital Rigshospitalet, and Faculty of Health and Medical Sciences, University of Copenhagen.
[b]Cell Therapy Facility, Blood Bank, Department of Clinical Immunology, Copenhagen University Hospital Rigshospitalet.
[c]Department of Plastic Surgery, Breast Surgery & Burns, Copenhagen University Hospital Rigshospitalet.
[d]Department of Oncology, Copenhagen University Hospital Rigshospitalet.
[e]Department of Oral Medicine, School of Dentistry, University of Copenhagen.

Received for publication Jun 21, 2013; returned for revision Oct 27, 2013; accepted for publication Nov 14, 2013.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2013.11.496

Biomaterials xxx (2014) 1–12

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Mesenchymal stem cell characteristics of dental pulp and periodontal ligament stem cells after *in vivo* transplantation

Ming Lei [a,b,1], Kun Li [a,b,1], Bei Li [b], Li-Na Gao [a,c], Fa-Ming Chen [a,c,*], Yan Jin [b,c,**]

[a] State Key Laboratory of Military Stomatology, Department of Periodontology & Biomaterials Unit, School of Stomatology, Fourth Military Medical University, Xi'an, PR China
[b] State Key Laboratory of Military Stomatology, Research and Development Center for Tissue Engineering, Fourth Military Medical University, Xi'an, PR China
[c] State Key Laboratory of Military Stomatology, Translational Research Team, School of Stomatology, Fourth Military Medical University, Xi'an, PR China

ARTICLE INFO

Article history:
Received 27 March 2014
Accepted 17 April 2014
Available online xxx

Keywords:
Dental pulp stem cells
Periodontal ligament stem cells
Stem cell therapy
Tissue engineering
In vivo transplantation

ABSTRACT

Mesenchymal stem cells (MSCs) isolated from human postnatal dental pulp and periodontal ligament (PDL) tissues can give rise to multilineage differentiation *in vitro* and generate related dental tissues *in vivo*. However, the cell properties of human dental pulp stem cells (DPSCs) and PDL stem cells (PDLSCs) after *in vivo* implantation remain largely unidentified. In this study, cells were re-isolated from *in vivo*-generated dental pulp-like and PDL-like tissues (termed re-DPCs and re-PDLCs, respectively) as a result of ectopic transplantation of human DPSC and PDLSC sheets. The cell characteristics in terms of colony-forming ability, cell surface antigens and multi-differentiation potentials were all evaluated before and after implantation. It was found that re-DPCs and re-PDLCs were of human and mesenchymal origin and positive for MSC markers such as STRO-1, CD146, CD29, CD90 and CD105; and, to some extent, re-DPCs could maintain their colony forming abilities. Moreover, both cell types were able to form mineral deposits and differentiate into adipocytes and chondrocytes; however, quantitative analysis and related gene expression determination showed that the osteo-/chondro-differentiation capabilities of re-DPCs and re-PDLCs were significantly reduced compared to those of DPSCs and PDLSCs, respectively ($P < 0.05$); re-PDLCs showed a greater reduction potential than re-DPCs. We conclude that DPSCs and PDLSCs may maintain their MSC characteristics after *in vivo* implantation and, compared to PDLSCs, DPSCs appear much more stable under *in vivo* conditions. These findings provide additional cellular and molecular evidence that supports expanding the use of dental tissue-derived stem cells in cell therapy and tissue engineering.

1. Introduction

In recent years, stem/progenitor cells have been characterized from a variety of dental-related tissues, such as gingiva [1], periodontal ligament (PDL) [2], papilla [3], follicle [4] and, indeed, dental pulp of exfoliated deciduous (children's) [5] and adult teeth [6], which represents a rich source of mesenchymal stem cells (MSCs) that are suitable for tissue engineering applications due to their accessibility and multilineage differentiation capacity [7–11]. In addition, dental stem cells display multifactorial advantages, such as a high proliferation rate, high viability and easy induction to distinct cell lineages [7,8]. Currently, dental stem cells are known to be of ectomesenchymal origin and are considered to share a common lineage of being derived from neural crest cells [9]. Most, if not all, dental stem cells identified thus far have generic MSC-like properties, including expression of marker genes and differentiation into mesenchymal cell lineages (osteoblasts, adipocytes and chondrocytes) *in vitro* and, to some extent, *in vivo* (reviewed in Refs. [8–11]). While extensive efforts have been and still are being made

* Corresponding author. State Key Laboratory of Military Stomatology, Department of Periodontology & Biomaterials Unit, School of Stomatology, Fourth Military Medical University, 145th West Chang-le Road, Xi'an 710032, PR China. Tel.: +86 29 84776093; fax: +86 29 84776096.
** Corresponding author. State Key Laboratory of Military Stomatology, Research and Development Center for Tissue Engineering, Fourth Military Medical University, 1st Kang-fu Road, Xi'an 710032, PR China. Tel.: +86 29 84776472; fax: +86 29 83218039.
E-mail addresses: cfmmunhb@fmmu.edu.cn (F.-M. Chen), yanjin@fmmu.edu.cn (Y. Jin).
[1] These authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.biomaterials.2014.04.071
0142-9612/

Hindawi Publishing Corporation
Stem Cells International
Volume 2016, Article ID 4709572, 12 pages
http://dx.doi.org/10.1155/2016/4709572

Review Article

Mesenchymal Stem Cells Derived from Dental Pulp: A Review

Edgar Ledesma-Martínez,[1] Víctor Manuel Mendoza-Núñez,[2] and Edelmiro Santiago-Osorio[1]

[1]*Hematopoiesis and Leukemia Laboratory, Research Unit on Cell Differentiation and Cancer, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, MEX, Mexico*
[2]*Research Unit on Gerontology, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, MEX, Mexico*

Correspondence should be addressed to Edelmiro Santiago-Osorio; edelmiro@unam.mx

Received 18 June 2015; Accepted 9 August 2015

Academic Editor: Daria Nurzynska

The mesenchymal stem cells of dental pulp (DPSCs) were isolated and characterized for the first time more than a decade ago as highly clonogenic cells that were able to generate densely calcified colonies. Now, DPSCs are considered to have potential as stem cell source for orthopedic and oral maxillofacial reconstruction, and it has been suggested that they may have applications beyond the scope of the stomatognathic system. To date, most studies have shown that, regardless of their origin in third molars, incisors, or exfoliated deciduous teeth, DPSCs can generate mineralized tissue, an extracellular matrix and structures type dentin, periodontal ligament, and dental pulp, as well as other structures. Different groups worldwide have designed and evaluated new efficient protocols for the isolation, expansion, and maintenance of clinically safe human DPSCs in sufficient numbers for various therapeutics protocols and have discussed the most appropriate route of administration, the possible contraindications to their clinical use, and the parameters to be considered for monitoring their clinical efficacy and proper biological source. At present, DPSC-based therapy is promising but because most of the available evidence was obtained using nonhuman xenotransplants, it is not a mature technology.

1. Introduction

The regenerative capacity of adult tissues depends on their stem cell populations, which stably self-renew and, in turn, give rise to progeny that possess the ability to differentiate into specialized cells. Stem cells have different names depending on the tissue of origin; thus there are hematopoietic, mesenchymal, endothelial, mammary, intestinal, neural, skin, muscle, and hair follicle stem cells, among others.

Among these stem cells, mesenchymal stem cells (MSCs) are noteworthy for their pluripotency, which means that they can differentiate into cells of any type, including those of the three embryonic germ layers. Because of their capacity for differentiation and wide tissue distribution and because their infusion has induced tissue repair in both preclinical and clinical models, MSCs are very attractive tools for tissue repair. Therefore, MSCs of dental origin have been tested as candidates for cellular therapy of stomatognathic disorders, such as periodontal disease (PD), and for maxillofacial reconstruction. In particular, it has been shown that human dental pulp stem cells (DPSCs) can generate mineralized tissue, an extracellular matrix and structures type dentin, dental pulp, and periodontal ligament in xenograft models. Herein, we review the general characteristics and immunophenotypes that define the DPSCs as MSCs, their isolation and cultivation, and their potential applications to tissue repair, emphasizing the possible administration routes, type of scaffold to use, and suggestions for their clinical applications.

2. Dental Pulp Stem Cells: General Characteristics

Teeth develop due to interactions between the oral ectodermal epithelial cells and MSCs, first forming the enamel organ

frontiers in PHYSIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 06 January 2014
doi: 10.3389/fphys.2013.00395

Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs

*Sriram Ravindran, Chun-Chieh Huang and Anne George**

Brodie Tooth Development Genetics and Regenerative Medicine Research Laboratory, Department of Oral Biology, University of Illinois at Chicago, Chicago, IL, USA

Edited by:
Jean-Christophe Farges, University Lyon 1, France

Reviewed by:
Anne Poliard, Université Paris Descartes, France
Javier Catón, Universidad Complutense de Madrid, Spain

***Correspondence:**
Anne George, Department of Oral Biology, University of Illinois at Chicago, 801 S. Paulina st., Chicago, IL 60612, USA
e-mail: anneg@uic.edu

Dental Caries affects approximately 90% of the world's population. At present, the clinical treatment for dental caries is root canal therapy. This treatment results in loss of tooth sensitivity and vitality. Tissue engineering can potentially solve this problem by enabling regeneration of a functional pulp tissue. Dental pulp stem cells (DPSCs) have been shown to be an excellent source for pulp regeneration. However, limited availability of these cells hinders its potential for clinical translation. We have investigated the possibility of using somatic mesenchymal stem cells (MSCs) from other sources for dental pulp tissue regeneration using a biomimetic dental pulp extracellular matrix (ECM) incorporated scaffold. Human periodontal ligament stem cells (PDLSCs) and human bone marrow stromal cells (HMSCs) were investigated for their ability to differentiate toward an odontogenic lineage. *In vitro* real-time PCR results coupled with histological and immunohistochemical examination of the explanted tissues confirmed the ability of PDLSCs and HMSCs to form a vascularized pulp-like tissue. These findings indicate that the dental pulp stem derived ECM scaffold stimulated odontogenic differentiation of PDLSCs and HMSCs without the need for exogenous addition of growth and differentiation factors. This study represents a translational perspective toward possible therapeutic application of using a combination of somatic stem cells and extracellular matrix for pulp regeneration.

Keywords: biomimetics, extracellular matrix, 3D scaffold, pulp tissue regeneration, dental pulp stem cells, periodontal ligament stem cells, human marrow stromal cells

INTRODUCTION

Dental caries is the most prevalent infectious disease among children and adults. Approximately 90% of the world's population has experienced dental caries (Petersen et al., 2005). Dental caries is characterized by infected and necrotic dental pulp tissue. The dental pulp tissue provides vitality and sensitivity to the tooth. The pulp tissue is highly vascularized, innervated and also serves as a source of stem cells. These characteristics enable the pulp to play a significant role in homeostasis and formation of reparative dentin (Schmalz and Galler, 2011). Current clinical treatment for dental caries is root canal therapy. This involves the cleaning and replacement of the infected and necrotic pulp tissue with a mineral trioxide compound. As a result of replacing a living tissue with a trioxide compound, the tooth looses its vitality and sensitivity and it is prone to secondary infections and the complications associated with it (Cordeiro et al., 2008). In adolescents, root canal treatment poses an even greater problem by preventing root maturation (Lentzari and Kozirakis, 1989; McTigue et al., 2013).

All of these deficiencies can be overcome by the use of tissue engineering strategies to regenerate the dental pulp. The identification of stem cells from several dental tissues has made pulp tissue regeneration a realistic clinical possibility. The identified dental stem cells include: Dental pulp stem cells (DPSCs) (Gronthos et al., 2000), periodontal ligament derived stem cells (PDLSCs) (Gould et al., 1977; Gronthos et al., 2006), stem cells from the root apical papilla (SCAP) (Sonoyama et al., 2006), and stem cells from exfoliated deciduous teeth (SHED) (Miura et al., 2003). All of these mesenchymal stem cells (MSCs) have been shown to be multi-potent and capable of differentiation into different cell types of the mesenchymal lineage.

In order to engineer the dental pulp tissue successfully, the choice of stem cells, scaffolds and growth factors is paramount. Several recent publications, have shown the ability of different dental cell types with both natural and artificial polymeric scaffolds and growth factors to regenerate dental pulp-like tissue in a subcutaneous implantation model (Cordeiro et al., 2008; Alsanea et al., 2011; Huang, 2011; Sakai et al., 2011). However, more recently, CD105 positive and CD31 negative dental pulp cells along with collagen and stromal derived factor 1 (SDF1) were used to regenerate the dental pulp in a canine pulpectomy model (Iohara et al., 2011; Ishizaka et al., 2012). Additionally, Wang et al. showed the preliminary potential of dental pulp cells in regenerating pulp-like tissue in canine immature teeth (Wang et al., 2013).

Although all of these studies demonstrate promise, from a clinical perspective, the prospect of retrieving autologous dental stem cells for multiple pulp regeneration therapies is daunting (Demarco et al., 2011). The DPSCs in adult humans are limited to the availability of the third molars and are not replenished after extraction like the bone marrow. Most clinical cases possess more than one carious tooth. Additionally, the prospect of obtaining a

TISSUE ENGINEERING: Part A
Volume 20, Numbers 1 and 2, 2014

DOI: 10.1089/ten.tea.2013.0192

Odontogenic Induction of Dental Stem Cells by Extracellular Matrix-Inspired Three-Dimensional Scaffold

Sriram Ravindran, PhD, Youbin Zhang, PhD, Chun-Chieh Huang, MS, and Anne George, PhD

Currently, root canal therapy is the only clinical treatment available to treat damaged or necrotic dental pulp tissue arising from caries. This treatment results in the loss of tooth vitality. Somatic dental stem cell-based tissue engineering approaches can alleviate this problem by preserving tooth vitality. Dental stem cells are multipotent and under appropriate conditions could be used for dental pulp tissue engineering. Successful use of these cells in pulp repair requires a combination of growth factors and appropriate scaffolds to induce cell differentiation. In this study, we demonstrate the odontogenic differentiation of human dental pulp stem cells (DPSCs) and the human periodontal ligament stem cells when cultured on a decellularized 3D extracellular matrix (ECM) scaffold without the need for exogenous addition of growth factors. Subcutaneous implantation of the ECM scaffolds containing DPSCs showed the formation of dental pulp-like tissue with cells expressing dentin sialoprotein (DSP) and dentin phosphophoryn (DPP). Additionally, we also show that the ECM scaffold can be exploited as a tool to study the extracellular function of multifunctional proteins. These promising results demonstrate the feasibility of developing these biomimetic scaffolds for treatment of dental caries.

Introduction

Dental caries is one of the most prevalent dental disease and a chronic disease in children aged 5–17.[1] According to the World Health Organization (WHO) report from 2003, approximately 90% of the world's population has experienced dental caries. Apart from poor oral hygiene, treatments like chemotherapy and radiation therapy also contribute toward formation of caries. The conventional treatment for pulp inflammation due to dental caries is root canal therapy. Approximately 24 million root canals are performed annually in the United States alone. This therapy involves the complete removal of the infected pulp followed by disinfection and filling up of the chamber with a trioxide component such as mineral trioxide culminating in capping up with a crown. The problem with this approach lies in the removal of the pulp. The pulp tissue offers vitality, sensitivity, and regenerative ability to the tooth. All of these properties are lost as a result of root canal therapy, which in many cases leads to secondary infections. As tooth sensitivity is lost, the secondary infections go unnoticed, until the infection spreads to the surrounding tissues. This condition can, in some cases, lead to sepsis and other serious complications leading to a significant reduction in the quality of life of the patient.

Tissue engineering is a promising therapy to regenerate dental tissues and can provide an excellent replacement for root canal therapy. Two types of approaches exist in the current regenerative dental research to restore normal function to damaged tissue or missing teeth. One is the engineering of individual components such as the dental pulp,[2,3] the periodontal ligament[4] and alveolar bone[5] and the other is engineering the entire functional tooth.[6] In the current study, we have focused on dental pulp tissue engineering using somatic dental stem cells.

Several different dental stem cell sources have been investigated for regenerating the dental pulp. Of these, the most common ones are dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and stem cells from human exfoliated deciduous teeth (SHED).[7] Although SHED have shown promise for banking and to be useful for tissue engineering applications,[8–12] in terms of immediate clinical relevance, the DPSCs and PDLSCs are well suited for engineering the dental pulp as they can be isolated from an adult patient. The multipotent ability of these stem cells has been well documented.[13–15] However, to trigger the differentiation of these stem cells into the required lineage for tissue engineering purposes, a combination of growth factors and differentiation agents is required.

In vivo, the extracellular matrix (ECM) dictates cellular proliferation, migration, and differentiation.[16] Mimicking the complexity of the ECM will result in cellular environments favorable for lineage-specific differentiation of stem cells.[17–22] In the present study, the possibility of utilizing odontoblast ECM embedded type I collagen/chitosan scaffolds for dental pulp tissue engineering using DPSCs and PDLSCs was

Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, Illinois.

TISSUE ENGINEERING: Part A
Volume 17, Numbers 15 and 16, 2011

DOI: 10.1089/ten.tea.2010.0615

Complete Pulp Regeneration After Pulpectomy by Transplantation of CD105$^+$ Stem Cells with Stromal Cell-Derived Factor-1

Koichiro Iohara, Ph.D.,[1] Kiyomi Imabayashi, Ph.D.,[1] Ryo Ishizaka, D.D.S.,[1,2] Atsushi Watanabe, Ph.D.,[3] Junichi Nabekura, Ph.D.,[4] Masataka Ito, Ph.D.,[5] Kenji Matsushita, Ph.D.,[1] Hiroshi Nakamura, Ph.D.,[6] and Misako Nakashima, Ph.D.[1]

Loss of pulp due to caries and pulpitis leads to loss of teeth and reduced quality of life. Thus, there is an unmet need for regeneration of pulp. A promising approach is stem cell therapy. Autologous pulp stem/progenitor (CD105$^+$) cells were transplanted into a root canal with stromal cell-derived factor-1 (SDF-1) after pulpectomy in mature teeth with complete apical closure in dogs. The root canal was successfully filled with regenerated pulp including nerves and vasculature by day 14, followed by new dentin formation along the dentinal wall. The newly regenerated tissue was significantly larger in the transplantation of pulp CD105$^+$ cells with SDF-1 compared with those of adipose CD105$^+$ cells with SDF-1 or unfractionated total pulp cells with SDF-1. The pulp CD105$^+$ cells highly expressed angiogenic/neurotrophic factors compared with other cells and localized in the vicinity of newly formed capillaries after transplantation, demonstrating its potent trophic effects on neovascularization. Two-dimensional electrophoretic analyses and real-time reverse transcription–polymerase chain reaction analyses demonstrated that the qualitative and quantitative protein and mRNA expression patterns of the regenerated pulp were similar to those of normal pulp. Thus, this novel stem cell therapy is the first demonstration of complete pulp regeneration, implying novel treatment to preserve and save teeth.

Introduction

Dental pulp has many functions, and it is essential for longevity of teeth and quality of life. The long-term goal of endodontic treatment after deep caries and/or pulp inflammation is the conservation and restoration of teeth including dental pulp. A promising approach for it is stem-cell-based therapy to regenerate the dentin-pulp complex for the conservation and total restoration of structure and function.[1] The regeneration and tissue engineering of pulp is based on morphogens and growth factors, responding stem/progenitor cells, and the extracellular matrix scaffold.[2] The regeneration of dental pulp in immature teeth with incomplete apical closure has been reported using fibrin in the blood clot or collagen.[3,4] However, there have been no reports concerning total pulp regeneration in mature teeth with complete apical closure by stem/progenitor cell therapy. There is an intimate association of innervation with vasculature of the dental pulp. Angiogenesis/vasculogenesis and neurogenesis are critical for total functional pulp regeneration. The type III receptor of the transforming growth factor-β receptor family cell surface antigen CD105 (endoglin) was selected on the basis of its wide expression on mesenchymal stem cells (MSCs).[5] The stromal cell-derived factor-1 (SDF-1)/CXCR4 axis is present and functional in MSC populations.[6,7] CD105$^+$ stem/progenitor cells from human pulp tissue containing CXCR4-positive cells demonstrated angiogenic/vasculogenic and neurogenic potential.[8] Endothelial cells release SDF-1 under hypoxic conditions and promote cell survival and neovascularization by recruitment and perivascular retention of CXCR4-positive bone marrow-derived cells.[9,10] Therefore, in this study, autologous pulp CD105$^+$ cells were transplanted with SDF-1 in a collagen scaffold into the root canal of mature teeth induced complete apical closure after pulpectomy, in dogs. Thus, we demonstrate for the first time complete pulp regeneration in the root canal, by protein profiles and mRNA expression patterns.

[1]Department of Dental Regenerative Medicine, Center of Advanced Medicine for Dental and Oral Diseases, National Center for Geriatrics and Gerontology, Research Institute, Obu, Aichi, Japan.
[2]Department of Pediatric Dentistry, School of Dentistry, Aichi-gakuin University, Nagoya, Aichi, Japan.
[3]Department of Cognitive Brain Science, National Center for Geriatrics and Gerontology, Research Institute, Obu, Aichi, Japan.
[4]Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki, Aichi, Japan.
[5]Department of Developmental Anatomy and Regenerative Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan.
[6]Department of Endodontology, School of Dentistry, Aichigakuin University, Nagoya, Aichi, Japan.

Biomaterials 33 (2012) 2109–2118

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue

Ryo Ishizaka [a,b,1], Koichiro Iohara [a,1], Masashi Murakami [a], Osamu Fukuta [b], Misako Nakashima [a,*]

[a] Department of Dental Regenerative Medicine, Center of Advanced Medicine for Dental and Oral Diseases, National Center for Geriatrics and Gerontology, Research Institute, 35 Gengo, Morioka, Obu, Aichi 474-8511, Japan
[b] Department of Pediatric Dentistry, School of Dentistry, Aichi-gakuin University, Nagoya 464-8651, Japan

ARTICLE INFO

Article history:
Received 15 October 2011
Accepted 21 November 2011
Available online 16 December 2011

Keywords:
Dental pulp stem cells (DPSC)
Adipose-derived stem cells (ADSC)
Bone marrow-derived stem cells (BMSC)
Transplantation
Pulp regeneration
Side population (SP) cells

ABSTRACT

Pulp stem/progenitor cells can induce complete pulp regeneration. However, due to the limited availability of pulp tissue with age, there is a need to examine other sources for fractions of side population (SP) cells. In the present investigation bone marrow and adipose tissues of the same individual were evaluated as alternate sources. Pulp CD31$^-$ SP cells have higher migration activity and higher expression of angiogenic/neurotrophic factors than bone marrow and adipose CD31$^-$ SP cells. Adipose tissue CD31$^-$ SP cell transplantation yielded the same amount of regenerated tissue as pulp derived cells. However, bone marrow CD31$^-$ SP cell transplantation yielded significantly less regenerated tissue in pulpectomized root canals in dogs. The rate of matrix formation was much higher in adipose CD31$^-$ SP cell transplantation compared to pulp CD31$^-$ SP cell transplantation on day 28. Microarray analysis demonstrated similar qualitative and quantitative patterns of mRNA expression characteristic of pulp in the regenerated tissues from all three cell sources. Expression of many angiogenic/neurotrophic factors in the transplanted cells demonstrated trophic effects. Our results demonstrate that bone marrow and adipose CD31$^-$ SP cells might be suitable alternative cell sources for pulp regeneration.

1. Introduction

Dental pulp has a significant role in homeostasis of teeth. The potential possibility of regeneration of pulp tissue by cell therapy is a promising approach for the future treatment of pulpitis or periapical disease assuring longevity of teeth and improved quality of life. We have recently demonstrated that autologous transplantation of pulp CD105$^+$ stem/progenitor cells or pulp CD31$^-$ side population (SP) cells with stromal cell-derived factor (SDF)-1 is capable of inducing complete pulp regeneration in a root canal after pulpectomy in dogs [1,2]. However, as availability of autologous pulp tissue declines with age, we sought alternative sources of mesenchymal stem/progenitor cells (MSCs) for clinical application in endodontic treatment. MSCs can be isolated from many other tissues, such as bone marrow, adipose tissue, placenta, umbilical cord and amnion although their heterogeneity is a challenge. Transcriptional and epigenetic analyses of different MSC populations reveal very similar profiles [3,4]. However, there is a difference in expressed trophic factors and growth factors among MSC populations [4–6]. It is also not known whether MSCs from different sources are comparable in their differentiation potential in vivo or whether their capabilities are influenced by the niche of their origin. A recent study has shown that intravenous injection of human umbilical cord tissue-derived MSCs into the murine model of Limb-Girdle Muscle Dystrophy 2B reached the muscle, but did not differentiate into muscle cells unlike human adipose MSCs, suggesting different efficiencies of MSCs in differentiation into specific cell lineage in vivo [7]. Comparative analysis of chondrogenic differentiation of MSCs isolated from bone marrow, adipose tissue, and synovial membrane shows the different requirement for the induction of chondrogenesis [4]. Therefore, requirements and preconditions of MSCs for effective induction of pulp regeneration needs to be further investigated.

Autologous MSCs derived from bone marrow and adipose tissues have neither ethical nor immunoreactive consideration and might be an alternative cell source for pulp tissue regeneration. Adipose-derived stem cells and bone marrow-derived MSCs share many characteristics [8] but also difference in protein and function [5,6]. We have recently compared the regenerative potential of canine pulp CD105$^+$ stem/progenitor cells with adipose tissue CD105$^+$ stem/progenitor cells; The pulp CD105$^+$ cells showed higher

* Corresponding author. Tel.: +81 562 44 5651x5065; fax: +81 562 46 8684.
E-mail address: misako@ncgg.go.jp (M. Nakashima).
[1] R. I. and K. I. contributed equally in experimental design and execution, data collection, analysis, and assembly, and preparation of figures for publication.

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.11.056

Bone grafts in dentistry

Prasanna Kumar, Belliappa Vinitha[1], Ghousia Fathima[2]

Department of Oral and Maxillofacial Surgery, Bhabha College of Dental Sciences, Bhopal, Madhya Pradesh, [1]Prosthodontics, Vydehi Institute of Dental Sciences and Research Centre, Bangalore, [2]Al Badar Rural Dental College and Hospital, Gulbarga, Karnataka, India

Address for correspondence:
Dr. Prasanna Kumar,
E-mail: jiadsjkk@gmail.com

Received : 02-05-13
Review completed : 04-05-13
Accepted : 04-05-13

ABSTRACT

Bone grafts are used as a filler and scaffold to facilitate bone formation and promote wound healing. These grafts are bioresorbable and have no antigen-antibody reaction. These bone grafts act as a mineral reservoir which induces new bone formation.

KEY WORDS: Allograft, autograft, bone reconstruction, bone repair, calcium sulphate, ceramic, hydroxyapatite, implant, polymer

Ridge defects develop as a result of surgery, trauma, infection, or congenital malformations. The goals of osseous replacement are maintenance of contour, elimination of dead space, and reduce postoperative infection; and thus enhance bony and soft tissue healing. The insufficient quantity of bone is due to tooth loss which results in rapid resorption of alveolar bone due to lack of intraosseous stimulation by periodontal ligament (PDL) fibers, for example, pneumatization of maxillary sinus following tooth loss.

Bone grafting is a surgical procedure that replaces missing bone with material from patient's own body, an artificial, synthetic, or natural substitute. Bone grafting is possible because bone tissue has the ability to regenerate completely if provided the space into which it has to grow. As natural bone grows, it generally replaces the graft material completely, resulting in a fully integrated region of new bone.

Access this article online

Quick Response Code:

Website: www.jpbsonline.org

DOI: 10.4103/0975-7406.113312

Classification of bone grafts based on material groups:[1]

a. Allograft-based bone graft involves allograft bone, used alone or in combination with other materials (e.g., Grafton, OrthoBlast).
b. Factor-based bone graft are natural and recombinant growth factors, used alone or in combination with other materials such as transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and bone morphogeneic protein (BMP).
c. Cell-based bone grafts use cells to generate new tissue alone or are added onto a support matrix, for example, mesenchymal stem cells.
d. Ceramic-based bone graft substitutes include calcium phosphate, calcium sulphate, and bioglass used alone or in combination; for example, OsteoGraf, ProOsteon, OsteoSet.
e. Polymer-based bone graft uses degradable and nondegradable polymers alone or in combination with other materials, for example, open porosity polylactic acid polymer.

The biologic mechanisms that provide a rationale for bone grafting are osteoconduction, osteoinduction, and osteogenesis.[2]

Osteoconduction

Occurs when bone graft material serves as a scaffold for new bone growth, which is perpetuated by the native bone. Osteoblasts

How to cite this article: Kumar P, Vinitha B, Fathima G. Bone grafts in dentistry. J Pharm Bioall Sci 2013;5:125-7.

Hindawi
Stem Cells International
Volume 2020, Article ID 8890875, 12 pages
https://doi.org/10.1155/2020/8890875

Research Article

Dental Pulp Mesenchymal Stem Cells as a Treatment for Periodontal Disease in Older Adults

Beatriz Hernández-Monjaraz,[1] Edelmiro Santiago-Osorio,[2] Edgar Ledesma-Martínez,[2] Itzen Aguiñiga-Sánchez,[2] Norma Angélica Sosa-Hernández,[2] and Victor Manuel Mendoza-Núñez[1]

[1]*Research Unit on Gerontology, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, Mexico*
[2]*Haematopoiesis and Leukaemia Laboratory, Research Unit on Cell Differentiation and Cancer, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, Mexico*

Correspondence should be addressed to Victor Manuel Mendoza-Núñez; mendovic@unam.mx

Received 18 June 2020; Revised 23 July 2020; Accepted 30 July 2020; Published 18 August 2020

Academic Editor: Sangho Roh

Periodontal disease (PD) is one of the main causes of tooth loss and is related to oxidative stress and chronic inflammation. Although different treatments have been proposed in the past, the vast majority do not regenerate lost tissues. In this sense, the use of dental pulp mesenchymal stem cells (DPMSCs) seems to be an alternative for the regeneration of periodontal bone tissue. A quasi-experimental study was conducted in a sample of 22 adults between 55 and 64 years of age with PD, without uncontrolled systemic chronic diseases. Two groups were formed randomly: (i) experimental group (EG) $n = 11$, with a treatment based on DPMSCs; and a (ii) control group (CG) $n = 11$, without a treatment of DPMSCs. Every participant underwent clinical and radiological evaluations and measurement of bone mineral density (BMD) by tomography. Saliva samples were taken as well, to determine the total concentration of antioxidants, superoxide dismutase (SOD), lipoperoxides, and interleukins (IL), before and 6 months after treatment. All subjects underwent curettage and periodontal surgery, the EG had a collagen scaffold treated with DPMSCs, while the CG only had the collagen scaffold placed. The EG with DPMSCs showed an increase in the BMD of the alveolar bone with a borderline statistical significance (baseline 638.82 ± 181.7 vs. posttreatment 781.26 ± 162.2 HU, $p = 0.09$). Regarding oxidative stress and inflammation markers, salivary SOD levels were significantly higher in EG (baseline 1.49 ± 0.96 vs. 2.14 ± 1.12 U/L posttreatment, $p < 0.05$) meanwhile IL1β levels had a decrease (baseline 1001.91 ± 675.5 vs. posttreatment 722.3 ± 349.4 pg/mL, $p < 0.05$). Our findings suggest that a DPMSCs treatment based on DPMSCs has both an effect on bone regeneration linked to an increased SOD and decreased levels of IL1β in aging subjects with PD.

1. Introduction

Periodontal disease (PD) is an infectious and inflammatory alteration that affects the supporting tissues of the teeth and, when treatment is not appropriate or adequate, it can cause the loss of these [1]. PD presents an immune and anti-inflammatory response caused by antigenic substances from bacteria in the subgingival biofilm; however, the exacerbated host response is ineffective, and therefore chronic inflammation is maintained [2].

During the acute phase of PD, the presence of bacteria, and especially the lipopolysaccharides in your cell wall, attracts macrophages, leukocytes, and neutrophils to the area of infection. The latter contain enzymes such as NADPH oxidase and myeloperoxidase to produce reactive oxygen species (ROS) that help fight pathogens [3, 4].

Under normal conditions, antioxidant mechanisms protect tissues from damage by ROS secreted by neutrophils. However, if the body's antioxidant capacity is insufficient, oxidative stress (OxS) occurs, which is an imbalance between

Local Administration of Stem Cells from Human Exfoliated Primary Teeth Attenuate Experimental Periodontitis in Mice

Yi Qiang QIAO[1*], Ling Su ZHU[1*], Sheng Jie CUI[2], Ting ZHANG[2], Rui Li YANG[2], Yan Heng ZHOU[2]

Objective: *To evaluate the therapeutic effect of local injection of stem cells from human exfoliated primary teeth (SHED) on periodontitis in mice.*

Methods: *Fifteen female mice were randomly divided into three groups: normal control group, periodontitis group and SHED treatment group. A periodontitis model was established by ligating a 0.2 mm orthodontic ligation wire to the maxillary first molar. The SHED group was injected with SHED at 3 weeks post-ligation. All mice were sacrificed and their maxillae were dissected five weeks post-ligation. Clinical assessments, micro-computed tomography (micro-CT) scanning, and histologic examination were used to evaluate the outcome of tissue regeneration.*

Results: *Micro-CT analysis showed that SHED administration significantly increased periodontal regeneration and decreased the distance between the cemento-enamel junction and the alveolar bone crest. In addition, histopathological photomicrographs showed new regenerated bone, the number of TNF-α-positive, IFN-γ-positive and $CD4^+$ cells decreased, and osteoclasts-positive decreased in the periodontal defect area in the SHED group compared with the periodontitis group.*

Conclusion: *SHED administration suppresses the expression of inflammatory factors, inhibits the production of osteoclasts, and promotes the regeneration of periodontal tissues.*

Key words: *periodontitis, stem cells from primary teeth, inflammation*

Chin J Dent Res 2019;22(3):157–163; doi: 10.3290/j.cjdr.a43109

Periodontitis is a chronic inflammatory disease caused by bacteria. The clinical manifestation is the loss of periodontal support tissue, including the periodontal ligament, alveolar bone and gingival tissue. It is the main pathogenic cause of adult tooth loss[1]. In addition, periodontitis is closely related to cardiovascular diseases, diabetes and other systemic diseases[2]. Therefore, there is an urgent need to find new and effective treatment strategies for periodontitis.

The goal of periodontitis treatment is to stop the progression of the disease, regenerate the damaged tissue and restore its original structure and function. At present, traditional treatment methods, whether surgical or non-surgical, are not effective in regenerating damaged periodontal tissues. Stem cell therapy is a promising therapeutic approach for the treatment of periodontitis[3]. A previous study showed periodontal regeneration in a rat periodontitis model after local injection of bone marrow mesenchymal stem cells (BMSCs) in suspension, and regeneration in periodontal defected areas in miniature pigs, after using periodontal ligament stem cells (PDLSCs)[4]. However, the limited sources of

1 Department of Orthodontics, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, P.R. China.
2 Department of Orthodontics, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Beijing, P.R. China.
* These two authors contributed equally to this work.

Corresponding authors: Dr Rui Li YANG and Prof. Yan Heng ZHOU, Department of Orthodontics, Peking University School and Hospital of Stomatology, 22# Zhongguancun South Avenue, Haidian District, Beijing 100081, P.R. China. Tel: 86 10 82195381; Fax: 86 10 62173402. Email: ruiliyangpku@163.com; yanhengzhou@vip.163.com

This work was supported by Beijing Natural Science Foundation (No.7182182 to Rui Li YANG); the Young Elite Scientist Sponsorship Programme by CAST (YESS, 2017QNRC001) and the National Natural Science Foundation of China (No. 81470717 to Yan Heng ZHOU and No. 81600865 to Rui Li YANG), and the National Science and Technology Major Project of the Ministry of Science and Technology of China (No. 2018ZX10302207).

Received: 24 October 2017 | Revised: 18 April 2018 | Accepted: 13 May 2018
DOI: 10.1111/jcpe.12931

RANDOMIZED CLINICAL TRIAL

WILEY Journal of Clinical Periodontology

Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial

Francesco Ferrarotti | Federica Romano | Mara Noemi Gamba | Andrea Quirico | Marta Giraudi | Martina Audagna | Mario Aimetti

Department of Surgical Sciences, C.I.R. Dental School, University of Turin, Turin, Italy

Correspondence
Mario Aimetti, C.I.R. Dental School, Via Nizza 230 10126 Turin (Italy).
Email: mario.aimetti@unito.it

Funding information
The study was self-funded by the authors and their institution, but Human Brain Wave srl (Turin, Italy) provided free material to be used in this study.

Abstract

Aim: The goal of this study was to evaluate if dental pulp stem cells (DPSCs) delivered into intrabony defects in a collagen scaffold would enhance the clinical and radiographic parameters of periodontal regeneration.

Materials and Methods: In this randomized controlled trial, 29 chronic periodontitis patients presenting one deep intrabony defect and requiring extraction of one vital tooth were consecutively enrolled. Defects were randomly assigned to test or control treatments which both consisted of the use of minimally invasive surgical technique. The dental pulp of the extracted tooth was mechanically dissociated to obtain micrografts rich in autologous DPSCs. Test sites (n = 15) were filled with micrografts seeded onto collagen sponge, whereas control sites (n = 14) with collagen sponge alone. Clinical and radiographic parameters were recorded at baseline, 6 and 12 months postoperatively.

Results: Test sites exhibited significantly more probing depth (PD) reduction (4.9 mm versus 3.4 mm), clinical attachment level (CAL) gain (4.5 versus 2.9 mm) and bone defect fill (3.9 versus 1.6 mm) than controls. Moreover, residual PD < 5 mm (93% versus 50%) and CAL gain ≥4 mm (73% versus 29%) were significantly more frequent in the test group.

Conclusions: Application of DPSCs significantly improved clinical parameters of periodontal regeneration 1 year after treatment.

KEYWORDS
dental pulp, periodontal pocket, periodontal regeneration, randomized controlled trial, stem cells, tissue engineering

1 | INTRODUCTION

The goal of periodontal therapy is to arrest disease progression and ultimately to regenerate lost periodontal tissues (Karring, Nyman, Gottlow, & Laurell, 1993). Several studies over the past 30 years had demonstrated that blood clot stability plays a pivotal role in regeneration of tooth-supporting tissues (Wikesjo & Nilveus, 1990; Wikesjo et al., 2003), avoiding apical migration of epithelial cells during the first healing phase. In this respect, new surgical techniques designed to optimize flap and clot stability (Cortellini & Tonetti, 2007, 2009; Harrel, Nunn, & Belling, 1999; Trombelli, Farina, Franceschetti, & Calura, 2009) and new

Published by John Wiley & Sons Ltd

Hindawi Publishing Corporation
International Journal of Dentistry
Volume 2014, Article ID 182513, 9 pages
http://dx.doi.org/10.1155/2014/182513

Review Article

Risk Factors of Periodontal Disease: Review of the Literature

Yousef A. AlJehani

Dental Health Department, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia

Correspondence should be addressed to Yousef A. AlJehani; aljehaniyousef@gmail.com

Received 3 April 2014; Accepted 7 April 2014; Published 20 May 2014

Academic Editor: Jagan Kumar Baskaradoss

Objectives. This paper aims to review the evidence on the potential roles of modifiable and nonmodifiable risk factors associated with periodontal disease. *Data.* Original articles that reported on the risk factors for periodontal disease were included. *Sources.* MEDLINE (1980 to Jan 2014), PubMed (using medical subject headings), and Google Scholar were searched using the following terms in different combinations: "periodontal disease," "periodontitis," "risk factors," and "causal." This was supplemented by hand-searching in peer-reviewed journals and cross-referenced with the articles accessed. *Conclusions.* It is important to understand the etiological factors and the pathogenesis of periodontal disease to recognize and appreciate the associated risk factors. As periodontal disease is multifactorial, effective disease management requires a clear understanding of all the associated risk factors.

1. Introduction

Periodontitis is one of the most ubiquitous diseases and is characterized by the destruction of connective tissue and dental bone support following an inflammatory host response secondary to infection by periodontal bacteria [1, 2]. Severe periodontitis, which may result in tooth loss, is found in 5–20% of most adult populations worldwide [3–5]. Children and adolescents can have any of the several forms of periodontitis such as aggressive periodontitis, chronic periodontitis, and periodontitis as a manifestation of systemic diseases [6–8].

It is now generally agreed that almost all forms of periodontal disease occur as a result of mixed microbial infections within which specific groups of pathogenic bacteria coexist [9–11]. Evidence is reviewed on the potential roles of modifiable and nonmodifiable risk factors associated with periodontal disease. An understanding of risk factors is essential for clinical practice.

1.1. Search Strategy. MEDLINE (1980 to Jan 2014), PubMed (using medical subject headings), and Google Scholar were searched using the following terms in different combinations: "periodontal disease," "periodontitis," "risk factors," and "causal." This was supplemented by hand-searching in peer-reviewed journals and cross-referenced with the articles accessed.

2. Risk Factors of Periodontal Disease

2.1. Modifiable Risk Factors

2.1.1. Microorganisms and Periodontal Disease. The oral bacterial microbiome includes over 700 different phylotypes, with approximately 400 species found in subgingival plaque [12, 13]. The subgingival microflora in periodontitis can harbor hundreds of bacterial species but only a small number has been associated with the progression of disease and considered etiologically important. Subgingival plaque from deepened periodontal pockets is dominated by gram-negative anaerobic rods and spirochetes [14, 15]. Strong evidence has implicated *Porphyromonas gingivalis* [16] and *Aggregatibacter actinomycetemcomitans* [17, 18] to the pathogenesis of adult periodontitis. In addition, *Bacteroides forsythus* [19], *Prevotella intermedia* [18], *Peptostreptococcus micros* [20], and *Fusobacterium nucleatum* [21] have been strongly linked with the progression of adult periodontitis.

2.1.2. Tobacco Smoking. There is accumulating evidence for a higher level of periodontal disease among smokers [22, 23]. Tobacco smoking exerts a substantial destructive effect on the periodontal tissues and increases the rate of periodontal disease progression [24]. Risk factors including tobacco smoking modify the host response to the challenge of bacteria

Published in final edited form as:
Cell Stem Cell. 2008 April 10; 2(4): 313–319. doi:10.1016/j.stem.2008.03.002.

Mesenchymal Stem Cells: Revisiting History, Concepts, and Assays

Paolo Bianco[1,2,5,*], **Pamela Gehron Robey**[3,5], and **Paul J. Simmons**[4,5]

1*Department of Experimental Medicine and Pathology, La Sapienza University, 00161 Rome, Italy*

2*Biomedical Science Park San Raffaele, 00128 Rome, Italy*

3*Craniofacial and Skeletal Diseases Branch, Department of Health and Human Services, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA*

4*Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA*

Abstract

The concept of mesenchymal stem cells has gained wide popularity. Despite the rapid growth of the field, uncertainties remain with respect to the defining characteristics of these cells, including their potency and self-renewal. These uncertainties are reflected in a growing tendency to question the very use of the term. This commentary revisits the experimental origin of the concept of the population(s) referred to as mesenchymal-stem cells and the experimental framework required to assess their stemness and function.

The concept of stem cells originated at the end of the 19th century as a theoretical postulate to account for the ability of certain tissues (blood, skin, etc.) to self-renew for the lifetime of an organism even though they are comprised of short-lived cells. Many years later, identification of stem cells as discrete cellular entities followed from the development of methods for prospective isolation of stem cell candidates, in parallel with the design of rigorous bioassays to test their potency after transplantation in vivo.

The currently popular concept of mesenchymal stem cells (MSCs, a term first coined in Caplan [1991]) can be traced to classical experiments demonstrating that transplantation of bone marrow (BM) to heterotopic anatomical sites results in de novo generation of ectopic bone and marrow. Whereas examples of such studies date back to the 19th century (Goujon, 1869), the work of Tavassoli and Crosby clearly established proof of an inherent osteogenic potential associated with BM (Tavassoli and Crosby, 1968). Because these experiments were conducted with entire fragments of bone-free BM, the precise identity of any cell functioning as a progenitor of differentiated bone cells (and therefore of nonhematopoietic, mesenchymal cells) could not be delineated. It was Friedenstein and coworkers, in a series of seminal studies in the 1960s and 1970s (reviewed in Friedenstein, 1990), who demonstrated that the osteogenic potential, as revealed by heterotopic transplantation of BM cells, was associated with a minor subpopulation of BM cells. These cells were distinguishable from the majority of hematopoietic cells by their rapid adherence to tissue culture vessels and by the fibroblast-like appearance of their progeny in culture, pointing to their origin from the stromal compartment of BM. In addition to establishing BM stroma as the haystack in which to search for the

*Correspondence: paolo.bianco@uniroma1.it.
[5]These authors contributed equally to this work.

International Journal of Oral Science www.nature.com/ijos

REVIEW ARTICLE OPEN

Stem cell-based bone and dental regeneration: a view of microenvironmental modulation

Chenxi Zheng[1], Ji Chen[1], Shiyu Liu[1] and Yan Jin[1]

In modern medicine, bone and dental loss and defects are common and widespread morbidities, for which regenerative therapy has shown great promise. Mesenchymal stem cells, obtained from various sources and playing an essential role in organ development and postnatal repair, have exhibited enormous potential for regenerating bone and dental tissue. Currently, mesenchymal stem cells (MSCs)-based bone and dental regeneration mainly includes two strategies: the rescue or mobilization of endogenous MSCs and the application of exogenous MSCs in cytotherapy or tissue engineering. Nevertheless, the efficacy of MSC-based regeneration is not always fulfilled, especially in diseased microenvironments. Specifically, the diseased microenvironment not only impairs the regenerative potential of resident MSCs but also controls the therapeutic efficacy of exogenous MSCs, both as donors and recipients. Accordingly, approaches targeting a diseased microenvironment have been established, including improving the diseased niche to restore endogenous MSCs, enhancing MSC resistance to a diseased microenvironment and renormalizing the microenvironment to guarantee MSC-mediated therapies. Moreover, the application of extracellular vesicles (EVs) as cell-free therapy has emerged as a promising therapeutic strategy. In this review, we summarize current knowledge regarding the tactics of MSC-based bone and dental regeneration and the decisive role of the microenvironment, emphasizing the therapeutic potential of microenvironment-targeting strategies in bone and dental regenerative medicine.

International Journal of Oral Science (2019)11:23 ; https://doi.org/10.1038/s41368-019-0060-3

INTRODUCTION

Bone and dental loss and defects caused by diseases and trauma have become a global concern with high incidence, which seriously affects the health and life quality of the whole population and lays a heavy financial burden on society.[1,2] Currently, autogenous bone transplantation is the gold standard treatment for bone defects.[3,4] For oral diseases, dental prostheses, periodontal treatment and dental implants remain the mainstream therapies.[2,5] However, the application of autogenous bone transplantation is seriously restrained by limitations of sources, difficulty in graft harvest and morbidity of donor site.[6,7] Moreover, present therapies for oral diseases can only improve clinical diagnostic parameters and halt disease progression but fail to regenerate lost tissue.[2,8] Therefore, new technologies are in high demand to achieve excellent regeneration of bone and dental tissues.

Mesenchymal stem cells (MSCs), which can be isolated from different tissues and possess self-renewal and multiple differentiation potential, play an essential role in organ development and postnatal repair.[9–11] A variety of studies, via animal models and clinical trials, have demonstrated that both endogenous and exogenous MSCs hold enormous promise in regenerative medicine for bone and tooth,[12–15] among which bone marrow MSCs (BMMSCs) have received much attention. In addition, adipose-derived MSCs (ADMSCs)[16,17] and dental stem cells (DSCs),[18,19] including dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from the apical papilla (SCAP) and dental follicle cells (DFCs), have emerged as attractive cell sources for bone and dental regeneration due to their ease of accessibility and relative abundance. In addition to differentiation potential, the ability of MSCs to regulate the function of other cells and to modulate the systemic inflammatory condition via cell–cell interaction or paracrine mechanism also contributes to their therapeutic efficacy.[20,21] Presently, there are two main strategies of MSC-based bone and dental regeneration: the rescue or mobilization of endogenous MSCs and the application of exogenous MSCs in cytotherapy or tissue engineering. Nevertheless, despite much progress, establishing safe, effective and simple stem cell-based approaches for bone and dental repair and regeneration remains a tremendous challenge,[8,14,15,22] especially considering the adverse effects of a diseased microenvironment.[21]

In recent years, the microenvironment has been uncovered to exert enormous influence on the physical functions and pathologic changes as well as the therapeutic effects of stem cells.[23–25] Physiologically, the niche where MSCs reside consists of a variety of tissue components, cell populations and soluble factors, which tightly regulate the behaviours of MSCs.[26–28] Under pathological conditions, such as osteoporosis and periodontitis, both the viability and differentiation of MSCs are seriously impaired, leading to disease aggravation and impaired tissue healing.[21,29–31] Furthermore, in cytotherapy and tissue engineering, the microenvironments of donors and recipients play a pivotal role in determining the regenerative efficacy of transplanted MSCs,[32,33]

[1]State Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi International Joint Research Center for Oral Diseases, Center for Tissue Engineering, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, China
Correspondence: Shiyu Liu (liushiyu@vip.163.com) or Yan Jin (yanjin@fmmu.edu.cn)

Received: 28 January 2019 Revised: 28 May 2019 Accepted: 12 June 2019
Published online: 19 August 2019

BioResearch Open Access
Volume 2, Number 2, April 2013

DOI: 10.1089/biores.2012.0291

Mesenchymal Stem Cells from Bichat's Fat Pad: *In Vitro* Comparison with Adipose-Derived Stem Cells from Subcutaneous Tissue

Eugenio Broccaioli,[1,*] Stefania Niada,[1,2,*] Giulio Rasperini,[1,3] Lorena Maria Ferreira,[1] Elena Arrigoni,[1] Vijay Yenagi,[1] and Anna Teresa Brini[1,2]

Abstract

Adipose-derived stem/stromal cells (ASCs) are progenitor cells used in bone tissue engineering and regenerative medicine. Since Bichat's fat pad is easily accessible for dentists and maxillo-facial surgeons, we compared the features of ASCs from Bichat's fat pad (BFP-ASCs) with human ASCs from subcutaneous adipose tissue (SC-ASCs). BFP-ASCs isolated from a small amount of tissue were characterized for their stemness and multidifferentiative ability. They showed an important clonogenic ability and the typical mesenchymal stem cell immunophenotype. Moreover, when properly induced, osteogenic and adipogenic differentiation markers, such as alkaline phosphatase activity, collagen deposition and lipid vacuoles formation, were promptly observed. Growth of both BFP-ASCs and SC-ASCs in the presence of human serum and their adhesion to natural and synthetic scaffolds were also assessed. Both types of ASCs adapted rapidly to human autologous or heterologous sera, increasing their proliferation rate compared to standard culture condition, and all the cells adhered finely to bone, periodontal ligament, collagen membrane, and polyglycol acid filaments that are present in the oral cavity or are commonly used in oral surgery. At last, we showed that amelogenin seems to be an early osteoinductive factor for BFP-ASCs, but not SC-ASCs, *in vitro*. We conclude that Bichat's fat pad contains BFP-ASCs with stemness features that are able to differentiate and adhere to biological supports and synthetic materials. They are also able to proliferate in the presence of human serum. For all these reasons we propose BFP-ASCs for future therapies of periodontal defects and bone regeneration.

Key words: amelogenin; biomaterials; buccal fat pad; mesenchymal stem/stromal cells; oral bone regeneration

Introduction

Mesenchymal stem/stromal cells (MSCs) represent important suitable candidates in regenerative medicine applications for the treatment of tissues damaged by trauma or pathological diseases. They have been isolated from bone marrow, adipose tissue, tendon, periodontal ligament, synovial membranes, trabecular bone, skin, periostium, and muscle.[1] Even though bone marrow represents the more used source of stem cells (BMSCs) in the clinical field, adipose tissue is a valid alternative source of MSCs. It is easily accessible in large quantities with a minimal invasive harvesting procedure and allow a high number of adipose-derived mesenchymal stem/stromal cells (ASCs) to be obtained.[2,3] ASCs show a multilineage differentiation capacity similar to that of BMSCs.[4–6] The growth factor secretome of MSCs was characterized by Wang et al.,[7] and the secretory activity of MSCs favors a regenerative microenvironment at sites of tissue injury.[8]

Adipose tissue withdrawn during plastic surgery is a discarded tissue, and the usual anatomical regions from which this tissue is collected are the abdomen, breast, buttock, knee, and thigh. In this study, we have characterized human ASCs isolated from the buccal fat pad, usually called Bichat's fat pad (BFP), one of the encapsulated fat masses in the cheek. It is a deep fat pad located on either side of the face between the buccinator muscle and several more superficial muscles, including the masseter, the zygomaticus major,

[1]Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy.
[2]Galeazzi Orthopedic Institute Research Hospital (IRCCS), Milan, Italy.
[3]Ca Granda Polyclinic Foundation Research Hospital (IRCCS), Milan, Italy.
*These authors contributed equally.

Periodontology 2000, Vol. 67, 2015, 251–267
Printed in Singapore. All rights reserved

Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering

ANTONIO R. SANZ, FLAVIO S. CARRIÓN & ALEJANDRA P. CHAPARRO

Periodontal disease is one of the most common conditions affecting humans, and the prevalence of advanced periodontitis in adults is about 15% (49). Periodontitis is a chronic inflammatory condition of the supporting tissues of the teeth, resulting in destruction of the attachment of the tooth to the surrounding bone. Untreated periodontitis may eventually lead to tooth loss. Fortunately, research has provided evidence that most types of periodontal disease can be successfully treated (63–110). Current treatment strategies focus on the removal of dental plaque and the long-term control of dental plaque accumulation, and these treatment strategies are generally successful in eliminating active disease and in promoting tissue repair. However, the complete regeneration of periodontal attachment lost to periodontal disease remains an elusive goal and a challenge (3); according to a position paper published by the American Academy of Periodontology in 2005 (3), the formation of new bone and cementum with supportive periodontal ligament is the ultimate objective that current periodontal-regeneration therapies are incapable of fulfilling. Regeneration is defined as the reproduction or reconstitution of a lost or injured part of the body, in such a way that the architecture and function of the tissues are completely restored (3). Thus, the aim of regenerative periodontal therapy is to restore the structure and function of the periodontium, which means regeneration of the supporting tissues, including alveolar bone, periodontal ligament and cementum, over a previously diseased root surface. Despite evidence that some regeneration can occur after therapy (3, 12, 20), this regeneration is usually only partial, in part because of the complexity of the biological events involved, such as signals for the synthesis of growth factors and the recruitment of specific cells for periodontal regeneration. Currently, bone grafts and guided tissue regeneration are the two techniques for which vast histological documentation of periodontal regeneration is available (12, 76, 87).

The periodontium is a highly complex organ consisting of epithelial tissue and soft and mineralized connective tissues, including gingiva, periodontal ligament, cementum and alveolar bone. The unique anatomy and composition of the periodontium makes periodontal wound healing a complex process because of the requirement for interaction of these three different tissues. Wound healing after conventional periodontal therapy, including surgical debridement, generally results in repair by the production of collagenous scar tissue accompanied by apical migration of gingival epithelium (11, 52, 83). In order for periodontal regeneration to occur, progenitor periodontal ligament cells must migrate to the denuded root surface, attach to it, proliferate and mature into an organized and functional fibrous attachment apparatus that inserts into a newly formed cementum. Similarly, progenitor bone cells must migrate, proliferate and mature in conjunction with the regenerating periodontal ligament (12, 58, 103). However, most of the current regenerative procedures, used either alone or in combination, have limitations in attaining complete regeneration, especially in deep periodontal defects (84, 87, 94, 106, 112).

Wound healing, or the regenerative process of a specific tissue, requires a combination of fundamental events, such as appropriate levels and sequencing of regulatory signaling pathways, presence and number of progenitor cells responding to biological signals, appropriate extracellular matrix or carrier, and adequate blood supply (11, 52, 80). Based on tissue-engineering concepts, the healing/regeneration process of a tissue may be manipulated at one of the following points: regulation of molecules, extracellular matrix or scaffold, and cellular availability (50, 80, 84; Fig. 1).

Annals of Medicine. 2005; 37: 469–479

SPECIAL SECTION ON STEM CELLS: REVIEW ARTICLE

Mesenchymal stem cells

H. KALERVO VÄÄNÄNEN

Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland

Abstract

About 40 years ago Friedenstein described stromal cells in the bone marrow that were spindle shaped and proliferate to form colonies. These cells attach to plastic and are able to differentiate under defined *in vitro* conditions into multiple cell types present in many different tissues, e.g. osteoblasts, chondroblasts, adipocytes, etc. Later on these cells, obtained from postnatal bone marrow, were called mesenchymal stem cells (MSC) or stromal stem cells. Recently the presence of somewhat similar cells has been demonstrated in many other tissues too. In spite of extensive attempts to characterize these cells we are still lacking definitive *in vivo* markers of MSC although retrospective functional data strongly support the existence of common adult stem cells that have the capacity to differentiate along various specific differentiation lineages. Since MSC can be rather easily isolated from the bone marrow and can also be expanded *in vitro* they have become a prime target for researchers of tissue regeneration. These cells have now been extensively used for transplantation experiments in animals and also for some therapeutic trials in humans. However, much new research is needed to learn enough on the molecular mechanisms of MSC differentiation to evaluate their full capacity for tissue regeneration.

Key words: *Bone, cartilage, differentiation, mesenchymal stem cell, tissue regeneration*

Introduction

Most mesenchymal tissues are remodelled throughout life. Complete regeneration of damaged tissue is an extreme example of remodelling. There are remarkable differences in the regeneration capacity between different species as well as different tissues in the same individual. Notwithstanding the tissue, the remodelling process in adults requires a continuous supply of new cells suggesting the existence of cells, either local or circulating, which have retained their capacity for proliferation and differentiation. It is of interest that the German pathologist Julius Cohnheim, back in 1867 (1) suggested that tissue repair in mammals was dependent on cells coming from the bloodstream. Cohnheim's hypothesis was either denied or forgotten for decades but now there are numerous *in vivo* and *in vitro* experiments demonstrating that the bone marrow (source of circulating cells), in addition to haematopoetic stem cells (HSC), contain a population (or populations) of stromal cells that under suitable conditions are able to differentiate towards a number of specific mesenchymal cell types including fibroblasts, osteoblasts, chondroblasts, adipocytes etc. (2–6). These cells are now widely called mesenchymal stem cells (MSC) and could be found even in the bone marrow of very old individuals although their number may decrease with age (7,8).

Since the original demonstration of MSC in bone marrow, several other tissues have also been shown to contain cells that have several features in common with MSC in the bone marrow. Although we still have rather modest understanding of the details of their proliferation and differentiation, they have become an important tool of tissue regeneration studies and are presently considered as potential candidates for several clinical applications (9). Some clinical trials using MSC have already been initiated and encouraging results have been reported, for instance, in osteogenesis imperfecta (10,11) and metachromatic leukodystrophy (12). MSC transplants have also been used to enhance engraftment of heterologous bone marrow transplants (13). In this review I will first discuss some issues concerning the biology of MSC and then evaluate their potential

Correspondence: H. Kalervo Väänänen, MD, Department of Anatomy, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, FI-20520 Turku, Finland. Fax: +358 2 333 7352. E-mail: kalervo.vaananen@utu.fi

ISSN 0785-3890 print/ISSN 1365-2060 online © 2005 Taylor & Francis
DOI: 10.1080/07853890500371957

STEM CELLS TRANSLATIONAL MEDICINE

PERSPECTIVES

Mesenchymal Stem Cells: Time to Change the Name!

ARNOLD I. CAPLAN

Skeletal Research Center, Department of Biology, Case Western Reserve University, Cleveland, Ohio, USA

Key Words. Medicinal signaling cells • Mesenchymal stem cells • MSCs • Regenerative medicine

SUMMARY

Mesenchymal stem cells (MSCs) were officially named more than 25 years ago to represent a class of cells from human and mammalian bone marrow and periosteum that could be isolated and expanded in culture while maintaining their in vitro capacity to be induced to form a variety of mesodermal phenotypes and tissues. The in vitro capacity to form bone, cartilage, fat, etc., became an assay for identifying this class of multipotent cells and around which several companies were formed in the 1990s to medically exploit the regenerative capabilities of MSCs. Today, there are hundreds of clinics and hundreds of clinical trials using human MSCs with very few, if any, focusing on the in vitro multipotential capacities of these cells. Unfortunately, the fact that MSCs are called "stem cells" is being used to infer that patients will receive direct medical benefit, because they imagine that these cells will differentiate into regenerating tissue-producing cells. Such a stem cell treatment will presumably cure the patient of their medically relevant difficulties ranging from osteoarthritic (bone-on-bone) knees to various neurological maladies including dementia. I now urge that we change the name of MSCs to Medicinal Signaling Cells to more accurately reflect the fact that these cells home in on sites of injury or disease and secrete bioactive factors that are immunomodulatory and trophic (regenerative) meaning that these cells make therapeutic drugs in situ that are medicinal. It is, indeed, the patient's own site-specific and tissue-specific resident stem cells that construct the new tissue as stimulated by the bioactive factors secreted by the exogenously supplied MSCs. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:1445–1451*

INTRODUCTION

Mesenchymal stem cells (MSCs) were officially named more than 25 years ago [1] to represent a class of cells from human [2] and mammalian bone marrow and periosteum [3] that could be isolated and expanded in culture while maintaining their in vitro capacity to be induced to form a variety of mesodermal phenotypes and tissues (Fig. 1, The Mesengenic Process). The in vitro capacity to form bone, cartilage, fat, etc., became an assay for identifying this class of multipotent cells [9] and around which several companies (including Osiris Therapeutics, which my colleagues and I started,) were formed in the 1990s to medically exploit the regenerative capabilities of MSCs. Initially, the driving concept that a multipotent progenitor or "stem cell" existed in adult marrow was not only challenged, but was actively disregarded, especially by the orthopedic industry. Fast-forward to today and there are hundreds of clinics [10] and hundreds of clinical trials [11] using human MSCs (hMSCs) with very few, if any, focusing on the in vitro multipotential capacities of these cells.

Unfortunately, the fact that MSCs are called "stem cells" is being used to infer that patients will receive direct medical benefit, because they imagine that these cells will differentiate into the regenerating tissue-producing cells (i.e., these "stem cells" will be incorporated into and these differentiated cells will fabricate the diseased or missing tissue). Such a stem cell treatment will presumably cure the patient of their medically relevant difficulties ranging from osteoarthritic (bone-on-bone) knees to various neurological maladies, including dementia. I long ago urged, in print, that we change the name of MSCs to Medicinal Signaling Cells [12] to more accurately reflect the fact that these cells home in on sites of injury or disease and secrete bioactive factors [13] that are immunomodulatory and trophic [14] (regenerative), meaning that these cells make therapeutic drugs [15] that are medicinal. It is, indeed, the patient's own site-specific and tissue-specific resident stem cells that construct the new tissue as stimulated by the bioactive factors secreted by the exogenously supplied MSCs [16, 17].

HISTORY OF MSCS FROM A CAPLAN PERSPECTIVE

In the early 1970s into the 1980s, my colleagues and I published a number of papers based on the culturing of stage 24, embryonic chick limb bud mesodermal cells (ECLBMCs) that were observed to differentiate into cartilage, muscle, and bone under certain culture conditions [18–22]. These in vitro studies were correlated with a variety of in vivo studies that focused on the cellular and molecular events associated with the formation of embryonic limb bone [23, 24], cartilage [25], and muscle [26] in which several very prominent dogmas-of-the-day were challenged. For example, the concept that "cartilage is replaced by bone" led to the implication that if one

Correspondence: Arnold I. Caplan, Ph.D., Skeletal Research Center, Department of Biology, Case Western Reserve University, 10600 Euclid Avenue, Cleveland, Ohio 44106, USA. Telephone: 216-368-3562; Fax: 216-368-4077; e-mail: arnold.caplan@case.edu.
Received March 3, 2017; accepted for publication March 10, 2017; published Online First on April 28, 2017. © AlphaMed Press 1066-5099/2017/$30.00/0
http://dx.doi.org/10.1002/sctm.17-0051

Review Article

Stem cells: A potential regenerative future in dentistry

Sumit Narang, Nidhi Sehgal

Department of Periodontics, Peoples College of Dental Science and Research Centre, Bhopal, Madhya Pradesh, India

In recent years, the field of dentistry has embossed its presence by taking major leaps in research and further bringing it into practice. The most valuable ongoing research in regenerative dentistry is the study on stem cells. It was instituted that stem cells grow rapidly and have the potential to form specialized dentin, bone, and neuronal cells. These neuronal cells can be used for dental therapies and can provide better treatment options for patients. The stem cells based therapies could help in new advances in treating damaged teeth, inducing bone regeneration and treating neural injury as well.

Key words: Dentistry, genetics, periodontal regeneration, stem cells

Introduction

Judging from the explosion of articles not only in scientific journals, but also in the mass media and on the internet, one could say the term "*stem cells*" has become linked to the word "cure."[1]

In the face of extraordinary advances in the prevention, diagnosis, and treatment of human diseases, devastating illnesses such as heart disease, diabetes, cancer, and diseases of the nervous system, such as Parkinson's disease and Alzheimer's disease, continues to deprive people of health, independence, and well-being. Research in human developmental biology has led to the discovery of human stem cells.

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.100749

Stem cells are primitive cells found in all multi-cellular organisms that are characterized by self-renewal and the capacity to differentiate into any mature cell type. These stem cells have the awesome potential for regeneration and may be used to replace or repair damaged cells, and have the potential to drastically change the treatment of conditions like cancer, Alzheimer's and Parkinson's disease and even paralysis.

There are 2 main types of stem cells – embryonic stem cells and adult stem cells – which are classified according to their origin and differentiation potential.[2]

Mesenchymal stem cells (MSCs), a type of the adult stem cells that can be harvested from bone marrow and other sources such as liver, umbilical cord, placenta, adipose tissue, synovial membrane, amniotic fluid and even teeth,[3] have increasingly played a central role in regenerative medicine. Their attractiveness is found in their multipotency to differentiate and develop into various types of tissues such as adipose, cartilage, and bone,[4] as well as their promising use in patient-specific gene therapy.[3]

Stem cells are defined as having the capacity for extensive self-renewal and for originating at least one type of highly differentiated descendant.[5]

Research in the stem cell field grew out of findings by Canadian scientists Ernest A. McCulloch and James E. Till in the 1960.[6,7]

Stem cells are cells that have the following capabilities: First, they are able to continuously produce daughter cells having the same characteristics as themselves (self-renewal); secondly, they can generate daughter cells that have different, more restricted properties, and finally, they can re-populate a host *in vivo* (differentiation).[8]

Address for correspondence: Dr. Sumit Narang, Professor and Head, Department of Periodontology, Peoples College of Dental Science and Research Centre, Bhopal, Madhya Pradesh, India. E-mail: drsumitnarang@rediffmail.com

International Journal of
Molecular Sciences

MDPI

Review

Therapeutic Functions of Stem Cells from Oral Cavity: An Update

Ji Won Yang [1,2,†], Ye Young Shin [1,2,†], Yoojin Seo [2,*,‡] and Hyung-Sik Kim [1,2,*,‡]

[1] Department of Life Science in Dentistry, School of Dentistry, Pusan National University, Yangsan 50612, Korea; midnightnyou@naver.com (J.W.Y.); bubu3935@naver.com (Y.Y.S.)
[2] Dental and Life Science Institute, Pusan National University, Yangsan 50612, Korea
* Correspondence: amaicat24@naver.com (Y.S.); hskimcell@pusan.ac.kr (H.-S.K.); Tel.: +82-51-510-8216 (Y.S.); +82-51-510-8231 (H.-S.K.)
† These authors contributed equally to this work.
‡ Authors share co-corresponding authorship.

Received: 1 June 2020; Accepted: 17 June 2020; Published: 19 June 2020

check for updates

Abstract: Adult stem cells have been developed as therapeutics for tissue regeneration and immune regulation due to their self-renewing, differentiating, and paracrine functions. Recently, a variety of adult stem cells from the oral cavity have been discovered, and these dental stem cells mostly exhibit the characteristics of mesenchymal stem cells (MSCs). Dental MSCs can be applied for the replacement of dental and oral tissues against various tissue-damaging conditions including dental caries, periodontitis, and oral cancers, as well as for systemic regulation of excessive inflammation in immune disorders, such as autoimmune diseases and hypersensitivity. Therefore, in this review, we summarized and updated the types of dental stem cells and their functions to exert therapeutic efficacy against diseases.

Keywords: dental mesenchymal stem cells; oral cavity; periodontitis; regeneration; immunomodulation

1. Introduction

Tissue-specific adult stem cells (ASCs) are the specialized cell population responsible for organ development, homeostasis, and regeneration throughout the lifetime. In general, ASCs have a great self-renew potential with lineage-specific differentiation capacity. For instance, a subset of transplanted hematopoietic stem cells can replenish the whole-blood system of lethally irradiated mice [1]. A single Lgr5+ intestinal stem cell (ISCs) can reconstitute the intestinal epithelial layer containing not only ISC itself but also other mature cell types [2]. Besides organs, connective tissues such as bone and fat are regarded as a rich source of multipotent mesenchymal stromal/stem cells (MSCs). Similar to other organ-derived ASCs, MSCs can self-renew and proliferate well. They are capable of mesenchymal lineage-specific differentiation into bone, adipose tissue, and cartilage both in vitro and in vivo; however, in addition to primary tissue replacement, MSCs are known to play pivotal roles in microenvironment regulation. MSCs contribute to stem cell niche formation and support region-specific ASCs to maintain their stemness and multipotency [3]. They communicate neighbors via direct cell-to-cell contact but also via indirect, secretory factor-dependent signaling so-called paracrine effect. According to the context, MSCs produce a plethora of bioactive molecules that can promote stem/progenitor cell proliferation, determine the direction of differentiation, enhance angiogenesis and even modulate immune responses, leading to wound healing and tissue repair [4,5]. Furthermore, it is widely accepted that MSCs are less immunogenic than other ASCs since they express a low level of MHC antigens and immune cell co-stimulatory molecules [6,7]. In these aspects, MSCs have drawn great interest in the fields of stem cell therapeutics and regenerative medicine.

Review Article

Current overview on dental stem cells applications in regenerative dentistry

Ramta Bansal, Aditya Jain[1]

Department of Conservative Dentistry and Endodontics, Institute of Dental Sciences, Sehora, Jammu and Kashmir, [1]Department of Physiology, Government Medical College, Patiala, Punjab, India

Address for correspondence:
Dr. Aditya Jain, House No. 13, Khalsa College Colony, Patiala, Punjab, India.
E-mail: dr_aditya82@rediffmail.com

Abstract

Teeth are the most natural, noninvasive source of stem cells. Dental stem cells, which are easy, convenient, and affordable to collect, hold promise for a range of very potential therapeutic applications. We have reviewed the ever-growing literature on dental stem cells archived in Medline using the following key words: Regenerative dentistry, dental stem cells, dental stem cells banking, and stem cells from human exfoliated deciduous teeth. Relevant articles covering topics related to dental stem cells were shortlisted and the facts are compiled. The objective of this review article is to discuss the history of stem cells, different stem cells relevant for dentistry, their isolation approaches, collection, and preservation of dental stem cells along with the current status of dental and medical applications.

Key words: Cell culture techniques, stem cells, stem cell research, tissue banks, tissue engineering

INTRODUCTION

Regenerative capacity of the dental pulp is well-known and has been recently attributed to function of dental stem cells. Dental stem cells offer a very promising therapeutic approach to restore structural defects and this concept is extensively explored by several researchers, which is evident by the rapidly growing literature in this field. For this review article a literature research covering topics related to dental stem cells was made and the facts are compiled.

METHODS

A web-based research on Medline (www.pubmed.gov) was done. To limit our research to relevant articles, the search was filtered using terms review, published in the last 10 years and dental journals. Various keywords used for research were "regenerative dentistry" (128 articles found), "dental stem cells" (111 articles found), "dental stem cells banking" (2 articles found), "stem cells from human exfoliated deciduous teeth (SHED)" (11 articles found). For each heading in the review, relevant articles were chosen and arranged in chronological order of publication date so as to follow the development of the research topic. This review screened about 250 articles to get the required knowledge update. Relevant data were then compiled with aim of providing basic information as well as latest updates on dental stem cells.

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.149074

HISTORY OF STEM CELLS

Stem cells also known as "progenitor or precursor" cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation.[1] In 1868, the term "stem cell" for the first time appeared in the works of German biologist Haeckel.[2] Wilson coined the term stem cell.[3] In 1908, Russian histologist, Alexander Maksimov, postulated existence of hematopoietic stem cells at congress of hematologic society in Berlin.[4] There term "stem cell" was proposed for scientific use.

Stem cells have manifold applications and have contributed to the establishment of regenerative medicine. Regenerative medicine is the process of replacing or regenerating human

Murray and Péault *BMC Biology* (2015) 13:99
DOI 10.1186/s12915-015-0212-7

BMC Biology

QUESTION AND ANSWER Open Access

Q&A: Mesenchymal stem cells — where do they come from and is it important?

Iain R. Murray[1] and Bruno Péault[1,2*]

Abstract

Mesenchymal stem — or *stromal* — cells (MSCs) have been administered in hundreds of clinical trials for multiple indications, making them some of the most commonly used selected regenerative cells. Paradoxically, MSCs have also long remained the least characterized stem cells regarding native identity and natural function, being isolated retrospectively in long-term culture. Recent years have seen progress in our understanding of the natural history of these cells, and candidate native MSCs have been identified within fetal and adult organs. Beyond basic knowledge, deciphering the biology of innate MSCs may have important positive consequences for the therapeutic use of these cells.

What are mesenchymal stem cells, and how are they conventionally isolated?

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of lineages, including osteocytes, adipocytes and chondrocytes. This differentiation capacity, in addition to their release of trophic factors and immunomodulatory properties, holds great promise for cell therapies and tissue engineering. MSCs are not a new phenomenon. In the late nineteenth century the German biologist Cohnheim hypothesized that fibroblastic cells derived from bone marrow were involved in wound healing throughout the body [1]. In the 1970s Alexander Friedenstein, who is generally credited with the discovery of MSCs, described a population of plastic-adherent cells that emerged from long-term cultures of bone marrow and other blood-forming organs, and that he showed to have colony forming capacity and osteogenic differentiation characteristics in vitro as well as in vivo upon re-transplantation [2–4]. In light of their capacity to differentiate into bone, fat, cartilage and muscle in culture and an emerging link to the embryonic development of various mesenchymal tissues, the term "mesenchymal stem cell" was coined in 1991 by Arnold Caplan to describe these cells [5]. Cells with similar characteristics have since been found to emerge from cultures of virtually all adult and fetal organs tested [6]. Observation of these cells in culture led to a definition of MSCs by the International Society of Cell Therapy (ISCT) that included a propensity to adhere to laboratory culture plastic and the capacity to differentiate into at least bone, cartilage and fat [7]. MSCs were subsequently found to have a characteristic, although not specific, set of surface markers, with additional functions including the secretion of immunomodulatory factors and support, albeit limited, of hematopoiesis.

This body of work suggested that MSCs natively resided in all the tissues from which they were isolated; however, their exact location (whether in the stroma or, for instance, in blood vessels) was still not known. An improved understanding of the native identity and biology of these cells has recently been sought.

Is it important to understand the native origin of MSCs?

Yes, a complete understanding of the native origin of MSCs will allow their therapeutic potential to be fully exploited. The documented multipotency, immunomodulatory and trophic effects of MSCs sparked great excitement and enthusiasm to explore the use of MSCs as progenitors in tissue engineering to replace damaged tissues of mesodermal and possibly other germ line origins, to promote regeneration, and to treat immune-mediated disease [8]. As such, the number of clinical trials using MSCs has been rising almost exponentially since 2004. However, with the "gold rush" to use MSCs in the clinical setting, the question of what MSCs naturally do in bone marrow and other tissues, and what intrinsic roles these populations may play in vivo, beyond how their

* Correspondence: bpeault@mednet.ucla.edu
[1]BHF Centre for Vascular Regeneration, Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK
[2]Orthopedic Hospital Research Center and Broad Stem Cell Center, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

S Shi
PM Bartold
M Miura
BM Seo
PG Robey
S Gronthos

The efficacy of mesenchymal stem cells to regenerate and repair dental structures

Authors' affiliations:
S. Shi, M. Miura, B.M. Seo, P.G. Robey, Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, MD, USA
P.M. Bartold, Colgate Australian Clinical Dental Research Centre, Dental School, University of Adelaide, Adelaide Dental Hospital, Adelaide, SA, Australia
S. Gronthos, Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA, Australia

Correspondence to:
Stan Gronthos
Mesenchymal Stem Cell Group
Division of Haematology
Institute of Medical and Veterinary Science
Frome Road, Adelaide 5000
SA, Australia
Tel.: +61-8-82223460
Fax: +61-8-82223139
E-mail: stan.gronthos@imvs.sa.gov.au

Dates:
Accepted 10 April 2005

To cite this article:
Orthod Craniofacial Res 8, 2005; 191–199
Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S:
The efficacy of mesenchymal stem cells to regenerate and repair dental structures

Structured Abstract

Authors – Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S

Objectives – Identification, characterization, and potential application of mesenchymal stem cells (MSC) derived from human dental tissues.

Methods – Dental pulp and periodontal ligament were obtained from normal human impacted third molars. The tissues were digested in collagenase/dispase to generate single cell suspensions. Cells were cultured in α-MEM supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 100 μM L-ascorbate-2-phosphate. Magnetic and fluorescence activated cell sorting were employed to characterize the phenotype of freshly isolated and *ex vivo* expanded cell populations. The developmental potential of cultured cells was assessed following co-transplantation with hydroxyapetite/tricalcium phosphate (HA/TCP) particles into immunocompromised mice for 8 weeks.

Results – MSC were identified in adult human dental pulp (dental pulp stem cells, DPSC), human primary teeth (stem cells from human exfoliated deciduous teeth, SHED), and periodontal ligament (periodontal ligament stem cells, PDLSC) by their capacity to generate clongenic cell clusters in culture. *Ex vivo* expanded DPSC, SHED, and PDLSC populations expressed a heterogeneous assortment of makers associated with MSC, dentin, bone, smooth muscle, neural tissue, and endothelium. PDLSC were also found to express the tendon specific marker, Scleraxis. Xenogeneic transplants containing HA/TCP with either DPSC or SHED generated donor-derived dentin-pulp-like tissues with distinct odontoblast layers lining the mineralized dentin-matrix. In parallel studies, PDLSC generated cementum-like structures associated with PDL-like connective tissue when transplanted with HA/TCP into immunocompromised mice.

Conclusion – Collectively, these data revealed the presence of distinct MSC populations associated with dental structures with

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Review: Hematopoietic Stem Cells and Tissue Stem Cells: Current Concepts and Unanswered Questions

DONALD METCALF

The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Key Words. Hematopoietic stem cells • Tissue stem cells

ABSTRACT

The term hematopoietic stem cells has at times been used to include a miscellany of precursor cells ranging from multipotential self-generating cells to lineage-restricted progenitors with little capacity for self-generation. It is probable that the stem cells of other tissues also vary widely in their multipotentiality and proliferative capacity. This review questions several dogmas regarding the self-generative capacity of various hematopoietic cells, the single episodic origin of hematopoietic cells, and the irreversible nature of progressive mature cell formation in individual hematopoietic lineages. STEM CELLS *2007;25: 2390–2395*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

It is now slightly more than 45 years since the discovery of spleen colony-forming cells (CFU-S) [1] and the belief that these were the sought for hematopoietic stem cells. Since then, hematopoietic stem cells (HSC) and their progressively committed progeny have become the prototypical examples of what might be expected of candidate stem cells in other tissues. It is useful, therefore, to review somewhat critically what actually has been firmly established about HSC, what is believed or assumed, perhaps incorrectly, and what skeletons are tucked away in the family tree because they do not fit well in an orderly model. This review is the written version of a recent lecture and is deliberately referenced only lightly. The objective is to allow workers with candidate stem cells in other tissues to compare the properties they have established about their stem cells with what is known about hematopoietic stem cells and in this way to assess what information remains in need of discovery about their cells.

The word "stem cell" needs some definition because, for different workers, the term can embrace quite different cells. My view is that a hematopoietic stem cell should be capable of self-generation and be multipotential and, thus, potentially be able to form maturing cells in all eight major hematopoietic lineages. What has become apparent is that cells more or less fitting this definition are likely to be quite heterogeneous. Some—a minority—*may* also be totipotent and able to form cells of other tissue types [2]. Most stem cells are quite heterogeneous in their proliferative and self-generative capacity. I suspect of particular relevance to workers on tissue stem cells is the fact that some cells with a claim to the term "stem cells" do not possess the complete properties of a stem cell. Some hematopoietic cells may have substantial self-generative capacity but no longer be multipotential. More numerous are lineage-committed hematopoietic cells with considerable clonogenic (proliferative) capacity but no capacity for self-generation. Finally, there are some mature cells with a quite surprising proliferative (self-generative?) capacity. Cells of all the above types are likely to be encountered in studies on tissue stem cells and, because the various cells are likely to be related ancestrally, they need to be fitted into some sort of family tree.

THE HEMATOPOIETIC FAMILY TREE

A typical family tree diagram for hematopoietic cells is shown in Figure 1. The rudiments of this family tree date back at least as far as Pappenheim and Maximov in the early 20th century. At that time it was guesswork based largely on an extrapolation between two more or less secure reference points—at one end, a likely common origin of hematopoietic precursors and endothelial cells and, at the other end, the quite evident orderly morphological progression in various lineages of blast cells through to mature end cells.

What the family tree in Figure 1 implies is that continuous cell formation occurs with individual stem cells being both self-generating and generating more mature progeny. The model further proposes that a succession of precursor cells exists with cell-generating capacity but a progressively lesser capacity for self-generation. Finally, a series of irrevocably lineage-committed cells is generated that ultimately loses all proliferative capacity as it progressively matures.

Some major reservations should be raised about the reality of this proposed sequence. Need multipotential stem cells be continually involved in hematopoiesis? These cells are agreed to be nondividing or very slowly dividing. How good is the evidence excluding the possibility that more mature progeny are perfectly capable of sustaining hematopoiesis in normal life with stem cells perhaps only becoming activated in extreme emergency? Furthermore, with much of the current interest in the possible plasticity of commitment, how secure are the dog-

Correspondence: Donald Metcalf, M.D., The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia. Telephone: +61-3-9345-2555; Fax: +61-3-9347-0852; e-mail: metcalf@wehi.edu.au Received July 17, 2007; accepted for publication August 8, 2007; first published online in STEM CELLS *EXPRESS* August 9, 2007; available online without subscription through the open access option. 1066-5099/2007/$30.00/0 doi: 10.1634/stemcells.2007-0544

OPEN ACCESS Freely available online

PLoS one

Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine

Wataru Sonoyama[1,2☯], Yi Liu[2☯], Dianji Fang[2], Takayoshi Yamaza[1], Byoung-Moo Seo[4], Chunmei Zhang[2], He Liu[5], Stan Gronthos[6], Cun-Yu Wang[7], Songtao Shi[2*], Songlin Wang[1*]

1 Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, California, United States of America, 2 Salivary Gland Disease Center and the Molecular Laboratory for Gene Therapy, Capital Medical University School of Stomatology, Beijing, China, 3 Department of Oral and Maxillofacial Rehabilitation, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, 4 Department of Oral and Maxillofacial Surgery, School of Dentistry, Dental Research Institute, Seoul National University, Seoul, Korea, 5 Peking University School of Stomatology, Beijing, China, 6 Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia, 7 Laboratory of Molecular Signaling and Apoptosis, Department of Biologic and Materials Sciences and Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, Michigan, United States of America

Mesenchymal stem cell-mediated tissue regeneration is a promising approach for regenerative medicine for a wide range of applications. Here we report a new population of stem cells isolated from the root apical papilla of human teeth (SCAP, stem cells from apical papilla). Using a minipig model, we transplanted both human SCAP and periodontal ligament stem cells (PDLSCs) to generate a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function. This work integrates a stem cell-mediated tissue regeneration strategy, engineered materials for structure, and current dental crown technologies. This hybridized tissue engineering approach led to recovery of tooth strength and appearance.

Citation: Sonoyama W, Liu Y, Fang D, Yamaza T, Seo B-M, et al (2006) Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine. PLoS ONE 1(1): e79. doi:10.1371/journal.pone.0000079

INTRODUCTION

Regeneration of a functional and living tooth is one of the most promising therapeutic strategies for the replacement of a diseased or damaged tooth [1–3]. Recent advances in dental stem cell biotechnology and cell-mediated murine tooth regeneration have encouraged researchers to explore the potential for regenerating living teeth with appropriate functional properties [4–6]. Murine teeth can be regenerated using many different stem cells to collaboratively form dental structures *in situ* [4,5,7]. In addition, dentin/pulp tissue and cementum/periodontal complex have been regenerated by human dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs), respectively, when transplanted into immunocompromised mice [8,9]. However, owing to the complexity of human tooth growth and development, the regeneration of a whole tooth structure including enamel, dentin/pulp complex, and periodontal tissues as a functional entity in humans not possible given available regenerative biotechnologies.

The spatially and temporally organized microenvironment of the tooth bud and its surrounding tissues permits growth and development of the crown and roots, resulting in formation and eruption of the tooth [10]. Root development involves dentin formation, cementum generation, instruction of epithelium, and tooth eruption. From a clinical perspective, the most important part of the tooth is the root which supports for a (natural or artificial) crown. The crown alone cannot fulfill normal tooth function without a viable root. In contrast, the wide use of synthetic crowns to replace a damaged natural crowns has been widely applied in dental clinics with excellent therapeutic outcomes [11].

Although dental implant therapies have achieved long-term success in the clinic for the recovery of tooth function, the dental implants require pre-existing high-quality bone structures for supporting the implants [12,13]. Reconstruction of teeth in patients without adequate bone support would be a major advance. Stem cell-mediated root regeneration offers opportunities to regenerate a bio-root and its associated periodontal tissues, which are necessary for maintaining the physiological function of teeth. The purpose of this study is to explore the potential for reconstructing a functional tooth in miniature pigs (minipigs), in which a bio-root periodontal complex is built up by postnatal stem cells including stem cells from root apical papilla (SCAP) and PDLSCs, to which an artificial porcelain crown is affixed. This hybrid strategy of autologous dental stem cell engineering may be applicable to human tooth regeneration. Furthermore, functional tooth restoration in swine may shed light on human tooth regeneration in the future because of the close similarities between swine and human dental tissues [14,15].

RESULTS

Isolation and transplantation of SCAP

The mechanism of the contribution of stem progenitors to root formation remains to be elucidated. Here, we found that human root apical papilla tissue on the exterior of the root foramen area demonstrated positive staining for mesenchymal stem cell surface

Academic Editor: Marie Csete, Emory University, United States of America

Received September 12, 2006; Accepted November 13, 2006; Published December 20, 2006

Funding: This study was sponsored by the University of Southern California School of Dentistry, grants from the National Natural Science Foundation of China (30428009), the Beijing Major Scientific program grant (D0906007000091), and the Division of Intramural Research, the National Institute of Dental and Craniofacial Research, the National Institutes of Health, Department of Health and Human Service.

Competing Interests: The authors have declared that no competing interests exist.

* To whom correspondence should be addressed. E-mail: songtaos@usc.edu (SS); songlinwang@dentist.org.cn (SW)

☯ These authors contributed equally to this work.

npj | Regenerative Medicine

www.nature.com/npjregenmed

REVIEW ARTICLE OPEN

Mesenchymal stem cell perspective: cell biology to clinical progress

Mark F. Pittenger[1*], Dennis E. Discher[2], Bruno M. Péault[3,4*], Donald G. Phinney[5], Joshua M. Hare[6] and Arnold I. Caplan[7]

The terms MSC and MSCs have become the preferred acronym to describe a cell and a cell population of multipotential stem/progenitor cells commonly referred to as mesenchymal stem cells, multipotential stromal cells, mesenchymal stromal cells, and mesenchymal progenitor cells. The MSCs can differentiate to important lineages under defined conditions in vitro and in limited situations after implantation in vivo. MSCs were isolated and described about 30 years ago and now there are over 55,000 publications on MSCs readily available. Here, we have focused on human MSCs whenever possible. The MSCs have broad anti-inflammatory and immune-modulatory properties. At present, these provide the greatest focus of human MSCs in clinical testing; however, the properties of cultured MSCs in vitro suggest they can have broader applications. The medical utility of MSCs continues to be investigated in over 950 clinical trials. There has been much progress in understanding MSCs over the years, and there is a strong foundation for future scientific research and clinical applications, but also some important questions remain to be answered. Developing further methods to understand and unlock MSC potential through intracellular and intercellular signaling, biomedical engineering, delivery methods and patient selection should all provide substantial advancements in the coming years and greater clinical opportunities. The expansive and growing field of MSC research is teaching us basic human cell biology as well as how to use this type of cell for cellular therapy in a variety of clinical settings, and while much promise is evident, careful new work is still needed.

npj Regenerative Medicine (2019)4:22; https://doi.org/10.1038/s41536-019-0083-6

INTRODUCTION

MSCs have become widely studied over the past ~30 years for their interesting cell biology, broad-ranging clinical potential, and as a central building block in the rapidly growing field of tissue engineering. MSCs grow readily in the culture dish, have intrinsic differentiation potentials not found previously in other cells, and produce an abundance of useful growth factors and cytokines. The isolation of MSCs from various tissues and their re-implantation at other sites raises questions about the natural in vivo MSCs and their ability to normally repair endogenous tissues, a process that clearly diminishes with age. Mesenchymal cell replacement in the large numbers needed to treat significant tissue injury requires engraftment, structural organization and cellular differentiation—a complex process that has made much progress but remains unperfected. Friedenstein was first to culture bone-forming cells from guinea pig and Owen re-energized this inquiry by expanding such work to rats.[1,2] The isolation and culture expansion of human bone marrow MSCs were reported in 1992 [3] and their infusion into patients was begun as early as 1993 as reported in 1995.[4] Over the past 25 years the infusion procedures have exhibited an excellent safety profile, so much so that there are now over 950 registered MSC clinical trials listed with the FDA. There have been over 10,000 patients treated in a controlled clinical setting, of which 188 early trials (phase 1 or phase 2) have been completed and ten studies have advanced to phase 3 (Mesenchymal stem cells search at www.clinicaltrials.gov and https://celltrials.org/public-cells-data/msc-trials-2011-2018/65). Worldwide, for the years 2011–2018, there were 1043 MSC trials planned with a targeted enrollment of 47,548 patients (Mesenchymal stem cells search at www.clinicaltrials.gov and https://celltrials.org/public-cells-data/msc-trials-2011-2018/65). For comparison, bone marrow and hematopoietic stem cell (HSC) transplantations have been practiced since 1957, and through 1983, the first 25 years, about 9000 patients were treated.[5]

The most common and longest utilized adult source tissues for human MSCs are bone marrow[3,6] and the adipose tissue stromal vascular fraction[7,8] and these sources form the foundation for most of the data in this field (Fig. 1a). These are harvestable human tissues that are thought to be renewable (bone marrow) or unwanted (adipose). There are also two young "adult" tissues, umbilical cord tissue[9] and placenta,[10,11] that are excellent sources of human MSCs, and these tissues are normally discarded at birth. The decision to use autologous MSCs from bone marrow or adipose, or an allogeneic source tissue to isolate MSCs is a fundamental clinical decision, but both have shown success producing large numbers of MSCs.[6,8] For example, a target dose of 100–150 million human MSCs can be produced from 25 ml of bone marrow by cell culturing in about 3 weeks and this number of packed cells has a volume of about 0.4–0.5 ml.[12] There are still surprisingly few animal research reports or clinical studies that use autologous MSCs and most studies use allogeneic MSCs. In humans, there is also a recognized drop-off with age in the number of isolatable MSCs found in bone marrow, suggesting a very different set of circumstances in the aging population with

[1]Department of Surgery, University of Maryland School of Medicine, 105. Pine Street, Baltimore, MD 21212, USA. [2]Biophysical Engineering Labs, University of Pennsylvania, 129 Towne Bldg, Philadelphia, PA 19104-6393, USA. [3]Orthopedic Hospital Research Center, UCLA/Orthopedic Surgery, 615 Charles E. Young Drive, Los Angeles, CA 90095, USA. [4]MRC Centre for Regenerative Medicine, University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU, UK. [5]Department of Molecular Medicine, A231, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458, USA. [6]Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Biomedical Research Building/Room 908PO Box 016960 (R-125), Miami, FL 33101, USA. [7]Department of Biology and Skeletal Research Center, Case-Western University, Millis Science Center, Room 118, 10900 Euclid Ave, Cleveland, OH 44106, USA. *email: mfpittenger@comcast.net; bpeault@mednet.ucla.edu

Published in partnership with the Australian Regenerative Medicine Institute

npj nature partner journals

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Reviews: Characteristics and Potential Applications of Human Dental Tissue-Derived Mesenchymal Stem Cells

JUNJUN LIU,[a] FANG YU,[b] YAO SUN,[b] BEIZHAN JIANG,[b] WENJUN ZHANG,[c] JIANHUA YANG,[a] GUO-TONG XU,* AIBIN LIANG,* SHANGFENG LIU*

Key Words. Mesenchymal stem cells • Dental stem cells • Tissue regeneration • Immunomodulation • Cell-based therapy • Dental stem cell banks

[a]Department of Ophthalmology, Shanghai Tenth People's Hospital and [c]Translational Center for Stem Cell Research, Tongji Hospital, Tongji University School of Medicine, Shanghai, People's Republic of China; [b]Laboratory of Oral Biomedical Science and Translational Medicine, School of Stomatology, Tongji University, Shanghai, People's Republic of China

Correspondence: Shangfeng Liu, Ph.D., Stem Cell Research Center and Department of Regenerative Medicine, Room 807, Tongji University School of Medicine, 1239 Siping Road, Shanghai 200092, People's Republic of China. Telephone: 86-21-65986073; Fax: 86-21-65986073; e-mail: shangfengliu@tongji.edu.cn; or Aibin Liang, Ph.D., Department of Hematology, 389 Xincun Road, Shanghai 200065, People's Republic of China. Telephone: 86-21-66111019; Fax: 86-21-66111019; e-mail: lab7182@tongji.edu.cn; or Guo-Tong Xu, Ph.D., Medical Building, Room 521, 1239 Siping Road, Shanghai 200092, People's Republic of China. Telephone: 86-21-65986358; Fax: 86-21-65986358; e-mail: gtxu@tongji.edu.cn

Received April 28, 2014; accepted for publication November 7, 2014; first published online in STEM CELLS *Express* December 2, 2014.

1066-5099/2014/$30.00/0

http://dx.doi.org/10.1002/stem.1909

ABSTRACT

Recently, numerous types of human dental tissue-derived mesenchymal stem cells (MSCs) have been isolated and characterized, including dental pulp stem cells, stem cells from exfoliated deciduous teeth, periodontal ligament stem cells, dental follicle progenitor cells, alveolar bone-derived MSCs, stem cells from apical papilla, tooth germ progenitor cells, and gingival MSCs. All these MSC-like cells exhibit self-renewal, multilineage differentiation potential, and immunomodulatory properties. Several studies have demonstrated the potential advantages of dental stem cell-based approaches for regenerative treatments and immunotherapies. This review outlines the properties of various dental MSC-like populations and the progress toward their use in regenerative therapy. Several dental stem cell banks worldwide are also introduced, with a view toward future clinical application. STEM CELLS *2015;33:627–638*

INTRODUCTION

Mesenchymal stem cells (MSCs) are spindle-shaped cells with the potential for donogenic proliferation. MSCs were initially reported as fibroblast-like cells that could be isolated from bone marrow via their adherence to plastic in culture and subsequently confirmed as a population (the colony-forming unit-fibroblast) of bone-marrow-derived nonhematopoietic cells [1].

MSCs can differentiate into all mesodermal lineages, which prompted the investigation into the role of MSCs in mediating tissue regeneration [2]. The capacity for the differentiation of MSCs into mesodermal [3], ectodermal [4], and endodermal [5] cell lineages has since been fully characterized and forms the basis for most current work on bone marrow-derived MSCs (BMMSCs). In 2006, the International Society for Cellular Therapy (ISCT) [6] proposed the minimal characterization criteria for human MSCs, including their propensity for adherence to plastic when maintained under standard culture conditions and their ability to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. In addition, most (≥95%) MSCs positively express CD105 (endoglin), CD73 (ecto-5′-nucleotidase), and CD90 (Thy1) while negatively expressing (≤2%) CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR [6].

Since the discovery and characterization of BMMSCs, MSC-like populations from other tissues have been characterized based on the standard criteria established for BMMSCs [1–3, 6, 7]. In addition to bone marrow, MSC populations can be readily obtained from skeletal muscle [8] and a variety of other tissues, such as umbilical cord blood [9], synovium [10], the liver [11], adipose tissue [12], the lungs [13], amniotic fluid [14], tendons [15], placenta [16], skin [17], and breast milk [18].

The search for MSC-like cells in specific tissues led to the discovery of a distinctive population of MSCs from a variety of human dental tissues during previous decades. To date, eight unique populations of dental tissue-derived MSCs have been isolated and characterized. Postnatal dental pulp stem cells (DPSCs) were the first human dental MSCs to be identified from pulp tissue [19]. Gradually, other dental MSC-like populations, such as stem cells from human exfoliated deciduous teeth (SHED) [20], periodontal ligament stem cells (PDLSCs) [21], dental follicle progenitor cells (DFPCs) [22], alveolar bone-derived MSCs (ABMSCs) [23], stem cells from apical papilla (SCAP) [24], tooth germ progenitor cells (TGPCs) [25], and gingival MSCs (GMSCs) [26], were also reported (Fig. 1).

Preliminary data suggest that these dental tissue-derived MSCs not only display self-renewal and multidifferentiation potential but

Hindawi
Stem Cells International
Volume 2020, Article ID 3670412, 15 pages
https://doi.org/10.1155/2020/3670412

Research Article

Mesenchymal Stem Cells from Human Exfoliated Deciduous Teeth and the Orbicularis Oris Muscle: How Do They Behave When Exposed to a Proinflammatory Stimulus?

Alessander Leyendecker Junior, Carla Cristina Gomes Pinheiro, Daniela Yukie Sakai Tanikawa, José Ricardo Muniz Ferreira, Mariane Tami Amano, and Daniela Franco Bueno

Hospital Sírio Libanês, São Paulo, SP, Brazil

Correspondence should be addressed to Daniela Franco Bueno; daniela.fbueno@hsl.org.br

Received 19 October 2019; Revised 4 January 2020; Accepted 1 February 2020; Published 25 February 2020

Guest Editor: Alireza Moshaverinia

Mesenchymal stem cells (MSCs) have been studied as a promising type of stem cell for use in cell therapies because of their ability to regulate the immune response. Although they are classically isolated from the bone marrow, many studies have sought to isolate MSCs from noninvasive sources. The objective of this study was to evaluate how MSCs isolated from the dental pulp of human exfoliated deciduous teeth (SHED) and fragments of the orbicularis oris muscle (OOMDSCs) behave when treated with an inflammatory IFN-γ stimulus, specifically regarding their proliferative, osteogenic, and immunomodulatory potentials. The results demonstrated that the proliferation of SHED and OOMDSCs was inhibited by the addition of IFN-γ to their culture medium and that treatment with IFN-γ at higher concentrations resulted in a greater inhibition of the proliferation of these cells than treatment with IFN-γ at lower concentrations. SHED and OOMDSCs maintained their osteogenic differentiation potential after stimulation with IFN-γ. Additionally, SHED and OOMDSCs have been shown to have low immunogenicity because they lack expression of HLA-DR and costimulatory molecules such as CD40, CD80, and CD86 before and after IFN-γ treatment. Last, SHED and OOMDSCs expressed the immunoregulatory molecule HLA-G, and the expression of this antigen increased after IFN-γ treatment. In particular, an increase in intracellular HLA-G expression was observed. The results obtained suggest that SHED and OOMDSCs lack immunogenicity and have immunomodulatory properties that are enhanced when they undergo inflammatory stimulation with IFN-γ, which opens new perspectives for the therapeutic use of these cells.

1. Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that have the ability to differentiate into mesodermal cell lineages, including chondroblasts, osteoblasts, and adipocytes [1, 2]. In addition, three basic characteristics must be present to classify a culture of cells isolated from neonatal or adult tissues as a culture of MSCs [3]. First, MSCs are able to adhere to the plastic of the cell culture flasks. Furthermore, at least 95% of an isolated and cultured cell population must express mesenchymal antigens (such as CD29, CD44, CD73, CD90, and CD105) and should not express hematopoietic or immune cell markers (such as CD14, CD19, and CD34) or endothelial cell markers (such as CD31). Finally, MSCs should be able to differentiate into osteoblasts, chondrocytes, and adipocytes in vitro under specific culture conditions [3].

Although they are more commonly isolated from the bone marrow and adipose tissue of donors, recent studies have demonstrated that MSCs can also be obtained from several other tissue types [4, 5] and that the cells from distinct tissues present considerable differences in their proliferative abilities and differentiation potentials [6]. For example, it has been suggested that MSCs obtained from neonatal tissues are more proliferative, have a higher differentiation potential, and can be maintained in culture for longer periods (before reaching cell senescence) than MSCs isolated from the bone

Cytotherapy, 2011; 13: 598–605

informa
healthcare

Stem cells from human exfoliated deciduous teeth (SHED) enhance wound healing and the possibility of novel cell therapy

YUDAI NISHINO[1], YOICHI YAMADA[2], KATSUMI EBISAWA[3], SAYAKA NAKAMURA[1], KAZUTO OKABE[1], ERI UMEMURA[1], KENJI HARA[1] & MINORU UEDA[1]

[1]*Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan,*
[2]*Center for Genetic and Regenerative Medicine, Nagoya University School of Medicine, Nagoya, Japan, and*
[3]*Department of Plastic and Reconstructive Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Abstract

Background aims. In recent years, stem cells from human exfoliated deciduous teeth (SHED) have received attention as a novel stem cell source with multipotent potential. We examined the effect on wound-healing promotion with unique stem cells from deciduous teeth as a medical waste. *Methods.* An excisional wound-splinting mouse model was used and the effect of wound healing among SHED, human mesenchymal stromal cells (hMSCs), human fibroblasts (hFibro) and a control (phosphate-buffered saline; PBS) was evaluated by macroscopy, histology and enzyme-linked immunosorbent assay (ELISA), and the expression of hyaluronan (HA), which is related to wound healing, investigated. *Results.* SHED and hMSCs accelerated wound healing compared with hFibro and the control. There was a statistically significant difference in wound healing area among hFibro, hMSCs and SHED compared with the control after day 5. At days 7 and 14 after cell transplantation, the histologic observation showed that transplanted PKH26-positive cells were surrounded by human HA binding protein, especially in hMSCs and SHED. HA expression volume values were 1558.41 ± 60.33 (control), 2092.75 ± 42.56 (hFibro), 2342.07 ± 188.10 (hMSCs) and 2314.85 ± 164.91 (SHED) ng/mg, respectively, and significantly higher in hMSCs and SHED compared with hFibro and control at days 7 and 14 ($P < 0.05$). *Conclusions.* Our results show that SHED hMSCs have similar effects of wound-healing promotion as hFibro and controls. This implies that SHED might offer a unique stem cell resource and the possibility of novel cell therapies for wound healing in the future.

Key Words: *cell therapy, human mesenchymal stromal cells, hyaluronic acid, stem cells from human exfoliated deciduous teeth, wound healing*

Introduction

An intractable wound such as a chronic wound causes a patient great stress and treatment is very difficult (1,2). Until now, such a wound has been treated by using surgical operations and medical treatments. But there are various problems, such as imperfect wound healing and scaring. They have also not resulted in dramatic changes in wound closure or outcome (3). Accordingly, establishment of a treatment is essential. Now various examinations as the treatment method of the wound such as the development of the ointment treatment, artificial skin, and skin substitute, are done. Therefore cell therapy toward promotion of wound healing and scar-less treatment has been paid to attention. Several lines of evidence have reported that fibroblasts and mesenchymal stromal cells (MSCs), applied to accelerate wound healing, have differentiation and paracrine effects (4–6). Fibroblast injections are based on the hypothesis that autologous fibroblasts are capable of producing collagens for ongoing improvement without immune or allergic reactions. But clinical reports have recommended repeated injections in order to continue longer rhytide correction, and the results are less than optimal (7). Fibroblast injections can reportedly increase inflammation and scar formation (7–10). On the other hand, MSCs are referred to as stromal progenitor, self-renewing and expandable stem cells and are able to differentiate into osteoblasts, adipocytes, chondrocytes, etc. (11). However, bone marrow aspiration is an invasive procedure for the donor. In addition, the number, proliferation and differentiation potential of MSCs declines with increasing age (12). In a recent study, dental pulp contained a population of multipotent stem cells with the capacity to differentiate into several different cells

Correspondence: **Yoichi Yamada**, DDS, PhD, Center for Genetic and Regenerative Medicine, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466–8550, Japan. E-mail: yyamada@med.nagoya-u.ac.jp

(Received 12 September 2010; accepted 12 November 2010)

ISSN 1465-3249 print/ISSN 1477-2566 online © 2011 Informa Healthcare
DOI: 10.3109/14653249.2010.542462

STEM CELLS TRANSLATIONAL MEDICINE

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Three Years After Transplants in Human Mandibles, Histological and In-Line Holotomography Revealed That Stem Cells Regenerated a Compact Rather Than a Spongy Bone: Biological and Clinical Implications

ALESSANDRA GIULIANI,[a,*] ADRIAN MANESCU,[a,*] MAX LANGER,[b,c,d] FRANCO RUSTICHELLI,[a] VINCENZO DESIDERIO,[e,f] FRANCESCA PAINO,[f] ALFREDO DE ROSA,[g] LUIGI LAINO,[g] RICCARDO D'AQUINO,[g] VIRGINIA TIRINO,[f] GIANPAOLO PAPACCIO[f]

[a]Dipartimento di Scienze Cliniche e Odontostomatologiche, Sezione di Biochimica, Biologia e Fisica, Università Politecnica delle Marche, Ancona, Italy; [b]Creatis, INSA-Lyon, Lyon, France; [c]Université CB, Lyon, France; [d]European Synchrotron Radiation Facility, Grenoble, France; [e]Department of Computational Medicine and Bioinformatics, Center for Organogenesis of Complex Tissues, University of Michigan, Ann Arbor, Michigan, USA; [f]Dipartimento di Medicina Sperimentale, Sezione di Istologia e Medicina Rigenerativa, Seconda Università degli Studi di Napoli, Naples, Italy; [g]Dipartimento di Odontostomatologia, Seconda Università degli Studi di Napoli, Naples, Italy

[*]Contributed equally as first authors.

Correspondence: Gianpaolo Papaccio, M.D., Ph.D., Department of Experimental Medicine, Section of Histology, Laboratory of Tissue Engineering and Regenerative Medicine, Second University of Naples, via L. Armanni 5-80138 Naples (I), Italy. Telephone: 39-08-15666015/7720/7715; Fax: 39-08-15666014; E-Mail: gianpaolo.papaccio@unina2.it

Received October 18, 2012; accepted for publication January 24, 2013; first published online in SCTM *Express* March 15, 2013.

1066-5099/2013/$20.00/0

http://dx.doi.org/10.5966/sctm.2012-0136

Key Words. Clinical trials • Differentiation • Stem cell transplantation • Tissue regeneration • Bone

ABSTRACT

Mesenchymal stem cells deriving from dental pulp differentiate into osteoblasts capable of producing bone. In previous studies, we extensively demonstrated that, when seeded on collagen I scaffolds, these cells can be conveniently used for the repair of human mandible defects. Here, we assess the stability and quality of the regenerated bone and vessel network 3 years after the grafting intervention, with conventional procedures and in-line holotomography, an advanced phase-imaging method using synchrotron radiation that offers improved sensitivity toward low-absorbing structures. We found that the regenerated tissue from the graft sites was composed of a fully compact bone with a higher matrix density than control human alveolar spongy bone from the same patient. Thus, the regenerated bone, being entirely compact, is completely different from normal alveolar bone. Although the bone regenerated at the graft sites is not of the proper type found in the mandible, it does seem to have a positive clinical impact. In fact, it creates steadier mandibles, may well increase implant stability, and, additionally, may improve resistance to mechanical, physical, chemical, and pharmacological agents. STEM CELLS TRANSLATIONAL MEDICINE *2013;2:316–324*

INTRODUCTION

Dental pulp stem cells (DPSCs) are mesenchymal stem cells embryologically derived from both neural crest and mesenchyme, like many other stem cells of the oro-maxillo-facial (OMF) body area, including dental follicle cells [1–5]. These cells display a variety of characteristics and differentiation potentials. In our previous studies, we demonstrated that DPSCs isolated from dental pulp coexpress CD34 and CD117, proliferate extensively, have a long lifespan, and maintain their multipotency for many generations [3, 6–9]. In addition, DPSCs are capable of differentiating into adipocytes, chondrocytes, and myocytes when cultured in adipo-chondro-myogenic media and into osteoblasts and bone when cultured in standard medium supplemented with 20% fetal bovine serum but no specific osteogenic morphogens and are capable of forming a complete and well-vascularized lamellar bone after grafting in immunosuppressed rats [1, 3]. The quality and quantity of the regenerated bone formed from DPSCs were demonstrated both in vitro and in vivo in experiments using stem cells and biomaterials [1, 3, 9–11].

Bone repair and regeneration are of major interest not only for the OMF area but also for the body in general. Bone loss is caused by many (congenital and degenerative) diseases, traumas, and surgical procedures; it leads to problems in functionality and is having an ever-increasing social impact, especially for the elderly. Thus, dental pulp is an interesting and potentially important source of ready-for-use autologous stem/progenitor cells for therapies aimed at the repair/regeneration of bone defects.

In our clinical studies on OMF bone repair, we used a biocomplex made of up DPSCs seeded onto an equine collagen I-based sponge scaffold. The enrolled patients had significant bone loss (alveolar loss of more than 7 mm in height, without walls) caused by extraction of their third molars. Six months from the intervention, we found that DPSCs were responsible for optimal vertical repair of the damage area and completely restored the periodontal tissue up to the second molars; regenerated bone was still evident 1 year

Hindawi
Stem Cells International
Volume 2020, Article ID 8890873, 12 pages
https://doi.org/10.1155/2020/8890873

Research Article

Dental Pulp Mesenchymal Stem Cells as a Treatment for Periodontal Disease in Older Adults

Beatriz Hernández-Monjaraz,[1] Edelmiro Santiago-Osorio,[2] Edgar Ledesma-Martínez,[2] Itzen Aguiñiga-Sánchez,[2] Norma Angélica Sosa-Hernández,[2] and Víctor Manuel Mendoza-Núñez[1]

[1]*Research Unit on Gerontology, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, Mexico*
[2]*Haematopoiesis and Leukaemia Laboratory, Research Unit on Cell Differentiation and Cancer, FES Zaragoza, National Autonomous University of Mexico, 09230 Mexico City, Mexico*

Correspondence should be addressed to Víctor Manuel Mendoza-Núñez; mendovic@unam.mx

Received 18 June 2020; Revised 23 July 2020; Accepted 30 July 2020; Published 18 August 2020

Academic Editor: Sangho Roh

Periodontal disease (PD) is one of the main causes of tooth loss and is related to oxidative stress and chronic inflammation. Although different treatments have been proposed in the past, the vast majority do not regenerate lost tissues. In this sense, the use of dental pulp mesenchymal stem cells (DPMSCs) seems to be an alternative for the regeneration of periodontal bone tissue. A quasi-experimental study was conducted in a sample of 22 adults between 55 and 64 years of age with PD, without uncontrolled systemic chronic diseases. Two groups were formed randomly: (i) experimental group (EG) $n = 11$, with a treatment based on DPMSCs; and a (ii) control group (CG) $n = 11$, without a treatment of DPMSCs. Every participant underwent clinical and radiological evaluations and measurement of bone mineral density (BMD) by tomography. Saliva samples were taken as well, to determine the total concentration of antioxidants, superoxide dismutase (SOD), lipoperoxides, and interleukins (IL), before and 6 months after treatment. All subjects underwent curettage and periodontal surgery, the EG had a collagen scaffold treated with DPMSCs, while the CG only had the collagen scaffold placed. The EG with DPMSCs showed an increase in the BMD of the alveolar bone with a borderline statistical significance (baseline 638.82 ± 181.7 vs. posttreatment 781.26 ± 162.2 HU, $p = 0.09$). Regarding oxidative stress and inflammation markers, salivary SOD levels were significantly higher in EG (baseline 1.49 ± 0.96 vs. 2.14 ± 1.12 U/L posttreatment, $p < 0.05$) meanwhile IL1β levels had a decrease (baseline 1001.91 ± 675.5 *vs.* posttreatment 722.3 ± 349.4 pg/ml, $p < 0.05$). Our findings suggest that a DPMSCs treatment based on DPMSCs has both an effect on bone regeneration linked to an increased SOD and decreased levels of IL1β in aging subjects with PD.

1. Introduction

Periodontal disease (PD) is an infectious and inflammatory alteration that affects the supporting tissues of the teeth and, when treatment is not appropriate or adequate, it can cause the loss of these [1]. PD presents an immune and anti-inflammatory response caused by antigenic substances from bacteria in the subgingival biofilm; however, the exacerbated host response is ineffective, and therefore chronic inflammation is maintained [2].

During the acute phase of PD, the presence of bacteria, and especially the lipopolysaccharides in your cell wall, attracts macrophages, leukocytes, and neutrophils to the area of infection. The latter contain enzymes such as NADPH oxidase and myeloperoxidase to produce reactive oxygen species (ROS) that help fight pathogens [3, 4].

Under normal conditions, antioxidant mechanisms protect tissues from damage by ROS secreted by neutrophils. However, if the body's antioxidant capacity is insufficient, oxidative stress (OxS) occurs, which is an imbalance between