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Grado en Odontología

UTILITY OF STEM CELLS IN DENTAL RESEARCH AND CLINICAL PRACTICE

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Summary

Introducción: las células madre han demostrado su potencial para su uso en odontología regenerativa y terapéutica. Se clasifican en diferentes grupos en función de sus características y su origen. En este trabajo se incluyen las más relevantes para la odontología.

Objetivo: El objetivo principal fue identificar y discutir diferentes aplicaciones de las células madre en la investigación y el uso clínico, mientras que el objetivo secundario fue analizar los usos futuros potenciales de las células madre.

Metodología: se utilizaron las bibliotecas en línea de Cochrane, Medline Complete y Dulce Chacón para obtener revisiones, ensayos clínicos y artículos que trabajaron con criterios de exclusión y que ayudaron a lograr los objetivos propuestos.

Discusión: se han recopilado y revisado una serie de ensayos clínicos y preclínicos para evaluar el efecto que sus resultados podrían tener en el trabajo en el mundo real. Las áreas de la odontología analizadas fueron la regeneración del tejido periodontal, la endodoncia regenerativa y la disfunción de las glándulas salivales, y se analizaron los ensayos clínicos de terapias con células madre relevantes para evaluar la importancia de los resultados y si tienen potencial para convertirse en tratamientos habituales. La mayoría de los ensayos mostraron aplicaciones exitosas de células madre, pero sufrieron por tener tamaños de muestra pequeños que se estudiaron durante períodos de seguimiento cortos. Los posibles desarrollos futuros fueron la regeneración dental completa, la ingeniería de la raíz biológica, las células madre derivadas no orofaciales para su uso en odontología y el tratamiento de inmunomodulación para afecciones inflamatorias.

Conclusión: La investigación y la utilidad de las células madre están en desarollo todavía y, por lo tanto, muchos de los ensayos deben limitarse a pequeños estudios preclínicos y clínicos. Sin embargo, muchos de los ensayos realizados muestran resultados prometedores en la mejora de los resultados de ciertos tratamientos, destacando el gran papel que podrían desempeñar las células madre en el futuro de la odontología.

Abstract

Introduction: stem cells have demonstrated their potential for use in regenerative and therapeutic dentistry. They have been classified into different groups based on their characteristics and their origin and those most relevant to dentistry have been included in this work.

Objective: the primary objective was to identify and discuss different applications of stem cells in research and clinical use, whilst the secondary objective was to analyse potential future uses for stem cells in the field of dentistry.

Methodology: the online libraries of Cochrane, Medline Complete and Dulce Chacón were used to source reviews, clinical trials and articles that worked within the exclusion criteria and that helped to accomplish the objectives.

Discussion: a number of pre-clinical and clinical trials have been collated and reviewed to assess what effect their results could have on real world working. The areas of dentistry looked at were periodontal tissue regeneration, regenerative endodontics and salivary gland dysfunction and clinical trials of relevant stem cell therapies, which were analysed to evaluate how significant the results were and if they have potential to become common treatments. Most of the trials showed successful applications of stem cells but they suffered from having

small sample sizes that were studied over short follow up periods. The potential future developments were complete tooth regeneration, bio-root engineering, non-orofacial derived stem cells for use in dentistry and immunomodulation treatment for inflammatory conditions

Conclusion: Stem cell research and utility is in its infancy and therefore many of the trials must be limited to small pre-clinical and clinical studies. However, many of the trials carried out show promising results in improving the outcomes of certain treatments, highlighting a significant role stem cells could play in the future of dentistry.

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1. Introduction

Stem cells are recognised in the medical world as having great potential for advancing reparative and regenerative therapeutic treatments. These unique cells have the ability of self-renewal and differentiation, meaning they can become cells from many different lineages (1). They are classified according to the variety of different cells into which they can differentiate and are as follows: embryonic stem cells, adult stem cells (further broken down into hematopoietic and mesenchymal stem cells) and induced pluripotent stem cells (2).

- Embryonic stem cells (ESCs) are pluripotent, being able to differentiate in vitro into every somatic cell lineage as well as all germ cells and therefore have great therapeutic potential (2). However, harvesting of these cells can only be done from a fertilised embryo and therefore their use is limited by laws and ethical concerns.
- Mesenchymal stem cells (MSCs) are multipotent and can be harvested from bone marrow, skin, oral and maxillofacial areas, and adipose tissue. Their potential for differentiation is more limited than pluripotent cells'. The cell types they can become are dictated by the cell type from which they originate. For instance, blood stem cells (example of hematopoietic adult stem cells) can become many different blood cell types but cannot become bone cells (3). A great advantage of their use is that they are not rejected by immune systems and can be used autologously (1).
- Hematopoietic stem cells (HSCs) are classified as being able to form blood and can differentiate into: red blood cells, white blood cells and platelets (4). There exist two types of HSCs; long term and short term repopulating cells. The former maintain self-renewal capabilities for the entirety of their life cycle and the latter has partial self-

renewal (5,6). Sources of HSCs include bone marrow, peripheral blood cells and umbilical cord blood (4).

- Induced pluripotent stem cells (iPSCs), as the name suggests, share the embryonic stem cells ability to become many different cell types but are not constrained by the same ethical and legal restrictions. This new development involves artificially modifying adult stem cells into an embryonic-like form (7).

The classification of MSC is the one most used and therefore most important to dentistry. MSCs are further categorised into groups based on their source, that is to say, the part of the body they are harvested from (as can be seen in figure 1).

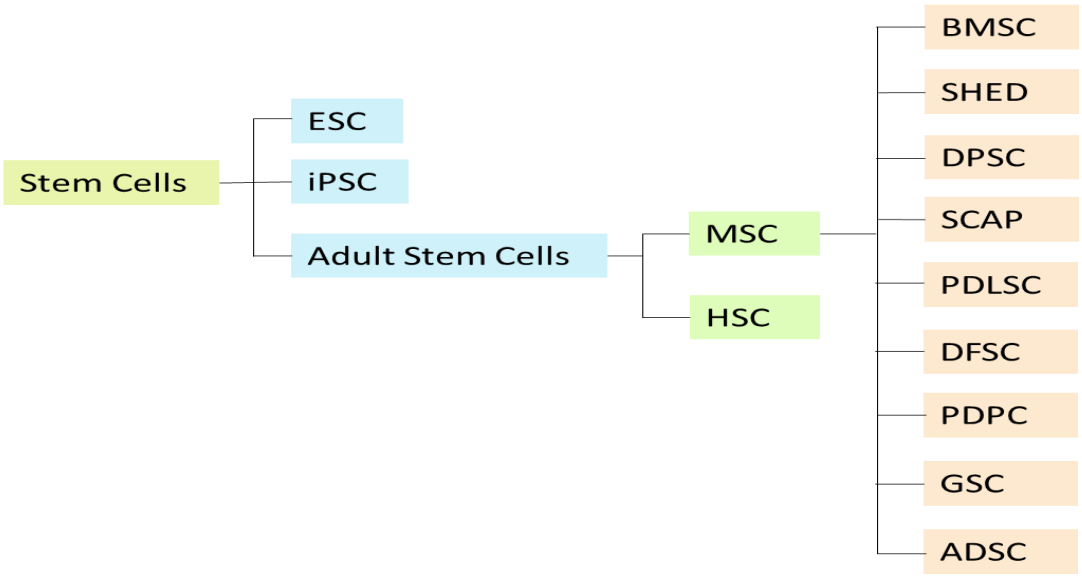

Figure 1. Categorisation of stem cells (14)

Stem Cells used in Dentistry

MSCs

In 2006 the International Society for Cellular Therapy (ISCT) established a minimum of three criteria for defining MSCs. Firstly, they must be able to adhere to plastic in standard culture conditions. Secondly, they need to present certain surface molecules: CD73, CD90 and CD105 whilst at the same time lacking the molecules: HLA-DR, CD79alpha, CD19, CD34, CD45, CD14 or CD11b. Thirdly, they require the ability to differentiate into osteoblasts, adipocytes and chondroblasts in vitro (8). Many MSCs used clinically today are derived from oromaxillofacial tissues (examples of which can be seen in figure 2).

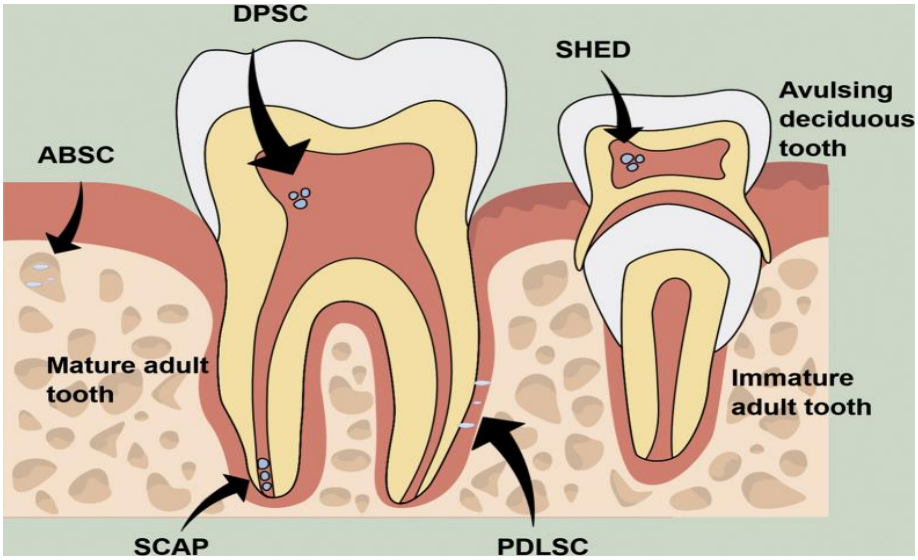

Figure 2. Visual depiction of stem cell sources (46)

(a) Bone Marrow-Derived Stem Cells (BMSCs)

Bone marrow contains two types of adult stem cells: HSCs and MSCs (9). This makes BMSCs very useful for regenerative treatments since the HSCs can become cells from blood cell lines and whilst MSCs, as previously mentioned, can become osteoblasts, adipocytes and chondroblasts, thus making them a useful source of multipotent stem cells. The varying cell

characteristics mean these stem cells can develop into bone, cartilage, tendon, muscle, adipose tissue and neuronal tissue (10,11). BMSCs are currently being used in a range of clinical applications as they help promote new bone growth, owing to their ability to develop into osteoblasts and form new areas of vascularisation (12). Most common delivery forms are to inject suspensions of single cell BMSCs into a site for regeneration or adding them to scaffolds or hydrogels for use in mandibular bone graft treatments (13). They have been shown to have some phenotypic variance between donors, which can make their efficacy and use somewhat unpredictable. BMSCs used in dentistry are typically harvested from two locations: orofacial bones and the iliac crest.

Bone marrow aspirate from the iliac crest yields some of the most commonly used BMSCs in dental regenerative treatments (14). They present great potential for differentiation into myogenic, osteogenic, chondrogenic, adipogenic and nonmesenchymal neurogenic cell lines. One main disadvantage of stem cells derived from the ilium is that it requires an invasive surgical treatment to harvest them. Another downside is that characteristics such as age and gender of the donor has some slight effects on outcome of use, with some studies reporting a decrease in chondrogenic differentiation potential for iliac crest BMSCs from older male populations when compared to female populations. Increasing age affects the viability, multilineage proliferation potentials, expression kinetics and immunoregulation. The age of the patient receiving the treatment, however, does not influence its regenerative capacity (15,16).

Orofacial bones can also serve as a source for BMSCs. Dental treatments such as: implants, surgical extractions of impacted teeth, enucleation of cysts and orthognathic surgery provide

opportunities to collect BMSCs from both the maxilla and the mandible (17). These cells can be extracted from donors of all ages, however, their gene expression can be affected by age (18). According to studies, orofacial bone marrow stem cells (OBMSC) produce better results than iliac crest stem cells when they are used for grafts in craniofacial areas, producing larger quantities of bone. A study in 1990 compared outcomes of bone grafts to bridge alveolar cleft defects using the ribs as a source versus bone from the mandibular symphysis. The group that received the rib graft showed higher levels of bone resorption and more complications such as wound dehiscence. This could be due to the embryological origins of facial bones compared to the ilium; the maxilla and mandible grow from the neural crest cells during embryo development whereas the iliac crest bone comes from the mesoderm (19). A 2004 study looked at how the biological features of BMSCs change depending on where in the body they came from by comparing alveolar bone with iliac crest bone. The results found that whilst the alveolar stem cells had great osteogenic potential, they presented worse chondrogenic and adipogenic potential when compared to iliac crest cells. This can mean less fat production while the bone is healing which is beneficial for regeneration (20). The main downside to OBMSCs is that extracting the marrow from craniofacial bones produces far less volume than from the iliac crest (21).

(b) Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

SHED were first isolated from the dental pulp of exfoliated primary teeth in 2003 (22). They represent an excellent source of postnatal stem cells. For instance, they are readily available and easily acquired as part of a natural process, not requiring any form of additional treatment to source them. Their accessibility is such that even carious primary teeth can have their stem

cells isolated (23). Furthermore, ethical and legal issues surrounding SHED are few, owing to the fact they are easily attained without the risk of harm to the donor, which results in them being very useful in the field of research and development. SHED are considered multipotent and have the ability to become adipocytes, endothelial cells, neurons, osteoblasts and can self-renew, making them similar to BMSCs and they therefore can be used in many regenerative procedures. Moreover, SHED can excel in bone regeneration specifically, having been shown to, in vivo, participate in osteogenesis by promoting osteogenic differentiation of the host's own cells (23). In 2008, experimental work revealed the potential SHED displays in regenerating dental pulp. The researchers attached SHED as scaffold material for xenologous teeth implants into a mouse to determine if the SHED had the capacity to specialise into odontoblasts capable of producing tubular dentin (24).

(c) Dental Pulp Stem Cells (DPSC)

Similar to exfoliated deciduous teeth, adult dental pulp tissues also represent a bank of useful stem cells and are the most frequently used source. First isolated in 2000, DPSCs have been shown to be osteogenic, odontogenic, myogenic, adipogenic, neurogenic and can contribute to pulp-dentin complex production (1,25). An advantage that DPSC share with SHED is that they have good accessibility, with only a small surgical access being necessary to harvest the tissue. Cryopreservation means that once extracted and isolated, the stem cells can be stored for later use in scaffolds for example (26). Adult dental pulp is different physiologically to deciduous pulp tissue and therefore differences between the stem cells derived from each are to be expected. SHED, by comparison, present higher proliferation rates, cluster formation and differentiation potential, resulting in higher levels of osteocalcin being produced as well

as greater alkaline phosphate activity when differentiating osteogenically (27). Despite this DPSC are still a very viable and commonly used option for research and clinical use.

(d) Stem Cells from Apical Part of Papilla (SCAP)

Stem cells were first discovered in the apical tissue of teeth when, upon inspection, they were seen to express the STRO-1 marker, with STRO being an indicator of mesenchymal stem cells. The regenerative potential of SCAP can be demonstrated by immature teeth with necrotic pulps still being able to complete their root development and, furthermore, root maturation stops when no SCAP is present (28,29). SCAP is harvested following tooth extractions when the tissue attached to the apex of the tooth is removed with tweezers. Then using collagenase and dispase, the tissue is isolated in a single cell suspension. SCAP have strong proliferation, migration and regeneration characteristics, being able to produce pulp-dentin complex in vivo (30).

(e) Periodontal Ligament Stem Cells (PDLSC)

Multipotent stem cells were first found in human periodontal ligament in 2004, again, recognised by their expression of the STRO-1 marker (31). In vitro they have adipogenic, chondrogenic and osteogenic potential, whilst in vivo they can differentiate into different components that make up the periodontium, such as cementum, periodontal ligament and alveolar bone (32,33). PDLSC are obtained after a tooth extraction and can be sourced from either the root of the extracted tooth or from the alveolar bone in the socket. In fact, the origin of the tissue from which the cells are harvested affects their behaviour. PDLSCs taken from the roots of deciduous teeth have stronger proliferation, adipogenic and osteogenic ability than from the roots of permanent teeth, whilst alveolar bone PDLSC have even greater

capacity for these characteristics than root tissue stem cells (34,35). More recently, infected granulation tissue and supernumerary teeth are newly discovered sources of PDLSC. With these being tissues requiring removal, the PDLSCs can be the by-product of otherwise necessary procedures (36).

(f) Dental Follicle Stem Cells (DFSC)

DFSC are derived from the follicle that surrounds a tooth during the cap stage of development. The dental sac (as it is otherwise known) is comprised of ectomesenchymal progenitor cells and for this reason it is suggested that DFSC could display more plasticity than other stem cells from dental tissues. These cells are typically harvested from the follicle surrounding third molars (37).

(g) Periosteum Derived Progenitor Cells (PDPC)

Periosteum is the tissue that surrounds bones, providing protection and a stream of nutrients and blood. Progenitor stem cells live within the specialized connective tissue that makes up the periosteum. Despite the osteogenic nature of periosteum first being suggested in 1932, it was not until 2009 that stem cells were shown to be present in periosteum (38,39). PDPC are characterised as multipotent, since they can differentiate into adipocytes, chondrocytes, osteoblasts and skeletal monocytes, making them suitable for bone regenerative therapies (40).

(h) Gingival Derived Stem Cells (GSC)

GSC represent an encouraging branch of dental stem cells since they have an abundant source, with collecting the tissue only requiring a minimally invasive practice, as opposed to tooth

extractions for instance. GSC have a greater proliferation than BMSCs and do not have tumorigenic potential (40). Moreover, they have been found to have immunomodulatory functions, making them good candidates for regenerative applications (41).

(i) Adipose Derived Stem Cells (ADSC)

Adipose tissue is one of the most readily available sources of stem cells and they have potential for proliferation, differentiation and expression of immunophenotypes (42). This is because adipose tissue has a higher concentration of MSCs than most other sites (43). A benefit of ADSC over BMSC is that the procedure to collect it is far less invasive and has a low donor site morbidity rate (44). Subcutaneous fat stores have adipose tissue amounts that are very plentiful, however the buccal fat pad is more commonly used as a harvest site for stem cells since it is highly accessible and the progenitor cells within the fat pad have been shown in animal studies to differentiate osteogenically. Furthermore, they have been used in bone regeneration procedures in jaw resections (45).

2. Objective

The main objective in this work is to analyse the different uses of stem cells in dental research and clinical practice in order to identify and discuss applications of stem cells in these situations.

As a secondary objective, the potential future developments of stem cell usage in dentistry will be evaluated.

3. Methodology

Study search analysis: the strategy employed was to use online libraries to source and locate publications relevant to the work. Online libraries used were Cochrane, Medline complete and Dulce Chacón. In order to obtain a brief and quick outline of the content surrounding the topic the keywords “dentistry AND stem cells” were used to give a broad array of works which then could have filters applied to them, allowing specific themes to be focused on. For example, the initial search was refined to display only reviews, since they provide an accessible summary of the topic. This meant an understanding of what was relevant to the objectives could be achieved in a time efficient manner. The search was then filtered to locate texts specific to stem cell utility and research in dentistry to help achieve the objective. To realise both objectives, clinical trials were collated for review and comparison using the online libraries mentioned previously.

The inclusion and exclusion criteria were all English publications released as recently as possible. The clinical trials used were from the last 10 years, since they represent what the current findings and knowledge is. However, some sources used for theoretical knowledge are older than that, since that knowledge is still pertinent to today. Publication types used were reviews, clinical trials, and articles.

4. Discussion

Following on from the discovery of stem cells, the attention of the scientific community turned to their potential uses and applications. Owing to their biological dynamism and variability many of the uses in clinical practice revolve around tissue regeneration and gene therapies. Since stem cells and their utility is a recent venture in scientific terms, numerous clinical trials

have been undertaken to evaluate the outcomes of their clinical use. Many of the studies involve tissue engineering by way of transplanting the stem cells into a site, to achieve this, 3 biological requirements are necessary: the stem cells, growth and homing factors and finally a scaffold to transport the cells to the site (62).

4.1 Periodontal Tissue Regeneration

Periodontitis is a chronic condition that involves inflammation and subsequent destruction of the supporting structures of the teeth. Based on global figures in 2016, it was the 11th most prevalent disease in the world, with a range of 20% - 50% of the global population having the condition. With it being one of the leading causes of tooth loss in adults the necessity for advances in therapeutic methods of combating the disease is undeniable (47). The main objective of the treatment of periodontitis is to halt its advancement and achieve periodontal tissue regeneration (PTR) (48). Traditional methods of treatment include scale and root planing (SRP) and then for more severe cases, periodontal surgery with guided tissue regeneration (GTR) and flap debridement techniques. Complete regeneration of the destroyed tissue thus far has not been possible using these procedures and has mostly only been successful in postponing the eventual loss of teeth (49). The advent of stem cell isolation and cultivation has opened new opportunities to attain PTR in a predictable and repeatable manner which is less technique sensitive.

i) A randomized control trial (RCT) conducted in 2018 looked at the efficacy of using DPSCs in the regeneration of intrabony defects. As seen previously, DPSCs have great osteogenic potential and therefore represent a fitting candidate for the

redevelopment of lost bone tissue when impregnated onto a scaffold and then implanted into the site of the defect, much like in figure 3 (1,23).

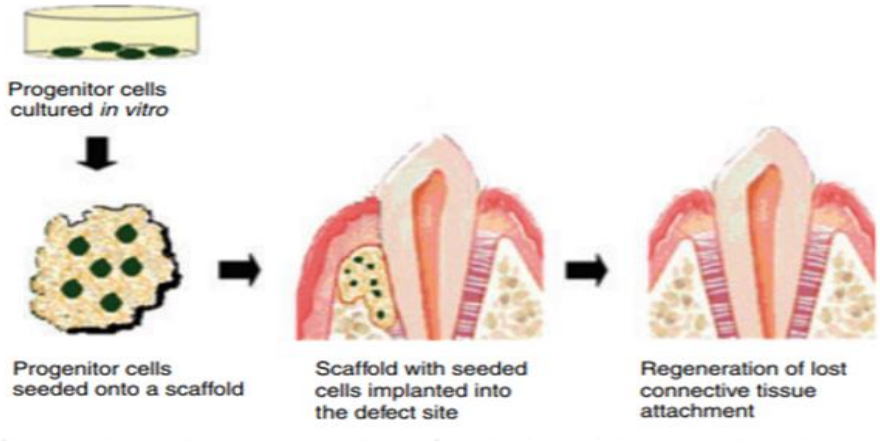

Figure 3. Theoretical example of stem cells being loaded onto a scaffold to treat an intrabony defect (51)

During the trial, 29 patients with intrabony defects related to periodontitis had autologous micro-grafts (containing the stem cells) surgically placed into the defects in a collagen sponge scaffold, with the results being recorded after half a year and then again after a whole year. The outcomes of the trial were compared against a control group which only received a standard collagen sponge at the area of bone loss. At both the 6 month and 12 months follow up periods, the trial group showed better radiographic and clinical responses. The probing depth parameter showed a recuperation of 4.8mm in the trial group against 3.3mm in the control group at 6 months and 4.9mm to 3.4mm respectively at 12 months whilst the parameter of radiographic intrabony defect depth showed an improvement of 3.7mm (trial) compared to 1.5mm (control) at 6 months and 3.9mm to 1.6mm at 12 months. Clearly

the difference in these results is not insignificant and therefore represents a promising area of study for further development of the treatment. This study, however, is not without its drawbacks. For instance, the study only includes 29 patients, split into two groups of 15 (trial group) and 14 (control group) so the actual sample size is quite small and therefore the precision of the results and the power to draw conclusions from the study are not ideal. Another drawback to the trial is that the autologous graft of the DPSCs came from a vital tooth that needed to be extracted. This reduces the applicability of the results to real world situations because not all patients that have bony defects will need an extraction of a vital tooth and therefore the results, whether positive or not, would apply to a smaller category of patients (50).

ii) Early preclinical findings have suggested that PDLSCs would be adequate for restoring periodontal tissues. The periodontal ligament shows an ability to regenerate itself and this is achieved by the progenitor cells that constitute part of the apparatus, owing to the propensity of the cells to produce bone, fibre, and cementum tissues (29). Motivated by these findings, a clinical RCT conducted in 2016 explored the safety and efficacy of PDLSC use as grafts into bone defects. Similar to the previous trial, this one used a control group that received bovine bone substitute (Bio-oss) that would be used in standard GTR and a trial group that received the same Bio-oss but combined with an autologous PDLSC graft material, with the group split being 21 teeth involved in the control group and 20 in the trial. The parameters measured were firstly the number of unwanted medical problems associated with the treatment counted over the course of the 12 month follow up period and then secondly, the amount of bone defect

regrowth in contrast to the baseline depth of the bone loss. In terms of the safety of using PDLSCs for this kind of treatment, the results showed that there were no significant risks or complications arising solely due to the inclusion of the stem cells. (Of course there were the usual side effects of pain and inflammation.) For the regenerative ability of the stem cell graft, the trial produced no significant differences in bone restoration between the control group and the trial group. Both groups showed a positive reaction to the GTR. However, the addition of PDLSCs did not improve the response when compared to that measured from the control Bio-oss group. Despite this trial not demonstrating the efficacy of PDLSC use in bone defect therapy, it was important in furthering the wealth of information for stem cell research and use. Many of the early studies in this field have been carried out on animals or in pre-clinical settings, so clinical trials like this are significant because they move the evidential findings into the next stage of research. Unlike other similar studies in this field, this trial investigated the safety of PDLSC utility in a clinical setting. This is a very important aspect to consider when trying to incorporate burgeoning medical techniques into the repertoire of solutions available to medical professionals. A new technique may achieve the desired result; however, it is of no practical use if unsafe. Thus, this study has accomplished a vital task in demonstrating the safety of PDLSC utility in the clinic. It should be noted that the drawbacks to this study are similar to the first periodontal GTR RCT looked at, these being small sample size and the isolation of autologous PDLSC required 3rd molars that needed to be extracted, meaning that the results would apply to a smaller niche of patients in real world situations (52).

iii) In 2016 a phase I/II study looked at the use of BMSC grafts in the treatment of periodontitis. This clinical trial featured 10 periodontitis patients who had osseus loss requiring restorative treatment. The tests measured the clinical attachment loss recovered, the difference in probing depth, tooth mobility, linear bone growth and any adverse effects experienced during the 36-month analysis period. The results of the study were compared against a previous trial that measured the same parameters and graft therapy, except without the inclusion of BMSCs (53). The bone regeneration outcomes were enhanced by the BMSCs with the pocket depth and clinical attachment loss presenting double the gains of the standard techniques and linear bone growth was four times better. In the case of the tooth mobility, however, the results gained were not statistically significant since the data analysis demonstrated p values of >0.05. Moreover, the safety of the treatment was sound as none of the problems postoperatively were causally related to the addition of the stem cells. The positive results of this trial can provide support for the hypothesis that BMSCs used in the treatment of periodontal bone defects can improve the restorative outcome. However this trial represents the first small steps in developing this field. The sample size (10 patients) is an obvious drawback and it is certain that more trials of this kind will be necessary. Nevertheless, this study is noteworthy for its length. The follow up period is 3 years, which is uncommonly long in stem cell clinical trials, most likely due to stem cell research being a relatively young endeavour, thus allowing for a more comprehensive understanding of the difference the stem cells can make in the treatment (54).

iv) In less severe cases of periodontitis, a non-surgical treatment plan is what is required. Scale and root planing (SRP) is the standard approach to rectify poor periodontal conditions that do not require surgery. However, in deeper pockets and hard to reach areas SRP does not always produce effective results so an adjuvant therapy can be helpful to achieve the desired effect (55). In 2018, Cairo University conducted an experiment in rats to assess the efficacy of using injected ADSCs as an adjunctive to SRP in non-surgical treatment of periodontitis. The study initiated periodontitis by encouraging plaque build-up in a group of 50 rats, with the group being divided into a control group (healthy periodontium), a group that would receive only SRP treatment, a group with SRP and ADSC injection and finally a group with SRP and the exosomes of ADSCs. Exosomes are extracellular vesicles that are given off by MSCs (and by many other cell types) that in recent years have been accredited with contributing to the reparative effect of stem cells (56). A third of the rats from each group were sacrificed at 3 different periods: after 2 days, 2 weeks and 4 weeks and then the samples were histologically analysed to reveal the amount of collagen and bone formation taking place. At each interval the results stayed consistent; the SRP group showed the least amount of tissue production, the ADSC group the second most and the ADSC exosome group showed the most positive results. An obvious drawback to this study is that it was carried out on rats and therefore the results will not necessarily be applicable to humans clinically. Nevertheless, this is a significant trial for various reasons; it is the first trial to use exosomes in the restoration of periodontitis and it is the first trial to evaluate the use of ADSCs as adjuvant therapy in SRP treatments (57). These pioneering trials using animals are important because they provide a basis for further

research into a certain field without risk of potential harm to human life. If the results gained show a positive correlation in favour of the hypothesis, then that is grounds to conduct more studies and hopefully develop theory into real world treatments. The recent advent of stem cell exosomes being found to be useful therapeutically demonstrates how stem cell research is continually evolving and making discoveries.

4.2 Regenerative Endodontics

Dental pulp plays an essential role in the maintenance and longevity of dental health and as such, significant effort is involved in preserving it with the ultimate aim of retaining natural teeth in the mouth for as long as possible. Traditionally, when affected by irreversible pulpitis or pulp necrosis the main goal of clinical treatment has been to remove the pulp, conform the canal and place an inert filling material to create a hermetic apical seal to, in theory, prevent bacteria from entering the canal space and possible apical infection from occurring. In practice, endodontic treatments do not always achieve this. Reasons for failure may be multifactorial and the technique sensitive and complex nature of the treatment can complicate outcomes. Worryingly success rates have not risen over the last 50 years, with figures staying between 68% - 85% (58), hence there is a desire to explore alternative methods of resolving affected pulp tissue. Regenerative endodontic procedures (REPs) represent a potential substitute for the traditional treatments. Regenerative medicine aims to replace lost structure or function and stem cells have been identified as being useful in accomplishing this aim.

i) In 2020 a phase I/II RCT took place to evaluate the safety and efficacy of using MSCs to treat periapical lesions. The study was conducted clinically and had a sample size of 36

patients presenting necrosis of the pulp and apical periodontitis, with the total sample being split into 2 groups, one receiving the MSC based REP and the other receiving traditional endodontic treatment. The parameters measured include: pulp vitality test responses, radiographic size of the apical lesion and perfusion units measured with laser doppler flowmetry (LDF). These measurements took place at 6 months and 12 months. The conductors of the trial also tracked any negative side effects experienced resulting from the therapy. The findings of this trial showed some statistically significant improvements made by the REPs over the standard treatments, meaning they reached the criteria of having $P<0.05$ (using the Mann-Whitney test). Specifically the parameters of vitality test responses (to cold, heat and electricity) and change in antero posterior apical lesion dimensions (as can be seen figure 4).

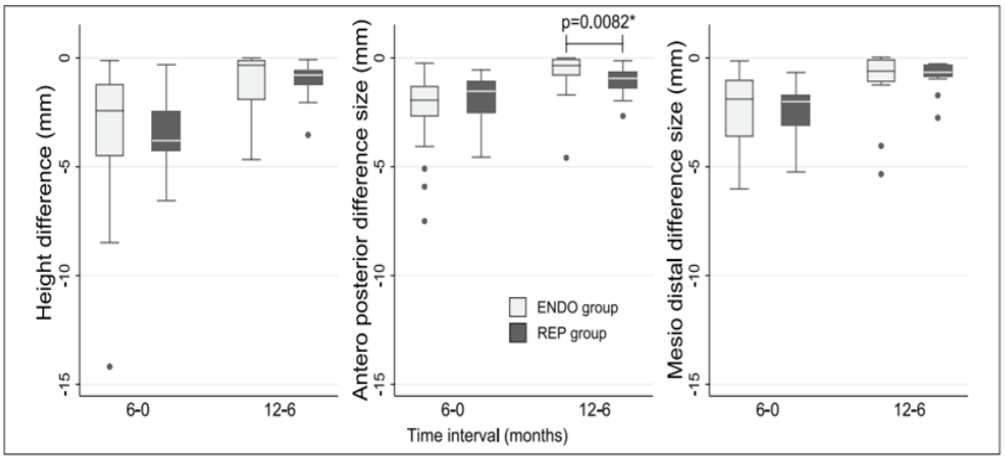

Figure 4. Results of the trial showing the differences in lesion sizes between the two groups (59)

Interestingly these statistically significant differences were only found at the 12 month follow up period and not at 6 months which holds promise for the long-term success rates of these particular treatments. A major conclusion drawn from this trial is that MSCs are safe to use in REPs, since every tooth subjected to the new treatment type

stayed in the mouth throughout the entire follow up period and by comparison to the other group did not present a deterioration in condition (59). The main drawback presented by this trial is the length of the follow up period. Typically, the success rates of endodontically treated teeth decline the more time passes since the treatment and 1 year is not enough time to be able to draw proper conclusions on the efficacy of new pulp treatments. This study is, however, looking into pioneering techniques that are in their infancy and have shown promising steps in the right direction based on the results of the trial. The evidence for clinical safety shown can serve as a foundation for further and more extensive clinical trials involving stem cell utility in REPs.

ii) In immature permanent teeth with an open apex, damaged or necrotic pulps can be regenerated using revascularisation techniques. The aim of the treatment is to allow the immature tooth root to develop into a mature one and complete the process of apical closure. In the past, artificial materials were used to create a man-made apical closure, but with advances in stem cell research, revascularisation has emerged as a new treatment option. It relies upon the ability of remnant stem cells within the pulp tissue to renew and proliferate, allowing for a natural maturation and closure of the root apex (60). This technique, unfortunately, is only indicated in immature teeth with an open apex and is therefore limited to a smaller subsect of patients. Nevertheless, using the success of these therapies, studies have emerged that are attempting to achieve full pulp restoration in mature teeth with a closed apex. One such study in 2011 delved into this innovative approach to REPs by focusing on the outcomes of inserting autologous DPSCs into the root canal of the teeth of dogs that had their pulp tissue completely removed. The stem cells were attached to a collagen scaffold which

occupied the space in the canal left behind by the original pulp tissue. The aim of the study was to induce new pulp tissue that was capable of forming new neurons and vessels, two requisite features of functional dental pulp (61). Promisingly, the findings showed complete pulp formation, with pulp that demonstrated vasculogenesis and neurogenesis. Of course, a limitation of this trial is that the stem cells used were autologous to the dogs and so performing the same investigation with humans and their autologous DPSCs might not yield the same results. However, the histological discussion of the study does highlight the CD105 cells isolated from the dog pulp stem cells as being the cells that bring the potential for neuron and vessel formation. Human CD105 pulp cells have shown that they also his capability, meaning that there is promising potential for this to be expanded onto human trials (62).

iii) Following on from ground-breaking animal trials involving complete pulp regeneration, the next step is to test the pulp formation capacity of transplanted stem cells in human subjects. Spurred on by the success of human DPSC demonstrating pulp renewal properties when grafted into the roots of mice, a 2017 pilot clinical study investigated the efficacy and safety of using autologous DPSCs to treat pulpitis in humans (63). The trial used 5 patients with irreversible pulpitis and for whom the only treatment option was the complete removal of the pulp tissue. Suspended in a collagen scaffold the stem cells were placed into the now clean canals and then the patient responses were monitored at 1, 2, 4, 12, 24, 28 ,32 weeks after the treatment. The safety of the technique was evaluated by the frequency of harmful side effects and the efficacy of the transplant was determined by patient response to electrical vitality tests and by MRI scanning to reveal newly formed tissue. The results of the

safety parameter showed no adverse effects linked to the transplant, whilst the electric vitality test demonstrated a change from negative vitality to positive vitality in four of the five patients and the MRI showed a pulp state that was consistent with normal healthy pulp. A drawback of this trial is that it was only performed on five patients and therefore the sample size is very small making conclusions hard to draw. A further drawback is that electrical vitality tests are not completely accurate as they rely on the subjective feeling of the patient and can give false positives (64). The electric tests combined with the MRI scanning does go some way to improving the accuracy of the claims, however. Within these limitations the results of this trial are incredibly promising for the future of regenerative endodontics and more larger scale trials of this nature will be necessary.

4.3 Salivary Gland Dysfunction

The body's saliva possesses a multitude of essential functions, without which the health of the oral cavity and quality of life would be compromised. It contributes towards phonation, mastication, gustation and protection and so a disruption to the production of saliva would have a great negative impact (65). In terms of dental health, saliva provides direct protection from bacterial tooth decay by diluting harmful acids, buffering acids to neutralise them, whilst salivary flow offers mechanical protection and the mineral content helps to remineralise the tooth after exposure to acidic conditions. Therefore, the importance of the body's saliva cannot be understated. The ability of the salivary glands to function properly can be affected by pathological conditions and syndromes, and by radiotherapy in cancer treatments, leading to xerostomia(66). Currently medical research is investigating the efficacy of using stem cells to restore function to salivary glands.

i) Radiotherapy treatment of head and neck cancer exposes the salivary glands to potentially damaging levels of radiation, often causing xerostomia as an adverse effect of the treatment (67). In 2018 a team conducted a randomised placebo-controlled phase I/II trial in which MSCs were transplanted into the submandibular glands of patients who had undergone radiotherapy of the head and neck. Like most trials of novel treatments, safety of use was one of the parameters measured, along with efficacy of outcome. The safety was measured by the appearance of unwanted harmful side effects and the effectiveness of the treatment was measured by the flow rate of unstimulated salivary glands. A sample size of 30 patients was split evenly into two groups: one receiving the MSC therapy and the other receiving a placebo. The periods of assessment were one month before the start of the trial (to get a baseline value to compare against) and then one and four months after the transplantation of the stem cells. Assessment of salivary changes were done by the passive drooling technique to measure the salivary flow; this was supplemented then by patients taking a visual analogue scale (VAS) questionnaire to record any feelings of hyposalivation on their part. The results of the trial concluded that no harmful side effects were experienced by the sample group and the response of the trial group receiving the MSCs was overall positive. A significant increase of saliva flow was found in the sample group when compared to the placebo group and the answers to the VAS revealed that the patients felt the symptoms of xerostomia less. This trial was a success in terms of achieving its aim and producing results that significantly supported the hypothesis (68). The methods used to obtain the data were sound, the passive drooling technique is considered the optimum method for measuring salivary flow rates since it can yield

large samples and has the lowest risk for error (69). The VAS questionnaire is a subjective evaluation of a patient's symptoms (70), so the data obtained from them is not totally reliable and the placebo effect could have some subconscious effect on the patient's subjective feeling of having a dry mouth sensation. The patients in the trial were blinded as to which treatment they were receiving, but they knew they were receiving some type of hyposalivation therapy, so a placebo effect could affect a qualitative assessment of the condition. The same cannot be said for the saliva flow measurements since they are purely quantitative. A possible improvement that could be made to this trial would be to replace the placebo group with a group that is receiving the typical xerostomia treatments used today, for example, sialagogue medication which induces saliva flow. This would allow the results of the new technique to be compared against contemporary standards.

ii) Sjogren's syndrome (SS) is an autoimmune disease that acts by targeting the body's own epithelial tissue causing damage to glands and disrupting their function. The disease is a slow acting progressive one that can take years for symptoms to appear. One symptom that is of interest to the dental profession is xerostomia. The sensation of dry mouth is caused as the disease destroys salivary glands. Currently, the only therapy for SS is to treat the symptoms and give immunosuppressant drugs. As things stand, the current treatment of SS' symptoms are not at the desired level and leaves patients with worse quality of life (71). For this reason, research is driven to find more successful alternatives. In 2014 an experiment was conducted on mice that had induced "sjogren's-like" disease to try and re-establish salivary gland function. The trial involved injecting BMSCs intravenously and then recording the salivary flow rates to

determine the effectiveness of the treatment. The trial did indeed find that that the flow rates were increased to regular values and thus the work served as a proof of concept (72). Following on from the success of this initial advance in the field a research group found that stem cells harvested from a sufferer of SS had immunoregulatory deficiencies, resulting in very high proliferation rates. In contrast, BMSCs taken from healthy patients were demonstrated to have lower proliferation rates (69). With higher rates comes an increased risk of tumour formation, posing a clear health risk and presenting a challenge to the progress of BMSCs used in this way because deploying stem cells autologously is often the most feasible method of use in practice. Consequently, using autologous stem cells to treat salivary gland dysfunction in an SS patient would carry a risk of tumour growth, owing to the dysfunctional proliferative nature of SS derived stem cells. These revelations demonstrate that stem cell research and clinical usage is still in its infancy and more studies must be undertaken to broaden current knowledge and potentially convert promising trial results into effective and safe treatments.

4.4 Potential Future Developments

As seen previously, there have been many encouraging outcomes in pre-clinical trials involving animals in addition to some human clinical trials. Owing to the relative recency of stem cell research, particularly in the field of dentistry, there is still much to be discovered and great potential for development. Some proposed uses for stem cells in the clinic hold great potential and could become the standard treatment in years to come.

4.5 Complete Tooth Regeneration

Dental stem cells have already demonstrated their efficacy in healing and replacing lost tissue in various trials, therefore one could see their prospective success in tissue regeneration. Bioengineering teeth represents a new field of dentistry that could rival the prosthetic replacement of lost dentition. For this to happen, techniques for inducing stem cells to form dentin and enamel that is consistent in its composition with natural teeth need to be developed. Research has demonstrated that, under the right conditions, it is possible for this to occur (73).

One study has proposed two methods for tooth generation, one which suggests growing the dental tissue on tooth shaped scaffolds that are loaded with the regenerative stem cells to grow the tooth. As with many tissue engineering methods, the scaffold materials need certain properties, such as biocompatibility and the ability to promote cell growth. The other proposed method involved placing tooth derived stem cells into an in vitro organ culture and guiding stem cell interactions to form tooth structure (74).

The framework for development of this idea has very much been set out by researchers. All that is required now is a series of appropriate pre-clinical and clinical trials to further the work done.

4.6 Bio-root Engineering

In recent years implants have increased in popularity and now are a viable option to accompany bridges and removable prosthesis, due to their growing accessibility. One drawback of implants is that they do not possess a periodontium tooth relationship in the form of periodontal ligament with the main structural reinforcement coming from

osseointegration. An alternative method to get around this problem has been proposed; to use PDLSCs to create a bio-root (a root generated by stem cells). This would allow for new ligament formation due to the PDLSCs and then once the root is secure an artificial crown can be attached (73). Early tests have placed gelfoam scaffolds carrying PDLSCs into minipig models. Periodontal ligament did indeed form around the bio-root, however its mechanical resistance was measured to be around two thirds of a normal root, thus the technique requires further inquiry if it is to one day rival implants as a treatment option (75).

4.7 Non-orofacial derived stem cells for use in dentistry

Orofacial derived stem cells, whilst very useful, carry with them some limitations. For instance, they can be difficult to harvest, sometimes requiring invasive techniques to access the tissue or in the cases where acquiring the tissue is easier because it comes as a by-product of a natural process (like SHED cells) or from a required treatment (PDLSCs harvested from extracted teeth) stem cells can still be difficult to isolate and cryogenically store. Therefore other, more accessible, sources of stem cells useful to dentistry are being investigated. Researchers specializing in regenerative medicine have suggested urine as a possible source of stem cells for growing teeth. Pluripotent stem cells isolated from human urine were manipulated into generating tissue with similar composition to tooth in a pre-clinical trial involving mice with the results yielding a 30% success rate in forming said tissue. If in the future urine derived stem cells can be developed further to offer a proper source of regenerative cells, this will promote greater research and utility in, since they would be low cost, non-invasive and autologous use of them will lower the chance of rejection (7).

4.8 Immunomodulation treatment for inflammatory conditions

Stem cell therapies are mostly centred around tissue engineering and regeneration. However, as more is learnt about the mechanisms of stem cell treatments and what makes them valuable to dentistry, new areas of study for potential clinical uses arise. For instance, modern research suggests that the effectiveness of stem cells in tissue regeneration is due to a modulatory effect they have on innate and adaptive immune responses of the host (76). Building upon this, GSCs have been used in several inflammatory diseases to test their effectiveness and give further evidence to the immunomodulatory effects of stem cells. The clinical signs of contact hypersensitivity have been shown to improve when GSCs are given prophylactically and as a therapeutic dose (77). Additionally, GSCs administered systemically exhibited a tolerance to the host's immune system, indicative of immunomodulation, achieving an improved survival of allogenic skin grafts (78). These first tentative results point towards the potentially useful immunomodulatory effects of GSCs in treating inflammatory conditions. This beneficial feature of stem cells suggests that in the future more treatments will be explored to see if their clinical outcome can be improved by stem cell immunomodulation.

5. Conclusion

Stem cell therapy represents an innovative and exciting branch of medical study and is a great avenue to advance the field. Having an intrinsic ability for regeneration means that there is the potential to restore not only structure but function too. For numerous pathologies faced in dentistry, the treatment method has not changed for many years and even in some cases

success rates have remained at an inadequate level, hence there is a great swell of interest in looking for alternative treatment options and stem cell research is at the forefront.

As with any novel treatments, trials must start small and then build on any successes. The majority of stem cell studies being carried out at present begin with pre-clinical animal trials as a way of proving a hypothesis whilst only a small number of human clinical trials have been conducted. In most of the cases reviewed, the results have been positive, firstly in determining if the treatment is safe and secondly in showing that the stem cell therapy has an efficacious outcome. However, a consistent factor across all the studies looked at is the restricted sample sizes and the relatively short follow up periods. This highlights the need for a greater number of adequate studies to increase the data pool. A large-scale, long term clinical trial has yet to take place for any treatment type, so this would be the next logical step in clinical research. In addition to this, more RCTs are required to increase the evidence pool and broaden understanding of the topic.

The future of stem cell research and utility is a bright one. Many new treatment ideas are being proposed and following proper testing could replace a number of the staple therapies used today in dentistry.

6. Responsibility

This review outlines the potential benefits that stem cells can have in improving dental treatments, whilst also focusing on the lack of significant clinical trials and the need to conduct more on a larger scale to validate the use of stem cells findings in practice. This review also indicates the need to advance from successful pre-clinical trials to verified clinical trials which provide the scientific community indisputable evidence to support the building and development of stem cell therapies thereby improving upon the success rates of already established treatments and improving patient quality of life.

As referenced, stem cell therapies allow for autologous individualized treatments meaning that many of the graft-based stem cell treatments in development benefit from having less risk of rejection. If less treatments fail due to improved practice then money, time, materials, and energy will be saved in the long term, thus increasing efficiency.

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Annexes

REVIEW ARTICLE

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Applications of stem cells in dentistry: A review

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ABSTRACT

Stem cells have the capacity to replicate and produce cell lines that differentiate into multiple cell lineages. Stem cells may be harvested from various sites and are named adult stem cells or embryonic stem cells based on their origin. Owing to their self renewing capabilities, they are used to correct large defects caused by diseases, trauma or surgery. However, they are limited by ethical and moral considerations as well as difficulty in isolation, culturing and implantation. Dental stem cells retain the property of differentiation into neurogenic, adipogenic and odontogenic components and are used in the reconstruction of orofacial structures. Scaffolding impregnated with bone morphogenic proteins and growth factors is essential prior to stem cell implantation. This 3D scaffolding with biomatrix is then introduced into the clinical site to facilitate regeneration of tissues. In the maxillofacial region, stem cells may be derived from the pulp, apical papilla, dental follicle, periodontal ligament, deciduous teeth and mucosa. They can be used for bioengineering of pulp and periapical tissues, soft tissues, bone, temporomandibular joint and periodontium. A multi-speciality approach involving cell biologists, pharmacologists and bioengineers is required to harness the vast potential of stem cell therapy and to obtain reliable treatment outcomes in the future.

Key words: *Stem cell, tissue engineering, dental, bioengineering*

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Introduction

Certain cells in the body retain their capacity to divide and produce progeny owing to their undifferentiated state. These cells are capable of self-renewal and future differentiation into multiple functional cell types and are called stem cells(1,2). Stem cells maybe adult (post-natal) stem cells and foetal (embryonic) stem cells based on their origin. As the name suggests, embryonic stem cells are derived from embryos following in-vitro fertilization while adult stem cells are derived from various sources such as bone marrow, umbilical cord, cord blood, peripheral blood, muscles, skin, adipose tissue, dental pulp or organs such as lungs, liver, breasts, eyes and brain(2,3). The first instance of therapy using adult stem cells was in 1968 when allogenic bone marrow transplant was carried out in a patient with combined immunodeficiency(4).

Classification of stem cells

Stem cells may be classified(5) as:

1.Embryonic stem cells

2.Adult stem cells

a.Hematopoetic stem cells

b.Mesenchymal stem cells

3.Induced pluripotent stem cells

Types of stem cells

1.Embryonic stem cells

Embryonic stem cells are derived from the blastocyst containing 50 to 150 cells(6). They are pluripotent and versatile and have the plasticity needed to differentiate into cells of all three germ layers(6,7). With appropriate stimulation, a large quantity of any particular adult cell type can be produced(6). However, since the inner cell mass of a fertilized embryo is used to produce these cell lines, their use is restricted by ethical and legal considerations(8). They have the added disadvantage of increased tumorigenesis potential which makes their use less favourable(2,5).

2.Adult stem cells

Adult stem cells are also called somatic or postnatal stem cells. They are multipotent and differentiate into a limited number of cell lines(9,10). Adult stem cells are easier to isolate and are not bound by the same legal and ethical constraints as embryonic stem cells. This, along with their rarer incidences of immune rejection and teratoma formation makes them suitable for use in most clinical practices(2,5).

3.Mesenchymal stem cells

Mesenchymal stem cells (MSC) are a type of adult stem cells and are also called mesenchymal stromal cells. They are

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Review Article

Stem Cells in Dentistry: Types of Intra- and Extraoral Tissue-Derived Stem Cells and Clinical Applications

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Stem cells are undifferentiated cells, capable of renewing themselves, with the capacity to produce different cell types to regenerate missing tissues and treat diseases. Oral facial tissues have been identified as a source and therapeutic target for stem cells with clinical interest in dentistry. This narrative review report targets on the several extraoral- and intraoral-derived stem cells that can be applied in dentistry. In addition, stem cell origins are suggested in what concerns their ability to differentiate as well as their particular distinguishing quality of convenience and immunomodulatory for regenerative dentistry. The development of bioengineered teeth to replace the patient's missing teeth was also possible because of stem cell technologies. This review will also focus our attention on the clinical application of stem cells in dentistry. In recent years, a variety of articles reported the advantages of stem cell-based procedures in regenerative treatments. The regeneration of lost oral tissue is the target of stem cell research. Owing to the fact that bone imperfections that ensue after tooth loss can result in further bone loss which limit the success of dental implants and prosthodontic therapies, the rehabilitation of alveolar ridge height is prosthodontists' principal interest. The development of bioengineered teeth to replace the patient's missing teeth was also possible because of stem cell technologies. In addition, a "dental stem cell banking" is available for regenerative treatments in the future. The main features of stem cells in the future of dentistry should be understood by clinicians.

1. Introduction

Stem cells are undifferentiated cells, capable of renewing themselves. Via differentiation, they have the potential to develop into many different cell lineages. There are different kinds of stem cells, depending on the type of cells they can create and the location in the body. In recent years, studies have shown that oral tissues are a source of stem cells. Structuring of tissue in dentistry has revealed promising results in the regeneration of oral tissue or organs. There are multiple factors that can produce alveolar bone resorption due to tooth extraction or loss because of severe cavities, trauma, or root fracture or even because of periodontal diseases. In edentulous patients, bone resorption continues throughout life particularly in the mandible, which makes it difficult to substitute the missing teeth with dental implants [1].

Tissue engineering therapies and stem cells are a promising way to achieve alveolar bone regeneration and solve large periodontal tissue defects and finally to substitute a lost tooth itself. Organs and tissues such as tongue, salivary glands, the temporomandibular joint condylar cartilage, and skeletal muscles are set to be used in regenerative dental medicine.

To develop the concept of oral tissue and organ regeneration for clinical application in dentistry, several studies have been carried out in animals including key elements of tissue engineering such as extracellular matrix scaffolds and stem cells [2]. Furthermore, clinical trials about jaw bone regeneration applied in dental areas such as implantology using stem cells and tissue engineering strategies have demonstrated positive results.

Considering the new role of regenerative biology and stem cells in dentistry, especially regarding the ideal stem cells for oral regeneration, some confusion can be made

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Review

What and where are the stem cells for Dentistry?

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ABSTRACT

Disinfection of root canals followed by the replacement of the infected or inflamed pulp tissues by inert materials is the foundation for treating irreversible damaged dental pulps. The management of pathological conditions of the periodontium is mainly based solely upon infection control via the reestablishment of oral hygiene, scaling and root planing to control inflammation which stops progressive bone loss. As one may see, the clinical management of endodontic and periodontal diseases has not changed drastically despite the development of new materials, techniques and medicaments. Tissue engineering is a multi-disciplinary field focused on the development of materials, techniques and strategies to improve or replace damaged or lost biological functions and tissues. As the tissue engineering field progresses, "scaffolds", "suggest pathways" and "stem cells" abandoned their role as technical words exclusively used by scientists and slowly assume a part in the language of students, educators, clinicians and patients. However the unfamiliarity with some of the concepts can lead to misinterpretations of the current status and overexcitement about future applications of stem cells for dental-related tissue regeneration. This paper will present a panorama and the future challenges on the path to use of stem cells for endodontic and periodontal tissue regeneration.

Contents

Introduction

Stem cells are clonogenic cells capable of self-renewal and are classified according to their potency, that is, the range of cell types they can differentiate into. This is directly related to the stages of cell division and differentiation of the human embryo during different stages of development which begin with a zygote, a totipotent cell that divides into identical totipotent cells in the first hours from the fertilization of an egg by a sperm. Totipotent stem cells can form embryonic

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Review

Hematopoietic stem cells: An overview

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ABSTRACT

Considerable efforts have been made in recent years in understanding the mechanisms that govern hematopoietic stem cell (HSC) origin, development, differentiation, self-renewal, aging, trafficking, plasticity and transdifferentiation. Hematopoiesis occurs in sequential waves in distinct anatomical locations during development and these shifts in location are accompanied by changes in the functional status of the stem cells and reflect the changing needs of the developing organism. HSCs make a choice of either self-renewal or committing to differentiation. The balance between self-renewal and differentiation is considered to be critical to the maintenance of stem cell numbers. It is still under debate if HSC can rejuvenate infinitely or if they do not possess "true" self-renewal and undergo replicative senescence such as any other somatic cell. Gene therapy applications that target HSCs offer a great potential for the treatment of hematologic and immunologic diseases. However, the clinical success has been limited by many factors. This review is intended to summarize the recent advances made in the human HSC field, and will review the hematopoietic stem cell from definition through development to clinical applications.

Contents

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Review

Haemopoietic stem cells

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Abstract

Considerable effort has been made in recent years in understanding the mechanisms that govern stem cell generation, proliferation, self-renewal, commitment and lately plasticity. In the development of the haemopoietic system during embryonic and fetal life the notion of different pools of stem cells arising from the endothelium is gaining consensus. Gene expression profiling of populations of stem cells is bringing to light categories of genes important for self-renewal or commitment. Besides the role of transcription factors in lineage decision, the role of soluble factors and transmembrane proteins, very active at the time of embryo development, are taking central stage in the maintenance and in vitro expansion of haemopoietic stem cells (HSCs). The hierarchical model of haemopoietic development is being questioned with reports of lineage switching and plasticity of haemopoietic stem cells to non-haemopoietic cells. Yet the understanding of the overall process is still very fragmented and hypothetical. This is mainly due to the absence of appropriate markers to enable selection of homogeneous stem cell populations and the need to rely on retrospective functional assays, able only to determine the overall behaviour of a population of cells. This review is intended to be an overview of the haemopoietic system and a critical re-visitation of issues such as plasticity and self-renewal important for therapeutic applications of haemopoietic stem cells.

Keywords: Self-renewal; Ontogeny; Commitment; Plasticity; Progenitor

Contents

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5.

Progress and prospects in hematopoietic stem cell expansion and transplantation

The fact that mice and humans have similar hematolymphoid systems and respond similarly to myeloablation and hematopoietic cell transplantation (HCT) has allowed research on mouse separated hematopoietic stem and progenitor cells to inform our progress on human HCT. Long-term engrafting hematopoietic stem cells (LT-HSC) and their multipotent progeny (short-term engrafting HSC [ST-HSC] and MPP), the lineage-committed common lymphoid progenitors (CLP) and common myeloid progenitors (CMP), downstream progenitors such as granulocye-monocyte progenitors (GMP) and megakaryocyte-erythrocyte progenitors (MEP), and unipotent megakaryocyte progenitors (MkP) were all first isolated in mice, and subsequently from human sources [1]. The direct transplantation of graded numbers of these mouse stem and/or progenitor cells in syngeneic hosts revealed many surprising and sometimes very useful findings, such as the fact that the content of HSC/MPP within a bone marrow (BM) transplant accounted entirely for the radioprotective capacity of the graft as well as the time to engraftment of neutrophils, platelets, and red blood cells [2]. In the context of bone marrow transplantation, therefore, the level of oligolineage progenitors (largely responsible for in vivo short-term colony-forming units (CFUs) and in vitro myeloerythroid colony-forming cells (CFCs) found in a minimally radioprotective dose of BM did not contribute measurably to restoration of blood cell counts or radioprotection [3]. In the mouse, transplantation of small numbers of HSC (as few as 1) gave rise to tens of thousands of HSC and the aforementioned progenitors, while the transplantation of any progenitors downstream of LT-HSC resulted in failed or minimal self-renewal. Thus, the only progenitors of mouse hematopoiesis that self-renew extensively in vivo are LT-HSC [4]. Similarly, the autologous transplantation of highly enriched human HSC resulted in more rapid engraftment of neutrophils and platelets than would be expected from their content in mobilized peripheral blood and were fully functional in protection from myeloablative doses of combination chemotherapy [5,6].

While it was hoped that self-renewing expansion of HSC in vitro could be accomplished with the set of cytokines cloned and generally available, such as steel factor (SLF), EPO, and interleukin (IL)-1, 3, 6, and 11, in fact, no single cytokine or combination of cytokines led to more than a modest expansion of HSC in mouse [6,7] or man [8], a sad fact that was verified by clinical transplantation experience [9–11]. Despite the massive expansion of hematopoietic and myeloerythroid cells that followed the optimal protocol combinations, the HSC content remained the same as the input HSC content [12]. It is not yet clear whether the number of oligolineage progenitors produced from HSCs in these studies mirrored the number expected from non-self-renewing expansions or if some cytokine combinations could lead to some progenitor self-renewal in vitro [13].

Therefore, the search for factors and pathways that could expand HSCs in vitro as they were obviously expanded in vivo continued. That search became even more significant for several reasons: 1) the requirement for more HSCs to achieve robust engraftment in allogeneic transplants than in syngeneic transplants in mouse studies became apparent [14]; 2) the looming specter that very large numbers of HSCs of a variety of HLA haplotypes might be required to radioprotect human populations threatened with radioactive or chemical warfare agents; and 3) the unexpected activities of oligolineage progenitors in situational transplants in mice revealed important and lifesaving properties. Some of these properties include 1) the ability of CMP and MEP given in very large doses in lethally irradiated mice to provide transient radioprotection until the few surviving host HSC could regenerate the hematolymphoid system [15]; 2) the ability of very large numbers of CLP to protect HSC-transplanted mice from otherwise lethal infections with murine cytomegalovirus (CMV) in both syngeneic and MHC minor antigen mismatch donor-host pairing [16]; and 3) the ability of very large numbers of CMP/GMP to protect HSC-transplanted mice from otherwise lethal infections with spores of the fungus *Aspergillus fumigatus* (obtained from a lethal human infection) or gram-negative rod bacteria *Pseudomonas aeruginosa* in both syngeneic and allogeneic circumstances [17,18]. If these properties of oligolineage progenitors extrapolate to human therapies, there would be no absolute requirement for donor-host matching. Such therapeutic potential underscores the importance of expanding human HSC in order to obtain (with the appropriate cytokine combinations, if necessary) substantial numbers of human CLP, CMP, GMP, MEP, and perhaps MkP.

The ex vivo expansion of hematopoietic stem cells is the next logical step in the field of hematopoietic cell transplantation. Despite remarkable progress in the field of HCT, current therapeutic limitations include the availability of donors, imprecision in immune reconstitution reflected by susceptibility to infectious pathogens or by graft-vs-host disease (GVHD), and relapse/persistent disease. Ex vivo expansion of hematopoietic stem and progenitor cells offers the potential for providing a graft that would more rapidly recapitulate the immune response and be free of malignant cells. Genetic engineering is also facilitated by ex vivo expansion of cells, offering the potential for enhanced immunotherapy or response to pathogens. Immediate scientific challenges to ex vivo expansion include 1) optimizing culture conditions, 2) identification of optimal human cell populations to expand, 3) identification and expansion of human long-term repopulating cells, and 4) concerns that expansion may lead to early senescence or decreased functional capacity after transfer, which may be related to cell cycle at a crucial time.

6.

Review Article

Current overview on dental stem cells applications in regenerative dentistry

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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.

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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

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POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

M Dominici[1], K Le Blanc[2], I Mueller[3], I Slaper-Cortenbach[4], FC Marini[5], DS Krause[6], RJ Deans[7], A Keating[8], DJ Prockop[9] and EM Horwitz[10]

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The considerable therapeutic potential of human multipotent mesenchymal stromal cells (MSC) has generated markedly increasing interest in a wide variety of biomedical disciplines. However, investigators report studies of MSC using different methods of isolation and expansion, and different approaches to characterizing the cells. Thus it is increasingly difficult to compare and contrast study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. *While these criteria will probably require modification as new knowledge unfolds, we believe this minimal set of standard criteria will foster a more uniform characterization of MSC and facilitate the exchange of data among investigators.*

Keywords

MSC, stem cells, adherent cells, immunophenotype, differentiation.

Biologic and clinical interest in MSC has risen dramatically over the last two decades, as shown by the ever-increasing number of research teams studying these cells. Not only are established laboratories focusing on MSC but new investigators are rapidly being attracted to the field, which will undoubtedly accelerate scientific discovery and the development of novel cellular therapies. However, this soaring interest has also generated many ambiguities and inconsistencies in the field.

To begin to address these issues, a recent report from the International Society for Cellular Therapy (ISCT) stated that 'multipotent mesenchymal stromal cells' (MSC) is the currently recommended designation [1] for the plastic-adherent cells isolated from BM and other tissues that have often been labeled mesenchymal stem cells [2].

The defining characteristics of MSC are inconsistent among investigators. Many laboratories have developed methods to isolate and expand MSC, which invariably have subtle, and occasionally quite significant, differences. Furthermore, investigators have isolated MSC from a variety of tissues with ostensibly similar properties [3]. These varied tissue sources and methodologies of cell

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8.

REVIEW

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The bone marrow niche for haematopoietic stem cells

Sean J. Morrison[1] & David T. Scadden[2]

Niches are local tissue microenvironments that maintain and regulate stem cells. Haematopoiesis provides a model for understanding mammalian stem cells and their niches, but the haematopoietic stem cell (HSC) niche remains incompletely defined and beset by competing models. Recent progress has been made in elucidating the location and cellular components of the HSC niche in the bone marrow. The niche is perivascular, created partly by mesenchymal stromal cells and endothelial cells and often, but not always, located near trabecular bone. Outstanding questions concern the cellular complexity of the niche, the role of the endosteum and functional heterogeneity among perivascular microenvironments.

Haematopoietic stem cell (HSC) niches are present in diverse tissues throughout development, beginning in the aorta–gonad–mesonephros (AGM) region and the yolk sac, followed by the placenta, fetal liver, spleen and bone marrow[1]. Postnatally, the bone marrow is the primary site of HSC maintenance and haematopoiesis, but in response to haematopoietic stress the niche can shift to extramedullary sites. Defining niche components and how they work in concert to regulate haematopoiesis provides the opportunity to improve regeneration following injury or HSC transplantation and to understand how disordered niche function could contribute to disease. In this Review, we focus on the nature of the HSC niche in bone marrow because it is the subject of most of the recent research and controversies.

Historical context

Following Darwin's contributions to evolutionary theory, there was much emphasis on defining hierarchical evolutionary relationships among organisms. Morphological similarities were used to construct ancestral trees that connected complex multicellular organisms to an original monocellular "stem cell"[2]. Lineage relationships were formulated, and biologist Ernst Haeckel proposed that cell organization in a developing organism was the recapitulation of events in the evolution of the species, with cells deriving from a stem cell equivalent[3]. Thirty years later, haematologist Artur Pappenheim proposed a less grand and more accurate formulation based on improved cell-morphology visualization techniques — that cells of the blood were related to one another, with mature cell types descending from a single cell type in a "unified view of haematopoiesis"[4]. In so doing, he articulated the hypothesis of tissue stem cells. This concept took about half a century to define experimentally through the inspired work of James Till and Ernest McCulloch, who showed that single cells could yield multilineage descendants while preserving the multipotency of the mother cell[5–7]. The researchers gave substance to the idea of a stem cell and gave us methods to define the cardinal properties of those cells — self-renewal and differentiation.

Till and McCulloch based much of their work on an *in vivo* spleen colony-forming (CFU-S) assay now known to measure mainly multipotent progenitors rather than long-term self-renewing HSCs[8,9]. The imprecise nature of that assay contributed to the formulation of the niche hypothesis by Ray Schofield in 1978. Recognizing that the putative CFU-S stem cells were less robust than cells of the bone marrow at reconstituting haematopoiesis in irradiated animals, he proposed that a specialized bone marrow niche preserved the reconstituting ability of stem cells[10]. His colleagues at the University of Manchester concurrently sought to define what made bone marrow a nurturing context for HSCs, and haematologist Michael Dexter showed that largely mesenchymal 'stromal' cell cultures could maintain primitive haematopoietic cells *ex vivo*[11]. Furthermore, another colleague, Brian Lord, progressively reamed long bone marrow cavities and showed that primitive cells tended to localize towards the endosteal margins, leading to the hypothesis that bone might regulate haematopoiesis[12] (Fig. 1).

These early studies were followed by *in vitro* evidence that osteoblasts differentiated in culture from human bone marrow stromal cells could produce haematopoietic cytokines and support primitive haematopoietic cells in culture[13]. This fostered the idea that bone cells might create the HSC niche, but it was essential to move to engineered mouse strains to test the hypothesis *in vivo*. Two studies followed, including a mouse model in which a promoter that was restricted in activity to osteoblastic cells was used to drive expression of a constitutively active parathyroid hormone receptor[14]. Along similar lines, Linheng Li's laboratory used a promoter that has since been shown to be restricted in bone marrow stroma to primitive and mature osteolineage cells[15], to delete the *BMPr1a* gene[16]. In both models, the number of endosteal osteoblasts and the number of primitive haematopoietic cells (scored as stem cells given the measures in use at the time) increased. These data provided the first evidence of specific heterologous cells regulating mammalian stem cells *in vivo*, although it remained unclear whether the regulation was direct or indirect. This demonstrated that the niche was experimentally tractable, prompting a series of studies that have since refined our understanding of the complexity of the bone marrow microenvironment.

Studies of the niche have now more precisely determined the components that regulate HSCs, and to some extent other haematopoietic progenitors, in the bone marrow. Like any interactive system there are complex regulatory relationships among cells in the bone marrow. A perturbation in one cell type that leads to an effect in another cell type does not necessarily require the interaction between the cells to be direct. The data now suggest that the early studies that observed effects on HSC frequency as a consequence of genetic manipulation in osteoblastic cells reflected indirect effects rather than the existence of an osteoblastic niche. Indeed, expression of constitutively active parathyroid hormone receptors in osteoblasts[14] probably causes widespread changes in many cell types of the bone marrow, including in the vasculature. Current data suggest

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Neuronal Differentiation of Bone Marrow-derived Stromal Stem Cells Involves Suppression of Discordant Phenotypes through Gene Silencing*

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Tissue engineering involves the construction of transplantable tissues in which bone marrow aspirates may serve as an accessible source of autogenous multipotential mesenchymal stem cells. Increasing reports indicate that the lineage restriction of adult mesenchymal stem cells may be less established than previously believed, and stem cell-based therapeutics await the establishment of an efficient protocol capable of achieving a prescribed phenotype differentiation. We have investigated how adult mouse bone marrow-derived stromal cells (BMSCs) are guided to neurogenic and osteogenic phenotypes. Naïve BMSCs were found surprisingly active in expression of a wide range of mRNAs and proteins, including those normally reported in terminally differentiated neuronal cells and osteoblasts. The naïve BMSCs were found to exhibit voltage-dependent membrane currents similar to the neuronally guided BMSCs, although with smaller amplitudes. Once BMSCs were exposed to the osteogenic culture condition, the neuronal characteristics quickly disappeared. Our data suggest that the loss of discordant phenotypes during BMSC differentiation cannot be explained by the selection and elimination of unfit cells from the whole BMSC population. The percent ratio of live to dead BMSCs examined did not change during the first 8–10 days in either neurogenic or osteogenic differentiation media, and cell detachment was estimated at <1%. However, during this period, bone-associated extracellular matrix genes were selectively down-regulated in neuronally guided BMSCs. These data indicate that the suppression of discordant phenotypes of differentiating adult stem cells is achieved, at least in part, by silencing of superfluous gene clusters.

Bone marrow represents an abundant source of renewing stem cells with potential for developing into multiple lineages (1–6). Bone marrow transplantations in humans and animals have led to an understanding that the donor's bone marrow-derived stromal cells (BMSCs)[1] can successfully integrate into a wide range of highly specialized tissues of the recipient *in vivo*. Transplanted BMSCs have been shown to fuse with differentiated resident cells, such as skeletal and cardiac muscles (7–13), liver (14, 15), and neuronal cells (16–21). However, Houghton *et al.* (22) have recently shown that the transplanted BMSCs can develop gastric epithelial cells that contain a single nucleus, suggesting that the formation of heterokaryons is not necessarily a prerequisite for BMSC differentiation. It must be noted that their study has further demonstrated that transplanted BMSCs can develop epithelial cancers under chronic *Helicobacter* infection (22). Regenerative medicine strategy using undifferentiated BMSC transplantation may thus require stringent monitoring.

Bone marrow aspirate can serve as an accessible source of cellular components for tissue engineering strategies. Increasing reports on *in vitro* differentiation protocols for adult mesenchymal stem cells indicate that the lineage restriction may be less stringent than previously believed. Experiments with multipotent adult stem cells *in vitro* suggest that the microenvironment contributes substantially to terminal differentiation (12, 14, 23). For example, BMSCs show the potential to adopt widely different end points not only of their well characterized mesenchymal derivatives, such as osteoblasts, chondroblasts, and adipocytes (24–26), but also of ectoderm-derived neural cells (27–33). The *in vitro trans*-lineage differentiation capability of stem cells should broaden the engineering application to a wide range of tissues, each of which awaits the establishment of an efficient protocol that stably guides them to a prescribed terminal differentiation.

Both rodent and human BMSCs can be rapidly induced to differentiate into neurons in a defined *in vitro* microenvironment (34). Shortly after the exposure to the neurogenic culture condition, BMSCs begin to develop characteristic neuron-like morphologies, such as processes resembling axons and dendrites (neurites). These cells also express genes and proteins that are normally associated with neuronal cells. We used the adult mouse BMSC model undergoing ectodermal/neurogenic or mesodermal/osteogenic differentiation to elucidate the molecular mechanism regulating the *in vitro trans*-differentiation

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[1] The abbreviations used are: BMSC, bone marrow-derived stromal cells; RT, reverse transcription; TTX, tetrodotoxin; TEA, tetraethylammonium chloride; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; ALP, alkaline phosphatase.

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Mesenchymal stem cells: Biology and potential clinical uses

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There has been an increasing interest in recent years in the stromal cell system functioning in the support of hematopoiesis. The stromal cell system has been proposed to consist of marrow mesenchymal stem cells that are capable of self-renewal and differentiation into various connective tissue lineages. Recent efforts demonstrated that the multiple mesenchymal lineages can be clonally derived from a single mesenchymal stem cell, supporting the proposed paradigm. Dexter demonstrated in 1982 that an adherent stromal-like culture was able to support maintenance of hematopoietic stem as well as early B lymphopoeisis. Recent data from in vitro models demonstrating the essential role of stromal support in hematopoiesis shaped the view that cell–cell interactions in the marrow microenvironment are critical for normal hematopoietic function and differentiation. Maintenance of the hematopoietic stem cell population has been used to increase the efficiency of hematopoietic stem cell gene transfer. High-dose chemotherapy and frequently cause stromal damage with resulting hematopoietic defects. Data from preclinical transplantation studies suggested that stromal cell infusions not only prevent the occurrence of graft failure, but they have an immunomodulatory effect. Preclinical and early clinical safety studies are paving the way for further applications of mesenchymal stem cells in the field of transplantation with respect to hematopoietic support, immunoregulation, and graft facilitation. © **2000 International Society for Experimental Hematology. Published by Elsevier Science Inc.**

Keywords: **Stroma—Transplantation—Hematopoiesis—Mesenchymal stem cell**

Stromal cell system

There has been an increasing interest in recent years in the stromal cell system, which includes the marrow-derived stromal cell that supports hematopoiesis, as well as the mesenchymal stem cell and its progeny, connective tissue cells such as osteocytes, chondrocytes, tenocytes, adipocytes, and smooth muscle cells. The stromal cell system, first described by Maureen Owen [1] in 1985, has been the subject of investigation in the fields of connective tissue engineering, cell transplantation, hematopoietic stem cell transplantation, and gene therapy.

What constitutes stroma?

There are three main cellular systems in the bone marrow: hematopoietic, endothelial, and stromal (with stromal cells loosely referring to the nonhematopoietic cells of mesenchymal origin). The stromal cell system as proposed by Owen was based on an analogy with the hematopoietic system, in which mesenchymal stem cells reside within the marrow, maintain a level of self-renewal, and give rise to cells that can differentiate into various connective tissue lineages, including the osteogenic lineage as described by Friedenstein [2] in 1980 as well as stromal tissues [3]. Four main cell types comprising the postnatal marrow stromal tissue are known to support hematopoiesis: macrophages, adipocytes, osteogenic cells, and "reticular cells." This is in contrast to the in vitro adherent layer derived from long-term in vitro bone marrow culture [4] and consisting of fibroblastic cells, macrophages, adipocytes, endothelial cells, and smooth muscle cells. The latter are not present in the extravascular space of the bone marrow and appear only in arteriolar walls.

Within the in vivo stromal environment, alkaline phosphatase positive (ALP^+) reticular cells associate closely with hematopoietic cells [5]. These ALP^+ reticular cells are thought to originate from cells that were destined to differentiate into osteoblasts but are capable of forming stroma. The presence of adipocytes in the postnatal stroma is dependent on a number of factors: 1) stage of skeletal development, because adipogenesis progresses from the diaphyses to the epiphyses; 2) age, because the number of adipocytes increases with age; and 3) the level of hematopoiesis, because adipogenesis appears to correlate indirectly with hematopoietic cell mass, which usually is reflected in the

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Neo-vascularization and bone formation mediated by fetal mesenchymal stem cell tissue-engineered bone grafts in critical-size femoral defects

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ABSTRACT

Tissue-engineered bone grafts (TEBG) require highly osteogenic cell sources for use in fracture repair applications. Compared to other sources of mesenchymal stem cells (MSC), human fetal MSC (hfMSC) have recently been shown to be more proliferative and osteogenic. We studied the functional performance of hfMSC-mediated TEBG in 7 mm rat femoral critical-sized bone defects (CSD). Dynamically-cultured and osteogenically-primed hfMSC seeded onto macroporous poly-ε-caprolactone tri-calcium phosphate scaffolds were transplanted into CSDs. After 12 weeks, hfMSC-mediated TEBG induced 2.1× more new bone formation (43.3 ± 10.5 vs. 21.0 ± 7.4 mm^3, $p < 0.05$), with greater compact and woven bone, and a 9.8× increase in stiffness (3.9 ± 1.7 vs. 0.4 ± 0.3 mNm/degree, $p < 0.05$) compared to acellular scaffolds, such that only animals transplanted with TEBG underwent full fracture repair of the CSD. Although hfMSC survived for <4 weeks, by 4 weeks they were associated with a 3.9× larger vasculature network in the defect area (35.2 ± 11.1 vs. 6.5 ± 3.6 mm^3 $p < 0.05$), suggesting an important role for hfMSC in the promotion of neo-vasculogenesis. We speculate that hfMSC-mediated healing of the CSD by stimulating neo-vascularization through as yet undetermined mechanisms. This proof-of-principle study demonstrates the utility of primitive MSC for bone regeneration, and may be of relevance to vascularization in other areas of regenerative medicine.

1. Introduction

Fracture injuries represent an increasing clinical burden, with >15 million fractures annually in the United States alone, of which around 10% are complicated by non-union despite the body's innate fracture repair mechanisms [1].

The usual strategy to prevent or treat fracture non-union is autologous bone grafting, delivering bone chips from a secondary site into the fracture, where they promote homing of bone-forming stem cells [2] and thus participation in the healing process. However, significant donor site morbidity such as chronic pain, hypersensitivity, infection and paraesthesia occur in up to a third of patients. In addition, the limited supply and unpredictable efficacy of autologous bone grafts has led to the use of alternatives such as allografts. Aside from the risks of immune rejection and disease transmission, allografts have been associated with reduced cellularity and vascularity compared to autologous grafts, and consequently, poorer bone healing. This in turn prompted the development of synthetic implants, which can be fashioned to different shapes and sizes, but thus far have been hampered by their poor speed of healing and inability to remodel in tandem with the natural healing process [1]. There is thus an urgent and unmet clinical need for alternative approaches.

Bone tissue engineering (BTE) provides an alternative way to develop an off-the-shelf engineered bone graft, which like allo- and synthetic grafts, is not only available in different shapes and sizes, but also possesses similar ability to stimulate rapid bone healing. Successful implementation of an effective BTE strategy requires the integrated contribution from scaffold, stem cell and dynamic culture technologies.

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The osteogenic differentiation of adult bone marrow and perinatal umbilical mesenchymal stem cells and matrix remodelling in three-dimensional collagen scaffolds

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ABSTRACT

Adult human mesenchymal stem cells from bone marrow (BM-MSC) represent a promising source for skeletal regeneration. Perinatal MSC from Wharton's Jelly of the umbilical cord (UC-MSC) are expected to possess enhanced differentiation capacities due to partial expression of pluripotency markers. For bone tissue engineering, it is important to analyse *in vitro* behaviour of stem cell/biomaterial hybrids concerning *in vivo* integration into injured tissue via migration, matrix remodelling and differentiation. This study compares the cell-mediated remodelling of three-dimensional collagen I/III gels during osteogenic differentiation of both cell types. When activated through collagen contact and subjected to osteogenic differentiation, UC-MSC differ from BM-MSC in expression and synthesis of extracellular matrix (ECM) proteins as shown by histology, immunohistochemistry, Western Blot analysis and realtime-RT-PCR. The biosynthetic activity was accompanied in both cell types by the ultrastructural appearance of hydroxyapatite/calcium crystals and osteogenic gene induction. Following secretion of matrix metalloproteinases (MMP), both MSC types migrated into and colonised the collagenous matrix causing matrix strengthening and contraction. These results indicate that UC-MSC and BM-MSC display all features needed for effective bone fracture healing. The expression of ECM differs in both cell types considerably, suggesting different mechanisms for bone formation and significant impact for bone tissue engineering.

1. Introduction

Large bone defects are a major clinical problem, because in up to 40% of patients autologous bone grafts are not available [1]. Thus, there is pressing need for effective tissue engineered solutions. Bone tissue engineering requires porous biodegradable scaffolds combined with a non-immunogenic cell source with osteogenic potential.

Three-dimensional (3D) scaffolds provide support for cells to attach, grow and differentiate, and define the spatial shape of the tissue engineered transplant. A variety of biomaterials have been investigated for stem cells in the context of bone tissue engineering [2]. A promising biomaterial for bone tissue engineering applications is a combination of collagen I and III, which are the most abundant proteins in the osteocyte environment and which are osteoinductive [3–5]. Collagen I fibres have great tensile strength and are broadly used for osteochondrocytic tissue engineering applications [2,6,7]. Collagen III as a quantitatively minor fibrillar collagen assembles into fibres with collagen I, thereby regulating the structures and properties of the fibres, thus enhancing the structural stability and the rate of resorption. The collagen gels used in the present study are composed of 90% collagen I and 10% collagen III. Combinations of collagen I and III are basically used for dermal tissue engineering [8–10]. Although collagen matrices have antigenic properties as an implant material, the smooth microgeometry and transmurale permeability as well as easy application, make them important for tissue engineering.

In recent years, mesenchymal stem cells (MSC) were shown to be an attractive cell source for tissue engineering [4,11,12]. They can be easily isolated from bone marrow (BM) and expanded through several passages while retaining their multipotent differentiation capacity [4,13]. Under permissive stimulation, MSC undergo osteogenic differentiation via a well-defined pathway [14], acquiring

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REVIEW ARTICLE

Adult Stem Cells of Orofacial Origin: Current Knowledge and Limitation and Future Trend in Regenerative Medicine

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Abstract Stem cell research is one of the most rapidly expanding field of medicine which provides significant opportunities for therapeutic and regenerative applications. Different types of stem cells have been isolated investigating their accessibility, control of the differentiation pathway and additional immunomodulatory properties. Bulk of the literature focus has been on the study and potential applications of adult stem cells (ASC) because of their low immunogenicity and reduced ethical considerations. This review paper summarizes the basic available literature on different types of ASC with special focus on stem cells from dental and orofacial origin. ASC have been isolated from different sources, however, isolation of ASC from orofacial tissues has provided a novel promising alternative. These cells offer a great potential in the future of therapeutic and regenerative medicine because of their remarkable availability at low cost while allowing minimally invasive isolation procedures. Furthermore, their immunomodulatory and anti-inflammatory potential is of particular interest. However, there are conflicting reports in the literature regarding their particular biology and full clinical potentials. Sound knowledge and higher control over proliferation and differentiation mechanisms are prerequisites for clinical applications of these cells. Therefore, further standardized basic and translational studies are required to increase the reproducibility and reduce the controversies of studies, which in turn facilitate comparison of related literature and enhance further development in the field.

Keywords Orofacial stem cells · Adult stem cell · Regenerative medicine · Stem cell therapy

1 Introduction to stem cells, types and potential applications

The stem cell engineering is a rapidly growing field in the area of regenerative medicine. Stem cells are being used extensively for understanding development and progression of diseases. Currently, stem cell therapy is one of the bravest and promising moves for successful treatment of various medical conditions. This field is rapidly expanding as different clinical trials reveal their tremendous therapeutic potentials. Stem cells have been investigated as potential therapy for various medical conditions and diseases such as; cerebral ischemia, parkinson's disease, alzheimer's disease, retinal disease, diabetes type 1 and 2, myogenic disease [1]. It is also applied for neuronal, cardiovascular and bone regeneration [1–3].

Although various stem cells have been isolated and defined, they share common general features which make them distinctive among other mammalian cells. The main interesting key feature of stem cells is their undifferentiated nature with a potential to either retain their stemness through self-renewal (symmetric division) or give rise to differentiated daughter cells (asymmetric division) [4]. In general, stem cells stay in a quiescent state inside adult tissue, where upon stimulation they enter the cell cycle for division [5, 6]. Two types of cell division mechanism

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Osteoarthritis and Cartilage

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Donor sex and age influence the chondrogenic potential of human femoral bone marrow stem cells

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SUMMARY

Objective: Damaged articular cartilage does not heal well and can progress to osteoarthritis (OA). Human bone marrow stem cells (BMC) are promising cells for articular cartilage repair, yet age- and sex-related differences in their chondrogenesis have not been clearly identified. The purpose of this study is to test whether the chondrogenic potential of human femoral BMC varies based on the sex and/or age of the donor.

Design: BMC were isolated from 21 males (16–82 years old (y.o.)) and 20 females (20–77 y.o.) during orthopaedic procedures. Cumulative population doubling (CPD) was measured and chondrogenesis was evaluated by standard pellet culture assay in the presence or absence of transforming growth factor beta 1 (TGFβ1). Pellet area was measured, and chondrogenic differentiation was determined by Toluidine blue and Safranin O-Fast green histological grading using the Bern score and by glycosaminoglycan (GAG) content.

Results: No difference in CPD was observed due to donor sex or age. The increase in pellet area with addition of TGFβ1 and the Bern score significantly decreased with increasing donor age in male BMC, but not in female BMC. A significant reduction in GAG content per pellet was also observed with increasing donor age in male BMC. This was not observed in female BMC.

Conclusions: This study showed an age-related decline in chondroid differentiation with TGFβ1 stimulation in male BMC, but not in female BMC. Understanding the mechanisms for these differences will contribute to improved clinical use of autologous BMC for articular cartilage repair, and may lead to the development of customized age- or sex-based treatments to delay or prevent the onset of OA.

Introduction

Articular cartilage is an avascular tissue with limited intrinsic healing capacity. Due to its inability to heal efficiently, focal cartilage injuries of the knee have an increased risk of progressing to osteoarthritis (OA), a leading cause of disability[1]. Current treatment modalities for articular cartilage repair include debridement, osteochondral grafting, autologous chondrocyte implantation, and microfracture[2–4]. While good to excellent clinical outcome scores have been reported for each technique, the repaired cartilage is biomechanically dissimilar to the surrounding native cartilage. This can lead to degradation of the cartilage over time, which would set the stage for the progression of OA. Thus, there is an urgent need to improve articular cartilage repair, as it may delay, or even prevent the onset of debilitating OA.

Microfracture is minimally invasive and the simplest of the treatment techniques, as it involves penetrating the subchondral bone to access repair cells from the bone marrow to infiltrate the defect. Microfracture is, however, inconsistent[4–6]. A high degree of variability in the amount of repair cartilage that fills the defect has been reported, indicating that there may be a subpopulation of patients that do not produce sufficient repair tissue after microfracture, leading to early failure[4]. Age has also been shown to affect the clinical outcome of microfracture, with younger patients (<40 years old (y.o.)) showing better clinical outcome scores[5]. Since bone marrow cells are the main repair cells recruited to the defect during microfracture, it suggests an important and continued role for the autogenous application of bone marrow stem cells (BMC) in articular cartilage repair. The variability seen in clinical outcome measures suggests that BMC from different individuals could differ in their capacity for chondrogenic differentiation.

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Research Article

The Influence of Aging on the Regenerative Potential of Human Adipose Derived Mesenchymal Stem Cells

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Tissue regeneration using human adipose derived mesenchymal stem cells (hASCs) has significant potential as a novel treatment for many degenerative bone and joint diseases. Previous studies have established that age negatively affects the proliferation status and the osteogenic and chondrogenic differentiation potential of mesenchymal stem cells. The aim of this study was to assess the age-related maintenance of physiological function and differentiation potential of hASCs in vitro. hASCs were isolated from patients of four different age groups: (1) >20 years ($n = 7$), (2) >50 years ($n = 7$), (3) >60 years ($n = 7$), and (4) >70 years ($n = 7$). The hASCs were characterized according to the number of fibroblasts colony forming unit (CFU-F), proliferation rate, population doubling time (PDT), and quantified parameters of adipogenic, chondrogenic, and osteogenic differentiation. Compared to younger cells, aged hASCs had decreased proliferation rates, decreased chondrogenic and osteogenic potential, and increased senescent features. A shift in favor of adipogenic differentiation with increased age was also observed. As many bone and joint diseases increase in prevalence with age, it is important to consider the negative influence of age on hASCs viability, proliferation status, and multilineage differentiation potential when considering the potential therapeutic applications of hASCs.

1. Introduction

Mesenchymal stem/stromal cells (MSCs) hold great promise as a novel therapeutic option for use in tissue regeneration. Because of their multilineage differentiation capacity, MSCs are considered as a potential therapeutic tool for treating a wide range of pathologies, especially bone and cartilage disorders such as osteoarthritis, osteoporosis, and osteonecrosis [1–3]. Numerous preclinical and clinical studies, using the ability of MSCs to repair bone and cartilage, have shown promising results in musculoskeletal regeneration of chondral and osteochondral lesions [4–7]. This lends hope for MSCs to be potentially used in daily clinical practice.

Beside proliferative and multilineage differentiation potential, MSCs have an immunomodulatory effect that is dependent on cell-cell contact or mediated through the secretion of immunosuppressive molecules [8, 9]. The ability of MSCs to secrete membrane derived vesicles (MVs), rich in a wide range of growth factors, antiapoptotic factors, and anti-inflammatory molecules, is currently considered as a novel molecular mechanism with significant therapeutic potential [10, 11]. Multipotent cells have been isolated from many sources including adipose tissue, bone marrow, and the umbilical cord [12, 13]. Traditionally, human MSCs are isolated from an aspirate of bone marrow harvested from the iliac crest or the acetabulum. However, the most common and effective way of acquiring the cells is from adipose tissue [14, 15]. Adipose derived mesenchymal stem cells (ASCs) are easily obtained from patients during surgery or through minimally invasive procedures. Results from a number of

16.

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Age-Related Decline in the Osteogenic Potential of Human Bone Marrow Cells Cultured in Three-Dimensional Collagen Sponges

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Abstract Studies with human and animal culture systems indicate that a sub-population of bone marrow stromal cells has the potential to differentiate into osteoblasts. There are conflicting reports on the effects of age on human marrow-derived osteogenic cells. In this study, we used a three dimensional (3D) culture system and quantitative RT-PCR methods to test the hypothesis that the osteogenic potential of human bone marrow stromal cells decreases with age. Marrow was obtained from 39 men aged 37 to 86 years, during the course of total hip arthroplasty. Low-density mononuclear cells were seeded onto 3D collagen sponges and cultured for 3 weeks. Histological sections of sponges were stained for alkaline phosphatase activity and were scored as positive or negative. In the group ≤ 50 years, 7 of 11 samples (63%) were positive, whereas only 5 of 19 (26%) of the samples in the group ≥ 60 years were positive ($p = 0.0504$). As revealed by RT-PCR, there was no expression of alkaline phosphatase or collagen type I mRNA before culture, however there were strong signals after 3 weeks, an indication of osteoblast differentiation in vitro. We performed a quantitative, competitive RT-PCR assay with 8 samples (age range 38–80) and showed that the group ≤ 50 years had 3-fold more mRNA for alkaline phosphatase than the group ≥ 60 years ($p = 0.021$). There was a significant decrease with age ($r = -0.78$, $p = 0.028$). These molecular and histoenzymatic data indicate that the osteogenic potential of human bone marrow cells decreases with age. J. Cell. Biochem. 82: 583–590, 2001. © 2001 Wiley-Liss, Inc.

Key words: bone marrow; aging; osteogenesis; in vitro; three-dimensional

Both in vivo and in vitro animal studies indicate that a sub-population of marrow stromal cells has the potential to differentiate to hard and soft connective tissue cells, including osteoblasts, chondrocytes, and adipocytes [Friedenstein, 1976, Owen, 1985]. More recently, studies with human marrow also indicate that marrow cells can give rise to these different tissues [Beresford, 1989, Long et al., 1990, Vilamitjana-Amedee et al., 1993, Cheng et al., 1994, Gronthos et al., 1994, Rickard et al., 1996, Pittenger et al., 1999]. Culture conditions are important for promoting differentiation of osteoblasts from marrow; standard osteoblastogenic medium contains dexamethasone, β-glycerophosphate, and vitamin C [Leboy et al., 1991, Beresford et al., 1994]. Alkaline phosphatase activity has become a useful index of the early commitment of cells to the osteoblast lineage [Ashton et al., 1980].

Studies with animal bone marrow cells [Perkins et al., 1982, Tsuji et al., 1990, Liang et al., 1992, Roholl et al., 1994, Kahn et al., 1995, Bergman et al., 1996, Frenkel et al., 1997] suggest that there is an age-related decrease in their osteogenic potential, but reports are not consistent for human cells. Shigeno and Ashton found a higher number of proliferative precursor cells in younger than in older subjects [Shigeno and Ashton, 1995]. In a study of

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17.

Membranous versus Endochondral Bone: Implications for Craniofacial Reconstruction

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Large volumes of bone are frequently required in craniofacial reconstruction. Clinical observation suggests that membranous bone undergoes less resorption than endochondral bone when grafted in the craniofacial region. Peer[1] noted that unlike endochondral bone grafts, membranous bone maintained its volume in soft tissue, and Smith and Abramson[2] demonstrated that membranous bone underwent less resorption than endochondral bone in the rabbit. In this report we confirm Smith and Abramson's findings in the rabbit, extend the experimental study to a monkey model, and quantitate differences in bone formation using fluorescent microscopy and point-counting techniques.

Materials and Methods

Rabbit

Identical-sized blocks of endochondral and membranous bone measuring 10 × 5 × 3 mm were taken from the iliac crest and zygomatic arch of 15 adult white New Zealand rabbits. The endochondral grafts consisted of mixed cortical-cancellous bone (one cortical and one cancellous layer), and the membranous grafts consisted of full-thickness zygomatic arch. After removing the periosteum, the bone blocks were autografted to the snout of each of the 15 rabbits. Grafts were placed in a subperiosteal pocket with good bone-to-bone contact. Endochondral grafts were placed with the endosteal surface against recipient bone and cortical surface against soft tissue. No fixation was used to immobilize the grafts.

In odd-numbered animals, the membranous graft was placed closest to the nasal tip and the iliac crest graft proximal to this. In even-numbered animals, the order was reversed (Fig. 1).

The animals were injected with vital stains at the following intervals: tetracycline (30 mg/kg IV) at 2 weeks, alizarin complexone (20 mg/kg IV) at 4 weeks, calcein blue (20 mg/kg IV) at 8 weeks, and xylenol orange (20 mg/kg IV) at 12 weeks.

Five animals were sacrificed at 5, 10, and 20 weeks, respectively. At sacrifice, the skulls and grafts were stripped of all soft tissue. The snout was separated from the rest of the skull and the fused grafts and snout removed en bloc. Graft volumes were calculated in millimeters by direct caliper measurement of the graft's length, width, and height.

After measurements were carried out, the specimens were divided in half. One-half was placed in formalin for H&E preparation, and the second half was placed in 70% ethanol for fluorescent studies. For fluorescent microscopic examination, undecalcified specimens were fixed in 70% ethanol and then embedded in a 2% carboxy methyl cellulose solution. The embedded specimens were frozen in liquid nitrogen and equilabrated to −25°C in a freezer overnight. Undecalcified sections were cut to a thickness of 5 μm using the Jung model K cryostat microtome. Sections were collected with adhesive tape (No. 810, 3M Company, St. Paul, Minn.), rinsed in Tris buffer, and dehydrated sequentially in 50%, 70%, 95%, and absolute alcohol. Specimens were mounted in euparal and examined using the fluorescent microscope with no further staining.

Volume measurements in all groups were compared when appropriate by application of the Student's *t* test. Changes in graft dimensions (length, width, and height) were compared using the paired *t* test.

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18.

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Early Secondary Bone Grafting of Alveolar Cleft Defects

A Comparison between Chin and Rib Grafts

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Summary

Since 1981 in cleft lip and palate patients a combined surgical-orthodontic procedure has been performed to eliminate the residual alveolar cleft. For early secondary bone grafting (before the eruption of the canine tooth) initially the graft tissue of choice was rib. Since 1984 chin bone has also been used. Sixty one patients with complete unilateral clefts were reviewed (mean age 9.5 years). 15.7% of the rib graft cases showed resorption of the graft of 50% and more. Such resorption was not found in any of the chin graft cases. No complications such as wound dehiscence, sequestration, excessive resorption of bone or recurrence of an oro-nasal fistula were found in the chin graft group. This leads to the conclusion that if enough bone is available in the chin region to bridge the defect, this graft is preferable to a rib graft.

Key words

Cleft palate – Alveolar reconstruction – Chin graft – Rib graft – Secondary bone grafting

Introduction

An important part in the treatment of children with clefts in the primary palate is the reconstruction of the alveolar process with bone, which nowadays is a well known and commonly used surgical procedure.

Reviews of the literature on grafting of alveolar clefts are given by *Koberg* (1973) and *Witsenburg* (1985).

For many years rib bone was the graft tissue of choice for secondary bone grafting in our department (*Prahl-Andersen* and *Lekkas*, 1977; *Prahl-Andersen* and *Litten*, 1980; *Witsenburg*, 1984; *Freihofer* and *Kuijpers-Jagtman*, 1987, 1989; *Witsenburg* and *Freihofer*, 1990). This bone grafting procedure is performed during the mixed dentition stage.

Our Cleft Palate Team defines early secondary bone grafting as the surgical bone grafting procedure performed after the eruption of the maxillary lateral incisors but prior to the eruption of the canine tooth on the cleft side through the alveolar bone.

The term late secondary bone grafting is used when the canine has erupted through the bone at the time of operation.

The optimal time for early secondary bone grafting is determined by the development of the root of the canine, i.e. about one-quarter to one half of the final root length, as indicated by the radiographic appearance of a root length equal to that of the crown (*El Deeb* et al., 1982). The crown of this canine should still be completely covered by bone.

Based on the results obtained by *Bosker* and *van Dijk* (1980), in 1984 we also started to use chin bone for secondary bone grafting also. The aim of this article is to describe our surgical procedure of bone grafting with chin bone and to give a preliminary report of the results with chin as compared to rib grafts.

Material and Methods

During the period 1981 to 1988, 61 patients with a complete unilateral cleft were operated on for early secondary reconstruction of the alveolar process. The boy – girl ratio is 7 to 3. The mean age was 9.5 years (range 8.0–13.1 years). The graft tissue chosen was rib in 39 cases and chin bone in 22 cases. All patients were operated on by experienced maxillofacial surgeons.

The result of the bone grafting procedure was assessed on the basis of clinical and radiological observation. For each patient, preoperative, postoperative and annual follow-up radiographs are available. The height of the alveolar bone in the cleft region after the bone grafting procedure was compared to the length of the root of the teeth adjacent to the former cleft. Together with previous exposures the percentage bone loss could be determined. The minimum follow-up period was one year.

The treatment schedule for patients with unilateral cleft lip and palate begins with presurgical dental orthopaedic treatment soon after birth. At 3 to 6 months of age the lip is closed primarily, using a *Millard* procedure in most cases. At the age of one year the soft palate is repaired by the von *Langenbeck* technique.

Before reconstruction of the alveolar process (about 9 years of age) orthodontic transverse maxillary expansion is often needed to correct a lateral crossbite of the lesser segment. All clefts are reconstructed before the canine has erupted into the cleft. If there are supernumerary teeth in the cleft region they are removed peroperatively.

The operations are performed under general anaesthesia and prophylactic antibiotic cover with penicillin is given. Patients allergic to penicillin receive erythromycin. This is given up to 5 days postoperatively. Chlorhexidine mouth rinses 3 times daily are used for at least 2 weeks after the operation. For the first 5 postoperative days an iodoform-vaseline gauze pack is applied to cover the palatal wound, to protect it as well as to prevent formation of a haematoma. This pressure pack is sutured to the teeth. If retention of the expanded maxilla is needed, a custom-made palatal bar with extensions along the entire upper arch is used also.

19.

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Alveolar Bone Marrow as a Cell Source for Regenerative Medicine: Differences Between Alveolar and Iliac Bone Marrow Stromal Cells

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ABSTRACT: We isolated and expanded BMSCs from human alveolar/jaw bone at a high success rate (70%). These cells had potent osteogenic potential in vitro and in vivo, although their chondrogenic and adipogenic potential was less than that of iliac cells.

Introduction: Human bone marrow stromal cells (BMSCs) have osteogenic, chondrogenic, and adipogenic potential, but marrow aspiration from iliac crest is an invasive procedure. Alveolar BMSCs may be more useful for regenerative medicine, because the marrow can be aspirated from alveolar bone with minimal pain.
Materials and Methods: In this study, alveolar bone marrow samples were obtained from 41 patients, 6–66 years of age, during the course of oral surgery. BMSCs were seeded and maintained in culture with 10% FBS and basic fibroblast growth factor. In addition, BMSCs were induced to differentiate into osteoblasts, chondrocytes, or adipocytes in appropriate medium.
Results and Conclusion: From a small volume (0.1–3 ml) of aspirates, alveolar BMSCs expanded at a success ratio of 29/41 (70%). The success rate decreased with increasing donor age, perhaps because of age-dependent decreases in the number and proliferative capacity of BMSCs. The expanded BMSCs differentiated into osteoblasts under osteogenic conditions in 21–28 days: the mRNA levels of osteocalcin, osteopontin, and bone sialoprotein, along with the calcium level, in alveolar BMSC cultures were similar to those in iliac cultures. However, unlike iliac BMSC, alveolar BMSC showed poor chondrogenic or adipogenic potential, and similar differences were observed between canine alveolar and iliac BMSCs. Subsequently, human alveolar BMSCs attached to β-tricalcium phosphate were transplanted into immunodeficient mice. In transplants, new bone formed with osteoblasts and osteocytes that expressed human vimentin, human osteocalcin, and human GAPDH. These findings suggest that BMSCs have distinctive features depending on their in vivo location and that alveolar BMSCs will be useful in cell therapy for bone diseases.
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Key words: bone marrow stromal cells, alveolar bone, osteogenesis, mesenchymal stem cells

INTRODUCTION

BONE MARROW STROMAL CELLS (BMSCs) can differentiate into a variety of tissues—bone, cartilage, tendon, muscle, adipose tissue, and neuronal tissue—and their transplantation promotes regeneration of various tissues.[1–4] BMSCs have been isolated from various bones, including the ilium, femur, tibia, and spine,[5–6] but whether their proliferative and differentiation potentials depend on their in vivo location is unknown. Furthermore, marrow aspiration from these bones is an invasive procedure. Considering these facts, we decided to try collecting BMSCs from alveolar bone during the course of dental surgery, because most young adults undergo wisdom tooth extraction. We examined whether BMSCs could be expanded ex vivo from a small volume of alveolar bone marrow aspirates, and we also examined the effects of age, sex, disease history, and the volume of aspirates obtained from patients on ex vivo expansion of alveolar BMSCs. Furthermore, we compared the proliferative and differentiation potentials of alveolar BMSCs with those of iliac BMSCs, using human and canine marrow aspirates.

Drs Kato and Tsuji own stock in Two Cells Co., Ltd. All other authors have no conflict of interest.

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20.

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Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals

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Abstract

Autologous grafts from axial and appendicular bones commonly used to repair orofacial bone defects often result in unfavorable outcome. This clinical observation, along with the fact that many bone abnormalities are limited to craniofacial bones, suggests that there are significant differences in bone metabolism in orofacial, axial and appendicular bones. It is plausible that these differences are dictated by site-specificity of embryological progenitor cells and osteogenic properties of resident multipotent human bone marrow stromal cells (hBMSCs). This study investigated skeletal site-specific phenotypic and functional differences between orofacial (maxilla and mandible) and axial (iliac crest) hBMSCs in vitro and in vivo. Primary cultures of maxilla, mandible and iliac crest hBMSCs were established with and without osteogenic inducers. Site-specific characterization included colony forming efficiency, cell proliferation, life span before senescence, relative presence of surface markers, adipogenesis, osteogenesis and transplantation in immunocompromised mice to compare bone regenerative capacity. Compared with iliac crest cells, orofacial hBMSCs (OF-MSCs) proliferated more rapidly with delayed senescence, expressed higher levels of alkaline phosphatase and demonstrated more calcium accumulation in vitro. Cells isolated from the three skeletal sites were variably positive for STRO 1, a marker of hBMSCs. OF-MSCs formed more bone in vivo, while iliac crest hBMSCs formed more compacted bone that included hematopoietic tissue and were more responsive in vitro and in vivo to osteogenic and adipogenic inductions. These data demonstrate that hBMSCs from the same individuals differ in vitro and in vivo in a skeletal site-specific fashion and identified orofacial marrow stromal cells as unique cell populations. Further understanding of site-specific properties of hBMSCs and their impact on site-specific bone diseases and regeneration are needed.
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Keywords: Bone marrow stromal cells; Orofacial; Site-specific; Life span; Regeneration

Introduction

Autologous bone grafts used to stimulate new bone formation at sites of orofacial osseous defects are commonly obtained from several donor sites including orofacial, axial and appendicular bones. Bridging orofacial defects with grafts obtained from an orofacial donor site are usually more successful than those from non-orofacial sites, indicating anatomic skeletal site-specific differences affect graft integration [18,30,37]. Added evidence that orofacial bone development differs from that of axial and appendicular bone formation is suggested by the existence of skeletal diseases such as cherubism [42] and hyperparathyroid jaw tumor syndrome [41], which affect only jaw bones. In addition, craniofacial fibrous dysplasia is histologically and radiologically distinct from fibrous dysplasia in axial and appendicular bones [1,35]. The existence of site-specific variation in bone cell responses has been suggested based on skeletal site-dependent differences in the production of Insulin-like Growth Factor (IGF) system components by cultured human bone cells at various skeletal sites [24]. It is also noteworthy that the basic anatomy of axial and appendicular skeletons has been

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21.

SHED: Stem cells from human exfoliated deciduous teeth

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To isolate high-quality human postnatal stem cells from accessible resources is an important goal for stem-cell research. In this study we found that exfoliated human deciduous tooth contains multipotent stem cells [stem cells from human exfoliated deciduous teeth (SHED)]. SHED were identified to be a population of highly proliferative, clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts. After *in vivo* transplantation, SHED were found to be able to induce bone formation, generate dentin, and survive in mouse brain along with expression of neural markers. Here we show that a naturally exfoliated human organ contains a population of stem cells that are completely different from previously identified stem cells. SHED are not only derived from a very accessible tissue resource but are also capable of providing enough cells for potential clinical application. Thus, exfoliated teeth may be an unexpected unique resource for stem-cell therapies including autologous stem-cell transplantation and tissue engineering.

odontoblast | bone regeneration | neural differentiation | adipocyte | dental pulp stem cell

Postnatal stem cells have been isolated from a variety of tissues including but not limited to bone marrow, brain, skin, hair follicles, skeletal muscle, and dental pulp (1–7). Recently, the extraordinary plasticity of postnatal stem cells has been suggested, in which neural stem cells may contribute to blood and skeletal muscle (8, 9), and bone marrow stem cells may contribute to muscle, liver, and neuronal tissue (10–13). Recent emerging evidence suggests that cell-fusion events may account for some of these observations (14, 15). It is necessary to gain further insight into the characteristics of postnatal stem cells and examine their full developmental potential *in vivo* (16).

The transition from deciduous teeth to adult permanent teeth is a very unique and dynamic process in which the development and eruption of permanent teeth coordinate with the resorption of the roots of deciduous teeth. It may take >7 years in humans to complete the ordered replacement of 20 deciduous teeth (17). In this study we isolated a distinctive population of multipotent stem cells from the remnant pulp of exfoliated deciduous teeth. The significance of this study is that it provides evidence indicating that a naturally occurring exfoliated deciduous tooth is similar in some ways to an umbilical cord, containing stem cells that may offer a unique stem-cell resource for potential clinical applications.

Materials and Methods

Subjects and Cell Culture. Normal exfoliated human deciduous incisors were collected from 7- to 8-year-old children under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. The pulp was separated from a remnant crown and then digested in a solution of 3 mg/ml collagenase type I (Worthington Biochem, Freehold, NJ) and 4 mg/ml dispase (Roche Molecular Biochemicals) for 1 h at 37°C. Single-cell suspensions were cultured in a regular medium as reported (7). These techniques resulted in a population that we have termed stem cells from human exfoliated deciduous teeth (SHED).

Conditions for the induction of calcium accumulation were as reported (7), and recombinant human BMP-4 (R & D Systems) was used to induce osteogenic differentiation. Calcium accumulation was detected by 2% Alizarin red S (pH 4.2) staining. The calcium concentration was measured by using a commercially available kit (calcium kit 587-A, Sigma). The induction of adipogenesis was performed as reported (18). For neural differentiation, Neurobasal A (GIBCO/BRL), B27 supplement (GIBCO/BRL), 1% penicillin, 20 ng/ml epidermal growth factor (BD Bioscience), and 40 ng/ml fibroblast growth factor (FGF) (BD Bioscience) were used to culture cells attached to 0.1% gelatin-coated dishes (StemCell Technologies, Vancouver). For sphere-like cell-cluster formation, 3% rat serum and B27 were added.

Antibodies. Rabbit antibodies included anti-HSP90 and basic FGF (bFGF) (Santa Cruz Biotechnology); anti-core-binding factor, runt domain, α subunit 1 (CBFA1) (Oncogene Research Products, Cambridge, MA); anti-endostatin, human-specific mitochondria, and glutamic acid decarboxylase (GAD) (Chemicon); and anti-alkaline phosphatase (ALP) (LF-47), bone sialoprotein (LF-120), matrix extracellular phosphaglycoprotein (MEPE) (LF-155), and dentin sialophosphoprotein (DSPP) (LF-151) (National Institute of Dental and Craniofacial Research/National Institutes of Health). Goat antibodies included anti-MAP2 and Tau (Santa Cruz Biotechnology). Mouse antibodies included anti-STRO-1 and CD146 (CC9); glial fibrillary acidic protein (GFAP), nestin, neurofilament M (NFM), neuronal nuclei (NeuN), and 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) (Chemicon); and anti-βIII-tubulin (Promega). Rabbit and murine isotype-matched negative control antibodies were also used (Caltag Laboratories, Burlingame, CA).

Transplantation. Approximately 2.0×10^6 SHED were mixed with 40 mg of hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder (Zimmer, Warsaw, IN) and then transplanted s.c. into immunocompromised mice (NIH-bg-nu-xid, Harlan–Sprague–Dawley) as described (19).

SHED were injected into the brain of immunocompromised mice according to specifications of an approved small-animal

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Abbreviations: SHED, stem cells from human exfoliated deciduous teeth; FGF, fibroblast growth factor; bFGF, basic FGF; CBFA1, core-binding factor, runt domain, α subunit 1; GAD, glutamic acid decarboxylase; MEPE, matrix extracellular phosphoglycoprotein; DSPP, dentin sialophosphoprotein; GFAP, glial fibrillary acidic protein; NFM, neurofilament M; NeuN, neuronal nuclei; CNPase, 2′,3′-cyclic nucleotide-3′-phosphodiesterase; HA/TCP, hydroxyapatite/tricalcium phosphate; BMSSC, bone marrow stromal stem cell; DPSC, dental pulp stem cell; ALP, alkaline phosphatase.

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22.

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ORIGINAL ARTICLE

Carious deciduous teeth are a potential source for dental pulp stem cells

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Abstract

Objective The objectives of this study are to isolate, cultivate, and characterize stem cells from the pulp of carious deciduous teeth (SCCD) and compare them to those retrieved from sound deciduous teeth (SHED—stem cells from human exfoliated deciduous teeth).

Material and methods Cells were obtained of dental pulp collected from sound (n=10) and carious (n=10) deciduous human teeth. Rate of isolation, proliferation assay (0, 1, 3, 5, and 7 days), STRO-1, mesenchymal (CD29, CD73, and CD90) and hematopoietic surface marker expression (CD14, CD34, CD45, HLA-DR), and differentiation capacity were evaluated.

Results Isolation success rates were 70 and 80 % from the carious and sound groups, respectively. SCCD and SHED presented similar proliferation rate. There were no statistical differences between the groups for the tested surface markers. The cells from sound and carious deciduous teeth were positive for CD29, CD73, and CD90 and negative for CD14, CD34, CD45, and HLA-DR and were capable of differentiating into osteogenic, chondrogenic, and adipogenic lineages.

Conclusion SCCD demonstrated a similar pattern of proliferation, immunophenotypical characteristics, and differentiation ability as those obtained from sound deciduous teeth. These SCCD represent a feasible source of stem cells.

Clinical relevance Decayed deciduous teeth have been usually discarded once the pulp tissue could be damaged and the activity of stem cells compromised. These findings show that stem cells from carious deciduous teeth can be applicable source for cell-based therapies in tissue regeneration.

Keywords Dental pulp stem cells · Deciduous tooth · Caries · Proliferation · Differentiation

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Introduction

Populations of mesenchymal human stem cells have been isolated from the pulp of permanent (dental pulp stem cells—DPSCs) [1] and deciduous sound teeth (stem cells from human exfoliated deciduous teeth—SHED) [2]. Although these groups of cells share similar characteristics, SHED shows higher proliferation rates and better differentiation potential than DPSCs [3]. Moreover, deciduous teeth are more attractive because they physiologically exfoliate and pulp tissue is usually discarded [4, 5]. Thus, sound deciduous teeth, especially those in advanced exfoliation process [6], are considered a feasible stem cell source.

The use of dental pulp stem cells from sound teeth is successfully reported in several strategies of regeneration in animal models, like treatment of muscular dystrophy [7], corneal reconstruction [8], and repair of the central nervous system [9]. Moreover, DPSC and SHED are focus of dental researches and have showed promising results for pulp regeneration [10, 11] and bone repair in animal [12] and human [13] models.

Recent studies have shown conflicting results regarding the differentiation and proliferation potentials of stem cells obtained from the pulp of permanent teeth with deep carious lesions, which can be associated with the pulp inflammation stage [14–17]. The questions remains whether stem cells are modified

23.

Dental Pulp Tissue Engineering with Stem Cells from Exfoliated Deciduous Teeth

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Abstract

Stem cells from human exfoliated deciduous teeth (SHED) have been isolated and characterized as multipotent cells. However, it is not known whether SHED can generate a dental pulp-like tissue in vivo. The purpose of this study was to evaluate morphologic characteristics of the tissue formed when SHED seeded in biodegradable scaffolds prepared within human tooth slices are transplanted into immunodeficient mice. We observed that the resulting tissue presented architecture and cellularity that closely resemble those of a physiologic dental pulp. Ultrastructural analysis with transmission electron microscopy and immunohistochemistry for dentin sialoprotein suggested that SHED differentiated into odontoblast-like cells in vivo. Notably, SHED also differentiated into endothelial-like cells, as demonstrated by B-galactosidase staining of cells lining the walls of blood-containing vessels in tissues engineered with SHED stably transduced with LacZ. This work suggests that exfoliated deciduous teeth constitute a viable source of stem cells for dental pulp tissue engineering. *(J Endod 2008;34:962–969)*

Key Words

Angiogenesis, endodontics, multipotency, odontoblast, scaffold

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Regenerative medicine offers exciting opportunities to replace or restore tissues of the body after disease and trauma. Tissue engineering approaches aim to fabricate new replacement body tissues, and such approaches commonly involve seeding of cells at various stages of differentiation within scaffolds, which can then be implanted (1). The complexity of architecture and function of many tissues, however, provides significant challenges to engineering replacement tissues resembling their physiologic counterparts. Use of stem cells, either of embryonic or postnatal derivation, for tissue engineering is attractive because it offers greater scope for cell fate to try and mimic physiologic tissue architecture. However, ethical constraints associated with use of embryonic stem cells (ESCs) and limitations of readily accessible sources of autologous postnatal stem cells with multipotentiality pose significant challenges for use of stem cells in tissue engineering. Furthermore, the requirement for good vascularization of any tissue construct is of paramount importance to its vitality.

The discovery of stem cells in the pulp of permanent teeth (2) and also in deciduous teeth (3) raised the intriguing possibility of using dental pulp stem cells for tissue engineering (4–6). The dental pulp stem cells have been shown to be capable of self-renewal and multilineage differentiation (7). These stem cells can be isolated from patients with relatively minimal morbidity, especially when they are retrieved noninvasively from the pulps of exfoliated deciduous teeth (3). The first successful attempt to engineer complex whole tooth structures used single-cell suspensions dissociated from porcine third molar tooth buds and suggested the existence of dental pulp stem cells in this tissue (8). Others have successfully used a similar approach for the bioengineering of organs for regenerative therapies (9). The concept of using stem cells for dental tissue engineering was explored by Sharpe and Young (10). They and others demonstrated that it is possible to engineer murine teeth by using adult stem cells of nondental or dental origin (10–12). Recently, mesenchymal stem cells isolated from the root apical papilla of human teeth were shown to be capable of mediating tooth regeneration with recovery of tooth strength and appearance (13).

Stem cells from human exfoliated deciduous teeth (SHED) have become an attractive alternative for dental tissue engineering (3). The use of SHED might bring advantages for tissue engineering over the use of stem cells from adult human teeth as follows: (1) SHED were reported to have higher proliferation rate and increase cell population doublings as compared with stem cells from permanent teeth (3). This might facilitate the expansion of these cells in vitro before replantation. (2) SHED cells are retrieved from a tissue that is "disposable" and readily accessible in young patients, ie, exfoliated deciduous teeth. (3) We have previously proposed that dental pulp tissue engineering with stem cells could be ideally suited for young patients who have suffered pulp necrosis in immature permanent incisors as consequence of trauma (14). Such treatment could potentially allow for the completion of vertical and lateral root development and perhaps improve the long-term outcome of these teeth. The fact that these patients are in mixed dentition, and therefore their deciduous molars are at various degrees of exfoliation, makes SHED a timely and opportune stem cell source for the engineering of dental pulps in immature permanent teeth.

Although the concept of engineering whole tooth structures offers exciting potential, significant clinical challenges still remain, and engineering or regeneration of component tissues of the tooth might be a more realistic shorter-term goal. In partic-

24.

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

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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

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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.

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25.

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Multilineage Differentiation Potential of Stem Cells Derived from Human Dental Pulp after Cryopreservation

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ABSTRACT

The current study aimed to prove that human dental pulp stem cells (hDPSCs) isolated from the pulp of third molars can show multilineage differentiation after cryopreservation. First, hDPSC were isolated via enzymatic procedures, and frozen in liquid nitrogen until use. After defrosting, cells were analyzed for proliferative potential and the expression of the stem cell marker STRO-1. Subsequently, cells were cultured in neurogenic, osteogenic/odontogenic, adipogenic, myogenic, and chondrogenic inductive media, and analyzed on basis of morphology, immunohistochemistry, and reverse transcriptase-polymerase chain reaction (RT-PCR) for specific marker genes. All data were replicated, and the results of the primary cells were compared to similar tests with an additional primary dental pulp stem cell strain, obtained from the National Institutes of Health (NIH). Results showed that our cell population could be maintained for at least 25 passages. The existence of stem/ progenitor cells in both cell strains was proven by the STRO-1 staining. Under the influence of the 5 different media, both cell strains were capable to advance into all 5 differentiation pathways. Still differences between both strains were found. In general, our primary culture performed better in myogenic differentiation, while the externally obtained cells were superior in the odontogenic/osteogenic and chondrogenic differentiation pathways. In conclusion, the pulp tissue of the third molar may serve as a suitable source of multipotent stem cells for future tissue engineering strategies and cell-based therapies, even after cryopreservation.

INTRODUCTION

ADULT STEM CELLS (ASCs) situated in differentiated tissues have the potential to produce the specialized cell types to maintain and repair the tissue they reside in. ASCs have been isolated from many tissues[1] such as bone marrow,[2,3] brain,[4] skin,[5] muscle,[6] and fat tissue.[7] Previously, it was generally accepted that the differentiation potential of ASCs was lineage restricted. This hypothesis currently faces challenge as more and more evidence demonstrates that ASCs have the potential to cross lineage boundaries, and also are able to differentiate into specific cells of tissues beyond their origin. For instance, it has been confirmed that bone marrow stem cells have the ability to differentiate into neurons,[8] osteoblasts,[9] adipocytes,[9] and myoblasts.[10] Stem cells that have been isolated from other differentiated tissues such as brain,[11] muscle,[12] and fat tissue,[13] also show similar multilineage potential. Currently, there is no well accepted definition of this property of ASCs; and it has been termed plasticity,[14] trans-differentiation,[15] or unorthodox differentiation.[16] This finding increases the potential use of organ- or tissue-specific stem cells, because it suggests that stem cells can regenerate multiple tissues in different locations.

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26.

RESEARCH REPORTS
Biological

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Stem Cell Properties of Human Dental Pulp Stem Cells

ABSTRACT

In this study, we characterized the self-renewal capability, multi-lineage differentiation capacity, and clonogenic efficiency of human dental pulp stem cells (DPSCs). DPSCs were capable of forming ectopic dentin and associated pulp tissue *in vivo*. Stromal-like cells were reestablished in culture from primary DPSC transplants and re-transplanted into immunocompromised mice to generate a dentin-pulp-like tissue, demonstrating their self-renewal capability. DPSCs were also found to be capable of differentiating into adipocytes and neural-like cells. The odontogenic potential of 12 individual single-colony-derived DPSC strains was determined. Two-thirds of the single-colony-derived DPSC strains generated abundant ectopic dentin *in vivo*, while only a limited amount of dentin was detected in the remaining one-third. These results indicate that single-colony-derived DPSC strains differ from each other with respect to their rate of odontogenesis. Taken together, these results demonstrate that DPSCs possess stem-cell-like qualities, including self-renewal capability and multi-lineage differentiation.

KEY WORDS: stem cell, odontoblasts, dentin, *in vivo* transplantation.

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A supplemental appendix to this article is published electronically only at http://www.dentalresearch.org

INTRODUCTION

Stem cells are generally defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation. Post-natal stem cells have been isolated from various tissues, including bone marrow, neural tissue, skin, retina, and dental epithelium (Harada *et al.*, 1999; Fuchs and Segre, 2000; Bianco *et al.*, 2001; Blau *et al.*, 2001). Recently, we have identified a population of putative post-natal stem cells in human dental pulp, dental pulp stem cells (DPSCs). The most striking feature of DPSCs is their ability to regenerate a dentin-pulp-like complex that is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth (Gronthos *et al.*, 2000).

Previous studies have demonstrated that, like osteoblasts, pulp cells express bone markers such as bone sialoprotein, alkaline phosphatase, type I collagen, and osteocalcin (Kuo *et al.*, 1992; Tsukamoto *et al.*, 1992; Nakashima *et al.*, 1994; Butler *et al.*, 1997; Shiba *et al.*, 1998; Buurma *et al.*, 1999; Buchaille *et al.*, 2000). Their differentiation is regulated by various potent regulators of bone formation, including members of the TGFβ superfamily and cytokines (Kettunen *et al.*, 1998; Shiba *et al.*, 1998; Onishi *et al.*, 1999). The similarity of the gene expression profiles between DPSCs and precursors of osteoblasts, bone marrow stromal stem cells (BMSSCs), has recently been reported (Shi *et al.*, 2001).

BMSSCs have been defined, by *in vitro* and *in vivo* studies, as pluripotential adult stem cells (Prockop, 1997; Bianco *et al.*, 2001). They possess the capacity to differentiate into different kinds of cells such as osteoblasts, chondrocytes, adipocytes, muscle cells, and neural cells (Azizi *et al.*, 1998; Fuchs and Segre, 2000; Bianco *et al.*, 2001). In contrast, DPSCs have not yet been extensively studied in terms of their stem cell properties. Here, we demonstrate that human DPSCs represent a novel adult stem cell population that possesses the properties of high proliferative potential, the capacity of self-renewal, and multi-lineage differentiation.

MATERIALS & METHODS

Subjects and Cell Culture

Normal human third molars were collected from adults (19-29 yrs 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. For multi-colony- and single-colony-derived cell cultures, human DPSCs and BMSSCs were isolated and cultured as previously reported (Kuznetsov *et al.*, 1997; Gronthos *et al.*, 2000). For the culture of re-isolated DPSCs, three-month-old DSPC transplants were minced and then digested in a solution of 3 mg/mL collagenase type I

27.

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

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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. *(J Endod 2008;34:645–651)*

Key Words

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

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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 into odontoblast-like cells forming dentin when implanted into the subcutaneous space of immunocompromised mice using hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier vehicle (4). Although SCAP show similar characteristics to dental pulp stem cells (DPSCs) that were discovered earlier (6), they also behave differently in a number of aspects that were assessed by histologic, immunohistochemical, cellular, and molecular analyses. Evidence is accumulating to support the hypothesis that SCAP appear to be the source of primary odontoblasts that are responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement odontoblasts. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to its completion. The goal of this review is to introduce the background of these recently described stem cells, their isolation, and characterization. The potential role of these stem cells in the contribution of the continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and in autotransplanted teeth are discussed. The possibility of using SCAP and other types of pulp stem cells for pulp/dentin regeneration and the combination of SCAP and periodontal ligament stem cells (PDLSCs) for bioroot engineering shown by Sonoyama et al. (4) in a swine model as a potential future clinical approach to replace dental implants will be thoroughly described and analyzed.

Dental Papilla, Apical Papilla, and Pulp in Developing Teeth

It is well known that dental papilla is derived from the ectomesenchyme induced by the overlaying dental lamina during tooth development (7, 8). This developing organ evolves into dental pulp after being encased by the dentin tissue produced by odontoblasts that come from this organ. The apical portion of the dental papilla during the stage of root development has not been described much in the literature. Most information regarding tooth development comes from studies using animal models. Recently, we described the physical and histologic characteristics of the dental papilla located at the apex of developing human permanent teeth and termed this tissue apical papilla (5). The tissue is loosely attached to the apex of the developing root and can be easily detached with a pair of tweezers (Fig. 1). Apical papilla is apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp. Importantly, there are stem/progenitor cells located in both dental pulp and the apical papilla, but they have somewhat different characteristics (4, 5). Because of the apical location of the apical papilla, this tissue may be benefited by its collateral circulation, which enables it to survive during the process of pulp necrosis.

The Discovery of Multipotent SCAP

Stem-cell biology has become an important field for the understanding of tissue regeneration. In general, stem cells are defined by having two major properties. First, they are capable of self-renewal. Second, when they divide, some daughter cells give rise

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Survival of the Apical Papilla and Its Resident Stem Cells in a Case of Advanced Pulpal Necrosis and Apical Periodontitis

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Abstract

Introduction: Apical papilla represents a source of an enriched mesenchymal stem cell (MSC) population (stem cells of the apical papilla [SCAPs]) that modulates root development and may participate in regenerative endodontic procedures in immature teeth with pulp necrosis. The characteristics and phenotype of this tissue in the presence of inflammation are largely unknown. The purpose of this study was to characterize a human apical papilla sample that was isolated from an immature tooth with pulp necrosis and apical periodontitis. **Methods:** Inflamed periapical tissue that included part of the apical papilla (apical papilla clinical sample [CS]) was collected from an immature mandibular premolar previously diagnosed with pulp necrosis and apical periodontitis during an apexification procedure. Harvested cells from this tissue (SCAP CS) were compared with inflamed periapical progenitor cells (IPAPCs) and normal SCAP (SCAP-RP89) in flow cytometry and quantitative osteogenesis experiments. Part of the issue was further processed for immunohistochemistry and compared with apical papilla and coronal pulp sections from normal immature teeth as well as inflamed periapical tissues from mature teeth. **Results:** Similar to SCAP-RP89, 96.6% of the SCAP CS coexpressed the MSC markers CD73, CD90, and CD105, whereas only 66.3% of IPAPCs coexpressed all markers. The SCAP CS showed a significantly greater mineralization potential than both SCAP-RP89 and IPAPCs. Finally, immunohistochemical analysis revealed moderate infiltration of cells expressing the inflammatory markers CD45/68 in the apical papilla CS and prominent CD24, CD105, and von Willebrand factor expression. **Conclusions:** Under inflammatory conditions, human apical papilla was found moderately inflamed with retained SCAP vitality and stemness and increased osteogenic and angiogenesis potential. *(J Endod 2016;■:1–7)*

Key Words

Apical papilla survival, apical periodontitis, characterization, periapical inflammation, regenerative endodontics, stem cell, stem cells of the apical papilla

Significance

The apical papilla may survive despite intense inflammatory infiltrate following pulp necrosis. In this report, SCAPs maintained their stemness and expressed increased osteogenic and angiogenesis potential. Regenerative strategies should focus on promoting the continued survival, recruitment, and differentiation of these cells to achieve predictable guided endodontic repair and regeneration.

The apical papilla consists of the apical portion of the dental papilla and, in conjunction with the Hertwig epithelial root sheath, is responsible for root development (1). Stem cells of the apical papilla (SCAPs) have been shown to have great proliferation and differentiation potential in addition to high motility (2). Studies have highlighted the potential role of SCAPs and the apical papilla in the continuation of root development and regeneration of the pulp-dentin complex (1, 3). Notably, in a pilot experiment, surgically removing the apical papilla resulted in the arrest of root development even in the presence of intact pulp (1). Huang et al (3) further showed that SCAPs have the ability to differentiate into odontoblastlike cells and lead to *de novo* dental pulp regeneration *in vivo*. These findings suggest the importance of maintaining the vitality of the apical papilla in immature teeth as a source of stem cells that contribute to and regulate root development.

In regenerative endodontic procedures (REPs), evoked bleeding from the periapical tissues has been shown to lead to a significant influx of mesenchymal stem cells (MSCs) in the root canal system of both immature and mature teeth (4, 5). Using this step as a method to introduce stem cells into the root canals is a significant concept in regenerative endodontics because it provides access to the most readily available sources of MSCs (ie, apical papilla, periodontal ligament, alveolar bone, and inflamed periapical tissues) for potential dental pulp regeneration (6). In immature teeth, the apical papilla represents an enriched pool of MSCs in direct contact with the tooth apex (2, 7). Even with the odontogenic differentiation potential of SCAPs, REPs do not always result in the formation of dentin and pulplike tissue (8–10). The root canal microenvironment including pulp status and infection control regimens seems to affect the regeneration phenotype (8, 11–16). REPs in teeth with pulp necrosis and harboring bacteria in the root canal system have been associated with

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29.

Toward biomimetic materials in bone regeneration: Functional behavior of mesenchymal stem cells on a broad spectrum of extracellular matrix components

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Abstract: Bone defect treatments can be augmented by mesenchymal stem cell (MSC) based therapies. MSC interaction with the extracellular matrix (ECM) of the surrounding tissue regulates their functional behavior. Understanding of these specific regulatory mechanisms is essential for the therapeutic stimulation of MSC *in vivo*. However, these interactions are presently only partially understood. This study examined in parallel, for the first time, the effects on the functional behavior of MSCs of 13 ECM components from bone, cartilage and hematoma compared to a control protein, and hence draws conclusions for rational biomaterial design. ECM components specifically modulated MSC adhesion, migration, proliferation, and osteogenic differentiation, for example, fibronectin facilitated migration, adhesion, and proliferation, but not osteogenic differentiation, whereas fibrinogen enhanced adhesion and proliferation, but not migration. Subsequently, the integrin expression pattern of MSCs was determined and related to the cell behavior on specific ECM components. Finally, on this basis, peptide sequences are reported for the potential stimulation of MSC functions. Based on the results of this study, ECM component coatings could be designed to specifically guide cell functions. © 2010 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 95A: 1114–1124, 2010.

Key Words: mesenchymal stem cells, MSC functional behavior, extracellular, matrix, biomimetic materials, bone regeneration

INTRODUCTION

Nonunions represent a major challenge in successful bone defect treatment.[1] Especially in large bone defects, biomaterials are required that ideally fill the defect and provide optimal mechanical stability, as well as osteoinductive and -conductive stimuli. Although autogenic bone remains the "gold standard" of bone graft material, alternatives are needed to overcome the problems of limited biological availability and donor site morbidity.[2]

Mesenchymal stem cells (MSCs) are fundamental to bone defect healing.[3] Because of their capacity for migration, proliferation and multilineage differentiation,[4] MSCs hold great promise for treating bone defects through cell-based therapeutic approaches. Indeed, several studies have already shown the regenerative capacity of transplanted MSCs in bone, after either systemic or local injection or seeding on biomaterials.[5] However, although these approaches are generally successful, they are only applicable to small defects. One reason is the patient MSC availability, since bone marrow contains only about 0.001%–0.01% nucleated cells.[6] Although this problem can be circumvented by *ex vivo* cell expansion, the procedure is still time-consuming. Thus, a secondary operation for the patient is inevitable, which is especially demanding on patients of advanced age or suffering multiple trauma. Another reason is the lack of nutrition for seeded MSCs in the interior of large biomaterials depending entirely on passive diffusion in the absence of vascularity.

Biomaterial surfaces represent the local micro-environment for cells, regulating cell attachment, migration, proliferation, differentiation, and secretion of paracrine factors, and thereby acting on the healing outcome. One way to improve performance in comparison to existing biomaterials is surface or bulk modification with bioactive molecules such as native, full-length extracellular matrix (ECM) components, as well as short peptide sequences derived from these.[7] Optimally modified biomaterials could imitate the natural ECM ("biomimetic"), and thus induce tissue-specific interactions with cell receptors, such as integrins, and elicit specific cellular responses (e.g. proliferation) *in vivo*. Thus, through appropriate modifications, biomimetic materials have the potential to become readily available off the shelf.

The natural ECM of bone and cartilage is composed of a great variety of molecules including members of the

Additional Supporting Information may be found in the online version of this article.
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30.

Investigation of multipotent postnatal stem cells from human periodontal ligament

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Summary

Background Periodontal diseases that lead to the destruction of periodontal tissues—including periodontal ligament (PDL), cementum, and bone—are a major cause of tooth loss in adults and are a substantial public-health burden worldwide. PDL is a specialised connective tissue that connects cementum and alveolar bone to maintain and support teeth in situ and preserve tissue homoeostasis. We investigated the notion that human PDL contains stem cells that could be used to regenerate periodontal tissue.

Methods PDL tissue was obtained from 25 surgically extracted human third molars and used to isolate PDL stem cells (PDLSCs) by single-colony selection and magnetic activated cell sorting. Immunohistochemical staining, RT-PCR, and northern and western blot analyses were used to identify putative stem-cell markers. Human PDLSCs were transplanted into immunocompromised mice (n=12) and rats (n=6) to assess capacity for tissue regeneration and periodontal repair.

Findings PDLSCs expressed the mesenchymal stem-cell markers STRO-1 and CD146/MUC18. Under defined culture conditions, PDLSCs differentiated into cementoblast-like cells, adipocytes, and collagen-forming cells. When transplanted into immunocompromised rodents, PDLSCs showed the capacity to generate a cementum/PDL-like structure and contribute to periodontal tissue repair.

Interpretation Our findings suggest that PDL contains stem cells that have the potential to generate cementum/PDL-like tissue in vivo. Transplantation of these cells, which can be obtained from an easily accessible tissue resource and expanded ex vivo, might hold promise as a therapeutic approach for reconstruction of tissues destroyed by periodontal diseases.

Introduction

The periodontal ligament (PDL) is a soft connective tissue embedded between the cementum (a thin layer of mineralised tissue covering the roots of the teeth) and the inner wall of the alveolar bone socket, to sustain and help constrain teeth within the jaw. PDL not only has an important role in supporting teeth, but also contributes to tooth nutrition, homoeostasis, and repair of damaged tissue.[1–3] PDL contains heterogeneous cell populations[4,5] that can differentiate into either cementum-forming cells (cementoblasts) or bone-forming cells (osteoblasts).[6–8] Recent findings suggest that PDL cells have many osteoblast-like properties, including the capacity to form mineralised nodules in vitro, expression of the bone-associated markers alkaline phosphatase and bone sialoprotein, and response to bone-inductive factors such as parathyroid hormone, insulin-like growth factor 1, bone morphogenetic protein 2, and transforming growth factor β1.[2,5,9–14] The presence of multiple cell types within PDL has led to speculation that this tissue might contain progenitor cells that maintain tissue homoeostasis and regeneration of periodontal tissue.[4,15–17] However, to date, there is no direct evidence that a putative stem-cell population exists within PDL.

Periodontal diseases are infectious diseases that are characterised by destruction of periodontium (supporting tissue for tooth) including PDL, cementum, alveolar bone, and gingiva. Periodontal diseases are the main cause of tooth loss and are a substantial public health burden worldwide.[18,19] The reconstruction of healthy periodontium destroyed by periodontal diseases is a major goal of periodontal therapy. On the basis of recent advances in postnatal stem-cell biology, we postulated that PDL might contain multipotent stem cells that could be used to generate cementum and periodontal ligament in vivo. We report the isolation and characterisation of a unique stem-cell population from PDL tissue.

Methods

Samples and cell culture

Normal impacted third molars (n=25) were collected from 16 individuals aged 19–29 years at the Dental Clinic of the National Institute of Dental and Craniofacial Research, USA, following approved guidelines set by the National Institutes of Health Office of Human Subjects Research. PDL was gently separated from the surface of the root and then digested in a solution of 3 mg/mL collagenase type I (Worthington Biochem, Freehold, NJ, USA) and 4 mg/mL dispase (Roche, Mannheim, Germany) for 1 h at 37°C. PDL samples from different individuals were pooled and single-cell suspensions were obtained by passing the cells through a 70 μm strainer (Falcon, BD Labware, Franklin Lakes, NJ, USA).

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RESEARCH ARTICLE

Plasticity of stem cells derived from adult periodontal ligament

Background: The neural crest contains pluripotent cells that can give rise to neurons and glial cells of the peripheral nervous system, endocrine cells, connective tissue cells, muscle cells and pigment cells during embryonic development. Stem cells derived from the neural crest may still reside in neural crest derivatives including the periodontal ligament (PDL). However, the pluripotency of PDL-derived stem cells has not been investigated. **Aim:** To identify subpopulations of stem cells from the adult PDL and study their pluripotency. Human PDLs were harvested from impacted wisdom teeth (patients aged 19–22 years). **Results:** This study demonstrated that subpopulations of PDL cells expressed embryonic stem cell markers (Oct4, Sox2, Nanog and Klf4) and a subset of neural crest markers (Nestin, Slug, p75 and Sox10). Such PDL cell subpopulations exhibited the potential to differentiate into neurogenic, cardiomyogenic, chondrogenic and osteogenic lineages. Furthermore, preliminary evidence suggesting insulin production of PDL cells might be indicative of the generation of cells of the endodermal lineage. **Conclusion:** These findings suggest that the PDL may contain pluripotent stem cells that originate from the neural crest. Our observations open the door to prospective autologous therapeutic applications for a variety of conditions.

KEYWORDS: adult stem cells neural crest periodontal ligament pluripotent stem cells

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In spite of its origin from the ectoderm at the dorsal region of the neural tube, the neural crest (NC) contains pluripotent cells that contribute to the development of a wide variety of organs and tissues in the body after extensive migration. Depending on their final location, NC cells can give rise to neurons and glial cells of the peripheral nervous system, endocrine cells, connective tissue cells (e.g., ligament, cartilage and bone), muscle cells and pigment cells [1,2]. Based on regional characteristics and functions, the NC can be divided into four domains: cranial, trunk, vagal and sacral, and cardiac. Previous studies have demonstrated that there may be an intrinsic disparity in the capability of cell differentiation among the NC regions, with the cranial NC region exhibiting a higher level of plasticity [1–3]. Stem cells derived from the NC may still reside in various types of NC derivatives and help tissue regeneration or repair throughout adulthood [4–12].

The periodontal ligament (PDL), which is derived from the cranial NC, is a soft connective tissue embedded between the tooth root and the alveolar bone socket. It contains heterogeneous cell populations including fibroblasts, endothelial cells, epithelial cell rests of Malassez, osteoblasts and cementoblasts [13]. Owing to the remarkable capability of PDL cells for renewal, it has been speculated that different cell types within the PDL may originate from progenitors already residing therein [10,13]. Recent studies have shown that the PDL contains multipotent stem cells that are able to differentiate into neural and mesenchymal lineages [10,14,15]. More recently, Ibi *et al.* were able to establish pluripotent cells lines from miniature swine PDL fibroblasts by gene transfection of a human telomerase reverse transcriptase [16]. However, pluripotency of human PDL cells has not yet been investigated.

Potential applications of pluripotent stem cells (e.g., embryonic stem cells [ESCs]) include the development of cell-based regenerative therapies to treat diseases such as Parkinson's and Alzheimer's, spinal cord injury, heart disease, diabetes and osteoarthritis. The transcription factors Oct4, Nanog and Sox2 have been shown to be the key genes that lie at the core of the genetic circuitry involved in maintaining pluripotency of human ESCs [17–19]. Recent studies also demonstrated that pluripotent stem cells can be induced by introducing these key ESC genes into human dermal fibroblasts [20–23]. Therefore, the objective of our study was to identify subpopulations of stem cells from the adult PDL with the gene expressions of ESC and NC markers and investigate their pluripotency.

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Efficacy of Periodontal Stem Cell Transplantation in the Treatment of Advanced Periodontitis

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Periodontitis is the most common cause for tooth loss in adults and advanced types affect 10–15% of adults worldwide. The attempts to save tooth and regenerate the periodontal apparatus including cementum, periodontal ligament, and alveolar bone reach to the dental tissue-derived stem cell therapy. Although there have been several periodontitis models suggested, the apical involvement of tooth root is especially challenging to be regenerated and dental stem cell therapy for the state has never been investigated. Three kinds of dental tissue-derived adult stem cells (aDSCs) were obtained from the extracted immature molars of beagle dogs ($n = 8$), and ex vivo expanded periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs), and periapical follicular stem cells (PAFSCs) were transplanted into the apical involvement defect. As for the lack of cementum-specific markers, anti-human cementum protein 1 (rhCEMP1) antibody was fabricated and the aDSCs and the regenerated tissues were immunostained with anti-CEMP1 antibody. Autologous PDLSCs showed the best regenerating capacity of periodontal ligament, alveolar bone, and cementum as well as peripheral nerve and blood vessel, which were evaluated by conventional and immune histology, 3D micro-CT, and clinical index. The rhCEMP1 was expressed strongest in PDLSCs and in the regenerated periodontal ligament space. We suggest here the PDLSCs as the most favorable candidate for the clinical application among the three dental stem cells and can be used for treatment of advanced periodontitis where tooth removal was indicated in the clinical cases.

Key words: Dental mesenchymal stem cell; Adult stem cell therapy; Advanced periodontitis; Tooth; Cementum

INTRODUCTION

Oral diseases are included among the World Health Organization (WHO) definition of chronic diseases, and the associations between oral health status and chronic systemic diseases have been observed recently (48,49). Among them, periodontitis is a periodontal tissue infectious disease and the most common cause for tooth loss in adults. This common disease is characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing, and gingival inflammation (20). Many researchers have tried to regenerate the periodontal apparatus, including cementum, periodontal ligament, and alveolar bone, but the regenerative treatment of periodontal disease has been a major challenge in clinical periodontics (57).

Conventional regeneration therapies such as guided tissue regeneration (GTR), topical application of enamel matrix derivative (EMD), or various growth factors can partially regenerate periodontal tissues (8,10,11,14,15, 27,30). The results in clinical applications vary greatly, depending on the different characteristics of the defects or amount of remaining surrounding alveolar bone and PDL. The strategy of periodontal tissue regeneration therapies is to control inflammation and stimulate stem progenitors to regenerate new periodontal tissues. However, the residual stem cells are limited in those patients with periodontitis because of long-term inflammation and limited cell source (38). Especially among many kinds of periodontal defects, apical involvement is known as one of the most challenging states to be regenerated and no satisfactory therapeutic approach is agreed except for tooth extraction (16,58–60).

The aim of this study was to evaluate the effective-

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In Vitro and *In Vivo* Characteristics of Stem Cells Derived from the Periodontal Ligament of Human Deciduous and Permanent Teeth

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In many studies, adult stem cells have been found in human periodontal ligament (PDL), but in most cases they were found in the permanent teeth. The aim of the present study was to characterize stem cells from the PDL of deciduous teeth (dPDLSCs) and compare them with those from the PDL of permanent teeth (pPDLSCs). Stem cell markers were examined by a flow cytometric analysis. The results of *in vitro* differentiation into adipogenic and osteogenic lineages were analyzed by histochemical staining and quantitative reverse transcription–polymerase chain reaction (RT-PCR). The results of *in vivo* transplantation were analyzed by histological staining, immunohistochemical staining, and quantitative RT-PCR. There were no significant differences in the proliferation rate, cell cycle distribution, expressions of stem cell markers such as Stro-1 and CD146, or *in vitro* differentiation. The pPDLSC transplants made more typical cementum/PDL-like tissues and expressed more cementum/PDL-related genes (CP23 and collagen XII) than did the dPDLSC transplants. Together, these results suggest that pPDLSCs are better candidates for use in reconstructing periodontium.

Introduction

There are several kinds of adult stem cells in teeth and tooth-related tissues such as dental pulp stem cells (DPSCs),[1] stem cells from the apical papilla,[2] dental follicle precursor cells,[3] periodontal ligament stem cells (PDLSCs),[4] and stem cells from human exfoliated deciduous teeth (SHED).[5] Most of these originate from permanent teeth or related tissues; however, SHED originate from the dental pulp of deciduous teeth.

Because deciduous teeth differ from permanent teeth with respect to their morphology, constituents, and life cycle, it is reasonable to assume that cells originating from deciduous and permanent teeth will behave differently. Some investigators have reported that SHED differ from DPSCs with regard to their proliferation rate (that of the former being greater than that of the latter) and the differentiation pattern (unlike DPSCs, SHED are unable to reconstitute a complete dentin/pulp-like complex *in vivo*).[5–7] It was recently reported that the periodontal ligament (PDL) of deciduous teeth also contains adult stem cells,[8,9] and it was found that the proliferation rate and potential to differentiate into adipogenic and osteogenic lineages of these stem cells were superior to those from permanent teeth.[9] However, *in vivo* transplantation has not yet been studied in this cell type.

There have been many attempts to use PDLSCs for tissue reconstruction, not only to replace destroyed periodontium in animal and human models,[10–14] but also for other applications such as the formation of bone around prosthetic implants,[15] and even plastic reconstruction.[16] However, the application of stem cells from the PDL of deciduous teeth to tissue engineering has not yet been reported.

Stem cells obtained from deciduous teeth have some advantages as a source of stem cells in regenerative medicine. This is not because deciduous teeth can be obtained easily and noninvasively; rather, it is because the proliferation and differentiation activities are higher for cells isolated from patients at a younger age.[7,17] PDLSCs obtained from the deciduous teeth can be cryopreserved, and later in the lifetime of the donor they could be used as a source of stem cells for reconstructing periodontium destroyed by periodontal diseases. In addition, on delayed replantation of avulsed teeth or on auto/allo-transplantation of teeth thawed from a tooth bank, PDL tissues engineered with those PDLSCs would be helpful for preventing replacement root resorption by odontoclasts/osteoclasts and for the successful reattachment to the alveolar bone.[18]

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Periodontal tissue engineering with stem cells from the periodontal ligament of human retained deciduous teeth

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Background and Objective: Periodontal ligament stem cells from human permanent teeth (PePDLSCs) have been investigated extensively in periodontal tissue engineering and regeneration. However, little knowledge is available on the periodontal ligament stem cells from human retained deciduous teeth (DePDLSCs). This study evaluated the potential of DePDLSCs in periodontal tissue regeneration.

Material and Methods: DePDLSCs were isolated and purified by limited dilution. The characteristics of DePDLSCs were evaluated and compared with PePDLSCs both *in vitro* and *in vivo*.

Results: DePDLSCs presented a higher proliferation rate and colony-forming capacity than PePDLSCs *in vitro*. During the osteogenic induction, alkaline phosphatase (ALP) activity, mineralized matrix formation and expression of mineralization-related genes, including runt-related transcription factor 2 (*RUNX2*), *ALP*, collagen type I (*COLI*) and osteocalcin (*OCN*) were significantly enhanced in DePDLSCs compared with PePDLSCs. Furthermore, DePDLSC cell sheets showed a stronger synthesis of collagen type I in the extracellular matrix than did PePDLSC cell sheets. After *in vivo* transplantation, DePDLSC cell sheets recombined with human dentin blocks were able to generate new cementum/periodontal ligament-like tissues.

Conclusion: Our findings suggest that DePDLSCs can be used as a promising candidate for periodontal tissue engineering.

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Key words: retained deciduous teeth; periodontal ligament; stem cell; cell sheet; periodontal regeneration

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The dental attachment apparatus consists of two mineralized tissues – cementum and alveolar bone – with an interposed fibrous, cellular and vascular soft connective tissue termed the periodontal ligament (PDL) (1). The main function of the PDL is to serve as a supporting tissue by connecting teeth to alveolar bone (2). However, periodontal diseases affect more than 10% of adults and are the main cause of tooth loss (3–5). Regeneration of a healthy periodontium that has been destroyed by periodontal disease is the major goal of periodontal therapy. Recent advances in stem cell biology and tissue engineering have presented opportunities for periodontal therapy

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RESEARCH ARTICLE

Expression of embryonic stem cell markers and osteogenic differentiation potential in cells derived from periodontal granulation tissue

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Abstract

The aim of this study was to identify if cells obtained from periodontal granulation tissue possess embryonic stem cell properties and osteogenic capacities in vitro. Periodontal granulation tissue was removed from one furcation and one infrabony defect (FGTC/IGTC-furcation/infrabony defect derived granulation tissue cells) of six patients. The extracted tissues were treated with collagenase/dispase solution, cultured and passaged twice, while a fraction of them was bacteriologically analyzed. Upon reaching confluence, total RNA was extracted, followed by cDNA synthesis and real-time PCR analysis. Gene expression levels of collagen type I, alkaline phosphatase (ALP), and the embryonic stem cell markers Nanog, Oct-4, Rex-1 and Sox-2 were measured, calibrated against the housekeeping gene GAPDH. Further, osteogenic differentiation was induced. Mineralized matrix formation was confirmed by von Kossa staining, and ALP activity was measured colorimetrically. The total bacterial load amounted to $9.4 \pm 14.6 \times 10^6$ counts/mg of tissue for IGTC, and $11.1 \pm 6.1 \times 10^6$ counts/of tissue for FGTC. Among the embryonic stem cell markers (FGTC/IGTC), Nanog was most highly expressed ($3.48 \pm 1.2/5.85 \pm 5.7$), followed by Oct-4 ($1.79 \pm 0.69/2.85 \pm 2.5$), Sox-2 ($0.66 \pm 0.3/1.26 \pm 1.4$) and Rex-1 ($0.06 \pm 0.0/0.04 \pm 0.0$). The osteogenic differentiation process was positive in both FGTC and IGTC, judged by increased von Kossa staining, and elevated ALP activity and gene expression. This study provides evidence that infected periodontal granulation tissue harbors cells expressing embryonic stem cell markers, and exhibiting osteogenic capacities when in culture in vitro.

Keywords: ALP; embryonic; periodontal granulation tissue; stem cell; bacteria; in vitro

Introduction

Periodontitis is an infectious disease leading to loss of supporting connective tissue and alveolar bone around teeth. The aim of regenerative periodontal therapy is the complete reconstitution of the lost periodontal structures, i.e. the new formation of root cementum, periodontal ligament and alveolar bone (Sculean et al., 2007). Ideally, in vitro-expanded cells in sufficient quantities and possessing the potential to regenerate periodontal structures could be used with appropriate biomaterials to engineer living tissues in vitro for subsequent transplantation into defect sites (Chen and Jin, 2010). Alternatively, it is possible to transform existing tissue harboring cells with stem-cell capacities by treatment with adequate differentiation factors.

Embryonic stem cell therapies have been proposed as a promising approach in regenerative medicine. Typical markers for identification of embryonic stem cells include Oct-4 (Boiani et al., 2004), Rex-1 (Ben-Shushan et al., 1998), NANOG (Zhang et al., 2009; Bais et al., 2012) and Sox-2 (Avilion et al., 2003), as they have been accountable for the pluripotent capacity of these special cells.

Several types of adult stem cells have been isolated from the oral cavity, including dental pulp stem cells (Gronthos et al., 2000), stem cells from the human exfoliated deciduous teeth (Miura et al., 2003), periodontal ligament stem cells (Seo et al., 2004), dental follicle progenitor stem cells (Morsczeck et al., 2005) and stem cells from the apical papilla (Sonoyama et al., 2006). The use of dental pulp stem cells for the regeneration of the dentine-pulp complex may be a promising approach for future dentistry. The underlying biological principles have been proved in various studies; however, further investigations are required to investigate the potential clinical application in humans (Caton et al., 2011).

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36.

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Review

Dental follicle stem cells and tissue engineering

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Abstract: Adult stem cells are multipotent and can be induced experimentally to differentiate into various cell lineages. Such cells are therefore a key part of achieving the promise of tissue regeneration. The most studied stem cells are those of the hematopoietic and mesenchymal lineages. Recently, mesenchymal stem cells were demonstrated in dental tissues, including dental pulp, periodontal ligament, and dental follicle. The dental follicle is a loose connective tissue that surrounds the developing tooth. Dental follicle stem cells could therefore be a cell source for mesenchymal stem cells. Indeed, dental follicle is present in impacted teeth, which are commonly extracted and disposed of as medical waste in dental practice. Dental follicle stem cells can be isolated and grown under defined tissue culture conditions, and recent characterization of these stem cells has increased their potential for use in tissue engineering applications, including periodontal and bone regeneration. This review describes current knowledge and recent developments in dental follicle stem cells and their application. (J Oral Sci 52, 541-552, 2010)

Keywords: bone regeneration; dental follicle; stem cells; tissue engineering; periodontal regeneration.

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Introduction

New approaches in tissue engineering are being developed to reconstruct and restore the function of damaged or diseased tissues and organs (1). The term "tissue engineering" was coined in 1993 by Langer and Vacanti (1) to describe the process by which tissues and organs are regenerated by cell transplantation with or without a scaffold. Almost 20 years later, tissue engineering has not advanced as hoped, despite many important achievements.

In dentistry, interest in tissue engineering research has increased rapidly among researchers and institutes. One reason for this is that dentists are familiar with tissue regeneration techniques, such as those that use tertiary dentin in dental pulp and periodontium in guided tissue regeneration, which is now common in dental hospitals (2-3). Furthermore, the development of techniques to generate whole teeth is ongoing (4-7). A key factor in perfecting this technology within the context of modern tissue engineering is the use of adult stem cells.

Stem cells are defined by their capacity to generate daughter cells with different and more restricted properties. In human postnatal dental tissue, five different sources of mesenchymal stem cells (MSCs) have been already identified: dental pulp (8-9), periodontal ligament (10), exfoliated deciduous teeth (11), dental follicle (DF) (12), and root apical papilla (13). These dental stem cells are derived from the neural crest, and thus have a different origin from bone marrow-derived MSCs, which are derived from mesoderm. On the other hand epithelial stem cells have not been identified in postnatal dental tissues. However, we recently demonstrated that the epithelial rest

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TOPIC HIGHLIGHT

WJSC 6th Anniversary Special Issues (2): Mesenchymal stem cells

Periosteum derived stem cells for regenerative medicine proposals: Boosting current knowledge

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Abstract

Periosteum is a thin fibrous layer that covers most bones. It resides in a dynamic mechanically loaded environment and provides a niche for pluripotent cells and a source for molecular factors that modulate cell behaviour. Elucidating periosteum regenerative potential has become a hot topic in orthopaedics. This review discusses the state of the art of osteochondral tissue engineering rested on periosteum derived progenitor cells (PDPCs) and suggests upcoming research directions. Periosteal cells isolation, characterization and migration in the site of injury, as well as their differentiation, are analysed. Moreover, the role of cell mechanosensing and its contribution to matrix organization, bone microarchitecture and bone stenght is examined. In this regard the role of periostin and its upregulation under mechanical stress in order to preserve PDPC survival and bone tissue integrity is contemplated. The review also summarized the role of the periosteum in the field of dentistry and maxillofacial reconstruction. The involvement of microRNAs in osteoblast differentiation and in endogenous tissue repair is explored as well. Finally the novel concept of a guided bone regeneration based on the use of periosteum itself as a smart material and the realization of constructs able to mimic the extracellular matrix features is talked out. Additionally, since periosteum can differentiate into insulin producing cells it could be a suitable source in allogenic transplantations. That innovative applications would take advantage from investigations aimed to assess PDPC immune privilege.

Key words: Periosteum; Mesenchymal stem cells; MicroRNA; Bone tissue engineering; Bone turn-over

Core tip: Periosteum provides a niche for pluripotent cells. Elucidating periosteum regenerative potential is a hot topic in orthopaedics. This review discusses the state of the art of osteochondral tissue engineering rested on periosteum derived cells and suggests upcoming research directions aimed to the development of new standards of care for the maintenance of bone mass both in post-trauma healing process and in physiological turn-over.

Ferretti C, Mattioli-Belmonte M. Periosteum derived stem cells for regenerative medicine proposals: Boosting current knowledge. *World J Stem Cells* 2014; 6(3): 266-277 Available from: URL: http://www.wjgnet.com/1948-0210/full/v6/i3/266.htm DOI: http://dx.doi.org/10.4252/wjsc.v6.i3.266

INTRODUCTION

The field of Tissue Engineering and Regenerative Medicine (TERM) has burgeoned in the last decade. The term "Regenerative Medicine" was first found in a 1992 Kaiser *et al*[1] paper as "a new branch of medicine that

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Skeletal Cell Fate Decisions Within Periosteum and Bone Marrow During Bone Regeneration

Céline Colnot

ABSTRACT: Bone repair requires the mobilization of adult skeletal stem cells/progenitors to allow deposition of cartilage and bone at the injury site. These stem cells/progenitors are believed to come from multiple sources including the bone marrow and the periosteum. The goal of this study was to establish the cellular contributions of bone marrow and periosteum to bone healing in vivo and to assess the effect of the tissue environment on cell differentiation within bone marrow and periosteum. Results show that periosteal injuries heal by endochondral ossification, whereas bone marrow injuries heal by intramembranous ossification, indicating that distinct cellular responses occur within these tissues during repair. Next, lineage analyses were used to track the fate of cells derived from periosteum, bone marrow, and endosteum, a subcompartment of the bone marrow. Skeletal progenitor cells were found to be recruited locally and concurrently from periosteum and/or bone marrow/endosteum during bone repair. Periosteum and bone marrow/endosteum both gave rise to osteoblasts, whereas the periosteum was the major source of chondrocytes. Finally, results show that intrinsic and environmental signals modulate cell fate decisions within these tissues. In conclusion, this study sheds light into the origins of skeletal stem cells/progenitors during bone regeneration and indicates that periosteum, endosteum, and bone marrow contain pools of stem cells/progenitors with distinct osteogenic and chondrogenic potentials that vary with the tissue environment.
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Key words: periosteum, bone marrow, bone graft, in vivo cell lineage, bone repair

INTRODUCTION

Bone regeneration is largely dependent on a successful inflammatory response, revascularization of the injury site, secretion of osteogenic and chondrogenic factors, and remodeling of the extracellular matrix within the damaged and new bone tissues.[1–6] Less is known about the origins of cells that produce bone and cartilage at the injury site. Several potential sources of skeletal stem cells/progenitors have been identified that may participate in bone repair. Cells may be delivered through the vasculature[7–9] and may be recruited from bone itself[10–14] or tissues immediately adjacent to bone, such as fat, tendon, and muscle.[15–17] Several lines of evidence suggest that the periosteum and the bone marrow are the main local sources of skeletal stem cells/progenitors for bone repair. Mechanical disruption of the periosteum or bone marrow delays healing,[18,19] presumably by removing the local source of cells. Although cells isolated from the periosteum or bone marrow can differentiate into chondrocytes and/or osteoblasts in vitro,[10,11,13,14] in vivo studies on the chondrogenic and osteogenic potentials of periosteum and bone marrow are limited.[20–22] Therefore, we still lack direct evidence showing the cellular contribution of periosteum and bone marrow to bone healing.

The difficulty in separating the role of various sources of cells during skeletal regeneration arises in part from the intricate structure of bone and the multiple tissues involved. In the majority of bone injuries, cortical bone is broken, thereby permitting communication between the periosteum, the bone marrow, and surrounding soft tissues. Moreover, when the physical barrier between bone compartments is disrupted, the healing response in one tissue may impinge on the response in the adjacent tissue through the diffusion of cells and growth factors. The goal of this study was to assess the extent to which periosteum and bone marrow contribute to osteogenic and chondrogenic lineages during bone repair and the extent to which the environment influences cell fate decisions in these tissues. In vivo cell lineage analyses were developed to track cells derived from periosteum, bone marrow, and endosteum, a specific compartment of the bone marrow lining the inner surface of bone. Bone grafts were collected from genetically labeled mice (Rosa26) and transplanted into wildtype hosts. Because the integrity of the periosteum, endosteum, and/or bone marrow was preserved during transplantation, labeled cells were recruited from their original niche and the fate of these cells was followed during bone healing. Results show that cells derived from the three tissues contribute differently to healing by

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Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine

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ABSTRACT

Mesenchymal stem cells (MSCs) are capable of self-renewal and differentiation into multiple cell lineages. Presently, bone marrow is considered as a prime source of MSCs; however, there are some drawbacks and limitations in use of these MSCs for cell therapy. In this study, we demonstrate that human gingival tissue-derived MSCs have several advantages over bone marrow-derived MSCs. Gingival MSCs are easy to isolate, homogenous and proliferate faster than bone marrow MSCs without any growth factor. Importantly, gingival MSCs display stable morphology and do not loose MSC characteristic at higher passages. In addition, gingival MSCs maintain normal karyotype and telomerase activity in long-term cultures, and are not tumorigenic. Thus, we reveal that human gingiva is a better source of MSCs than bone marrow, and large number of functionally competent clinical grade MSCs can be generated in short duration for cell therapy in regenerative medicine and tissue engineering.

Introduction

Human adult mesenchymal stem cells (MSCs) are non-hematopoietic, adherent fibroblast-like cells with intrinsic ability of self-renewal and potential for multilineage differentiation [1–3]. In vitro-derived MSCs express a panel of characteristic surface markers such as Thy-1 (CD90), SH-2/endoglin (CD105), SH-3/4 (CD73), β-1-integrin (CD29), and CD44; and are negative for hematopoietic markers such as CD34, CD14, and CD45. MSCs differentiate in vitro primarily into the cells of mesenchyme lineage such as bone, cartilage, and adipose tissue [4–7]. MSCs are immunoprevileged and display immunomodulatory properties both in vitro and in vivo [8,9].

MSCs are isolated from bone marrow (BM) and other tissues including umbilical cord blood, adipose tissue, and amniotic fluid suggesting that MSC niche may not be restricted to BM [10–12]. In spite of various sources, to date, BM is considered as a main source of MSCs for cell therapy. However, the major drawback of BM is extremely low yield of MSCs ranging from 0.001 to 0.01%. This poses the limitation of availability, and also harvesting large volume of human BM is relatively difficult [13]. BM-MSCs contain hematopoietic and other cells in initial few passages resulting in cellular heterogeneity. BM-MSCs have highly variable and limited self-renewal capacity and differentiation potential, and also exhibit a variable morphology and replicative senescence during in vitro serial propagation [14–16]. Moreover, to prolong the lifespan and differentiation potential of BM-MSCs specific growth factors are needed [17]. Thus, the number of biologically competent MSCs derived from BM is limited for cell therapy. A variety of clinical applications need large number of functionally competent MSCs with stable phenotype to achieve successful results.

In this study, we characterized in detail the human gingival tissue-derived stem cells for MSCs properties and compared with BM-MSCs. We demonstrate that human gingiva-derived MSCs are more superior to BM-MSCs. Gingival MSCs are easy to isolate, uniformly homogenous, proliferate faster than BM-MSCs and display stable phenotype and maintain normal karyotype and telomerase activity in long-term cultures, and are not tumorigenic. Thus, large number of functionally competent clinical grade MSCs can be generated in short duration from human gingiva for cell therapy in regenerative medicine.

Materials and methods

Human clinical samples. Human gingival tissues and BM were obtained from healthy volunteers after approval of ethics

Abbreviations: BM, bone marrow; CFU-F, colony-forming unit-fibroblast; GT, gingival tissue; IBMX, isobutyl methyl xanthine; MSCs, mesenchymal stem cells; PD, population doubling; PPARγ2, peroxisome proliferator-activated receptor γ2; TPVG, trypsin phosphate versene glucose

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40.

Mesenchymal Stem Cells Derived from Human Gingiva Are Capable of Immunomodulatory Functions and Ameliorate Inflammation-Related Tissue Destruction in Experimental Colitis[1]

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Aside from the well-established self-renewal and multipotent differentiation properties, mesenchymal stem cells exhibit both immunomodulatory and anti-inflammatory roles in several experimental autoimmune and inflammatory diseases. In this study, we isolated a new population of stem cells from human gingiva, a tissue source easily accessible from the oral cavity, namely, gingiva-derived mesenchymal stem cells (GMSCs), which exhibited clonogenicity, self-renewal, and multipotent differentiation capacities. Most importantly, GMSCs were capable of immunomodulatory functions, specifically suppressed peripheral blood lymphocyte proliferation, induced expression of a wide panel of immunosuppressive factors including IL-10, IDO, inducible NO synthase (iNOS), and cyclooxygenase 2 (COX-2) in response to the inflammatory cytokine, IFN-γ. Cell-based therapy using systemic infusion of GMSCs in experimental colitis significantly ameliorated both clinical and histopathological severity of the colonic inflammation, restored the injured gastrointestinal mucosal tissues, reversed diarrhea and weight loss, and suppressed the overall disease activity in mice. The therapeutic effect of GMSCs was mediated, in part, by the suppression of inflammatory infiltrates and inflammatory cytokines/mediators and the increased infiltration of regulatory T cells and the expression of anti-inflammatory cytokine IL-10 at the colonic sites. Taken together, GMSCs can function as an immunomodulatory and anti-inflammatory component of the immune system in vivo and is a promising cell source for cell-based treatment in experimental inflammatory diseases. *The Journal of Immunology*, **2009, 183: 7787–7798.**

Mesenchymal stem cells (MSCs)[3] have the capacity to self-renew and differentiate into different cell lineages, including mesodermal, endodermal, and ectodermal cells (1, 2). Originally isolated from bone marrow (3), similar subsets of multipotent MSCs have also been identified in skin (4, 5), adipose tissue (6), tendon (7), lung, heart, liver (8), placenta (9), amniotic fluid (10), and umbilical cord blood (11). In addition, several populations of MSCs have been identified in various dental tissues (12), including dental pulp stem cells (DPSC) (13, 14), stem cells of human exfoliated deciduous teeth (15), periodontal ligament stem cells (PDLSCs) (16), dental follicle precursor cells (17, 18), and stem cells from apical papilla (19). Aside from the abilities of self-renewal and multipotent differentiation, MSCs commonly express specific genes for embryonic stem cells, such as Octamer-4 (Oct-4) and stage-specific embryonic Ag 4 (SSEA-4) (20, 21), and share a similar expression profile of cell surface molecules, such as Stro-1, SH2 (CD105), SH4 (CD73), CD90, CD146, CD29, but typically lack hematopoietic stem cell markers, such as CD34 and CD45 (22). At the functional level, MSCs display chemotactic properties similar to immune cells in response to tissue insult and inflammation, thus exhibiting tropism for the sites of injury (23–25) via production of anti-inflammatory cytokines and antiapoptotic molecules. These unique characteristics of MSCs make them attractive candidates for the development of novel allogeneic cell-based therapeutic strategies in harnessing inflammation in the repair or regeneration of a variety of damaged tissues (26).

A growing body of evidence has demonstrated that bone marrow-derived MSCs (BMSCs) are nonimmunogenic and, more importantly, display profound immunomodulatory and anti-inflammatory capabilities (25, 27, 28). BMSCs exhibit immunomodulatory effects via inhibiting the proliferation and function of innate and adaptive immune cells such as NK, dendritic cells, and T and B lymphocytes, as well as promoting the expansion of $CD4^+CD25^+FoxP3^+$ regulatory T cells

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[3] Abbreviations used in this paper: MSC, mesenchymal stem cell; GMSC, gingiva-derived mesenchymal stem cell; BMSC, bone marrow-derived mesenchymal stem cell; Oct-4, octamer-4; SSEA-4, stage-specific embryonic Ag 4; hTERT, human reverse telomerase transcriptase; DAPI, 4′,6-diamidino-2-phenylindole; LPL, lipoprotein lipase; PPARγ2, peroxisome proliferator-activated receptor γ-2; 1-MT, 1-methyl-L-tryptophan; iNOS, inducible NO synthase; L-NAME, *N*-nitro-L-arginine methyl ester; COX-2, cyclooxygenase 2; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; MPO, myeloperoxidase; DPSC, dental pulp stem cell; PDLSC, periodontal ligament stem cell; HGF, hepatocyte growth factor; CFU-F, CFU fibroblast; HA/TCP, hydroxyapatite/tricalcium phosphate; GFAP, glial fibrillary acidic protein; Treg, regulatory T cell; hASC, human adipose-derived stem cell.

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Review Article

Trophic Activity and Phenotype of Adipose Tissue-Derived Mesenchymal Stem Cells as a Background of Their Regenerative Potential

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There has been an increased interest in mesenchymal stem cells from adipose tissue, due to their abundance and accessibility with no ethical concerns. Their multipotent properties make them appropriate for regenerative clinical applications. It has been shown that adipose-derived stem cells (ASCs) may differ between the origin sites. Moreover, a variety of internal and external factors may affect their biological characteristics, as what we aimed to highlight in this review. It has been demonstrated that ASCs secrete multiple trophic factors that are capable of stimulating cell proliferation and differentiation and migration of various cell types. Particular attention should be given to exosomes, since it is known that they contribute to the paracrine effects of MSCs. Secretion of trophic agents by ASCs is thought to be in a greater importance for regenerative medicine applications, rather than cells engraftment to the site of injury and their differentiation ability. The surface marker profile of ASCs seems to be similar to that of the mesenchymal stem cells from bone marrow, although some molecular differences are observed. Thus, in this review, we have attempted to define trophic activity, as well as phenotypic characterization of ASCs, as crucial factors for therapeutic usage.

1. Introduction

Stem cells reside in almost all tissues within the human body where they exhibit various potential. These cells reveal self-renewal capacity, long-term viability, and ability to undergo multiple lineage differentiation in an appropriate microenvironment. They are of great importance for application in regenerative medicine because they control homeostasis, regeneration, and healing [1–3]. The stem cells should be accessible in large quantities, and the procedure of collection and harvesting of them should be non or minimally invasive, so then they can be used in regenerative medicine approaches. In addition, the differentiation of stem cells along multilineage pathways can be carried out in a reproducible manner. Then, the transplantation of them to autologous or allogeneic host is safe and effective, and their manufacturing is performed in accordance with current Good Manufacturing Practice guidelines [1, 3]. According to the origin, classification of stem cells is the following: embryonic stem cells (ES cells) [4], fetal stem cells [5], and adult (postnatal) stem cells [2, 6]. Although embryonic stem cells display enormous potential related to their pluripotency, many restrictions as well as ethical concerns are hindering their clinical applications. Facing such limitations, the need to generate an alternative source of pluripotent stem cells has emerged. The efforts succeeded in 2006, when Takahashi

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materials

MDPI

Review

Adipose Tissue-Derived Stem Cells: The Biologic Basis and Future Directions for Tissue Engineering

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Abstract: Mesenchymal stem cells (MSCs) have been isolated from a variety of tissues using different methods. Active research have confirmed that the most accessible site to collect them is the adipose tissue; which has a significantly higher concentration of MSCs. Moreover; harvesting from adipose tissue is less invasive; there are no ethical limitations and a lower risk of severe complications. These adipose-derived stem cells (ASCs) are also able to increase at higher rates and showing telomerase activity, which acts by maintaining the DNA stability during cell divisions. Adipose-derived stem cells secret molecules that show important function in other cells vitality and mechanisms associated with the immune system, central nervous system, the heart and several muscles. They release cytokines involved in pro/anti-inflammatory, angiogenic and hematopoietic processes. Adipose-derived stem cells also have immunosuppressive properties and have been reported to be "immune privileged" since they show negative or low expression of human leukocyte antigens. Translational medicine and basic research projects can take advantage of bioprinting. This technology allows precise control for both scaffolds and cells. The properties of cell adhesion, migration, maturation, proliferation, mimicry of cell microenvironment, and differentiation should be promoted by the printed biomaterial used in tissue engineering. Self-renewal and potency are presented by MSCs, which implies in an open-source for 3D bioprinting and regenerative medicine. Considering these features and necessities, ASCs can be applied in the designing of tissue engineering products. Understanding the heterogeneity of ASCs and optimizing their properties can contribute to making the best therapeutic use of these cells and opening new paths to make tissue engineering even more useful.

Keywords: adipose-derived stem cells; heterogeneity; tissue engineering

1. Introduction

The defining characteristics of mesenchymal stem cells (MSCs) are their capacity to self-renew and their multipotency to differentiate into more than one cell type and remain in this state for long periods [1]. Furthermore, MSCs produce growth factors and cytokines that are involved in immunomodulation and regeneration. This immunomodulatory capacity of MSCs enables them to be used in cell therapies, especially in autoimmune diseases, host grafting and organ transplantation [2].

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Review Article

Mesenchymal Stem Cells Isolated from Adipose and Other Tissues: Basic Biological Properties and Clinical Applications

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Mesenchymal stem cells (MSCs) are adult stem cells that were initially isolated from bone marrow. However, subsequent research has shown that other adult tissues also contain MSCs. MSCs originate from mesenchyme, which is embryonic tissue derived from the mesoderm. These cells actively proliferate, giving rise to new cells in some tissues, but remain quiescent in others. MSCs are capable of differentiating into multiple cell types including adipocytes, chondrocytes, osteocytes, and cardiomyocytes. Isolation and induction of these cells could provide a new therapeutic tool for replacing damaged or lost adult tissues. However, the biological properties and use of stem cells in a clinical setting must be well established before significant clinical benefits are obtained. This paper summarizes data on the biological properties of MSCs and discusses current and potential clinical applications.

1. Introduction

A stem cell is an undifferentiated cell with the capacity for multilineage differentiation and self-renewal without senescence. Totipotent stem cells (zygotes) can give rise to a full viable organism and pluripotent stem cells (embryonic stem (ES) cells) can differentiate into any cell type within in the human body. By contrast, trophoblasts are multipotent stem cells that can differentiate into some (e.g., mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs)), but not all, cell types.

Adult tissues have specific stem cell niches, which supply replacement cells during normal cell turnover and tissue regeneration following injury [1–3]. The epidermis, hair, HSCs, and the gastrointestinal tract all present good examples of tissues with niches that contribute stem cells during normal cellular turnover [3]. The exact locations of these stem cell niches are poorly understood, but there is growing evidence suggesting a close relationship with pericytes [1, 4, 5] (Figure 1). MSCs have been isolated from adipose tissue [6], tendon [7], periodontal ligament [8], synovial membranes [9], trabecular bone [10], bone marrow [11], embryonic tissues [12], the nervous system [13], skin [14], periosteum [9], and muscle [15]. These adult stem cells were once thought to be committed cell lines that could give rise to only one type of cell, but are now known to have a much greater level of plasticity [16, 17]. Despite the vast variety of source tissues, MSCs show some common characteristics that support the hypothesis of a common origin [1, 18]. These characteristics are: fibroblast like shape in culture, multipotent differentiation, extensive proliferation capacity, and a common surface marker profile (e.g., $CD34^-$, $CD45^-$ (HSC markers), $CD31^-$ (endothelial cell marker), $CD44^+$, $CD90^+$, and $CD105^+$ (Table 1)). However, there is no surface marker that uniquely defines MSCs.

The same general approaches are used to isolate all kinds of MSCs, including the use of Dulbecco's Modified Eagle Medium (DMEM) to dissolve collagenase, digestion times limited to a maximum of 1 hour at 37°C, isolation of stem cells as soon as possible following euthanasia, and the use of culture medium at temperatures not lower than room temperature [1].

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Clinical Study of the Efficiency of Combined Cell Transplant on the Basis of Multipotent Mesenchymal Stromal Adipose Tissue Cells in Patients with Pronounced Deficit of the Maxillary and Mandibulary Bone Tissue

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The use of synthetic osteoplastic materials not always provides the required amount of the bone tissue. Transplantation of tissue-engineering constructs containing osteogenic precursor cells can be an alternative high-technology implantation method. Here we present the results of a pilot clinical study demonstrating safety of this method, accelerated healing of the operation wound, formation of young bone tissue after transplantation, and the possibility of mounting implants after 3 months in case of sufficient amount of the bone for primary fixation.

Key Words: *oral surgery; multipotent mesenchymal stromal cells from the adipose tissue; tissue engineering; sinus lifting; osteoplastics*

Reconstruction of the bone tissue is an actual problem of modern surgical dentistry and oral surgery. The search for new and improvement of known materials for replacement of bone tissue defects and stimulation of bone growth is now in progress [1,2, 12]. The use of bone materials or bone substitutes is based on the assumption that they promote the formation of a new bone at the site of implantation. Autogenous bone transplants are considered as golden standard for reconstructive craniomaxillofacial surgery [4]. However, obtaining a sufficient amount of automaterial and the presence of an additional operation field considerably limit the use of these methods [7]. The existing methods for bone tissue reconstruction do not provide complete recovery and stimulation of bone regeneration. In most cases they perform an osteoconductive function [3,9].

Regeneration of the bone tissue is the most studied field in tissue engineering. Equivalents of the bone tissue can be obtained by targeted osteogenic differentiation of multipotent mesenchymal stromal cells (MMSC) of the bone marrow (BM) or adipose tissue [6,11,13]. MMSC predifferentiated towards osteogenic lineage are applied on biocompatible materials maintaining osteoinduction and possessing sufficient osteoconductive properties [6]. The resultant tissue-engineering construct is transplanted into the bone defect area.

Creation of tissue equivalents of the bone tissue is now beyond the scope of experimental studies. Numerous experimental and clinical studies demonstrated the possibility of effective reconstruction of the bone tissue using various biodegradable materials and MMSC [5,8,14].

A clinical study of reconstruction of bone defects and maxillary and mandibulary bone deficit

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Review Article

Banking on teeth – Stem cells and the dental office

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ABSTRACT

Science and commerce advance together and the stem cell field is no exception. With the promise of cures for conditions as diverse as cancer, autism, neural degeneration, organ replacement and addiction, long-term preservation of dental stem cells is a growth market. The discovery nearly twenty years ago, of viable, multipotent, stem cells in dental pulp from both baby and adult teeth initiated, and drives, this market.The dental stem cell preservation services, "tooth banks", focus on the collection of a child's baby teeth, as they are shed naturally, and storage of the stem cells from within the pulp for therapeutic use in later years should the child require them. This review focuses on the procedures related to these stem cell storage services and may serve as an introduction for many to the practice of "tooth banking".

Dentists have been changing lives for hundreds of years - removing pain, enabling people to eat normally, returning faces to their original splendor - but modern dentists are not only changing lives, they are saving them.

Over the last decade a service once solely the purview of hospital clinics, is becoming more and more popular in the dental office. The collection of stem cells for long-term storage for therapeutic use is a service now provided by dentists. More accurately, the dentist collects teeth and "tooth banking" services extract and preserve the stem cells within the pulp for the future benefit of the patient (Fig. 1).

Stem cell collection from bone marrow, blood, fetal material and umbilical cords present unique practical and conflicting ethical challenges [1,2]. However, the discovery of postnatal stem cell populations in the tooth pulp by Gronthos and Shi [3,4], around two decades ago, opened up new horizons to stem cell research and propelled the dental profession further into the exciting field of regenerative medicine. Post-natal stem cells are present in pulp from deciduous teeth (baby or milk teeth) lost - or exfoliated - by all children during the first 6–12 years of development and are also commonly available from orthodontic extraction of third molars (wisdom teeth) in adults.

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Research Article

Global Prevalence of Periodontal Disease and Lack of Its Surveillance

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Background. Periodontal disease is a public health problem and is strongly associated with systemic diseases; however, its worldwide distribution is not fully understood. *Objective.* To evaluate global data of periodontal disease: (1) among adolescents, adults, and older population and (2) in low-, middle-, and high-income countries. *Methods.* This ecological study included data of periodontal disease from the World Health Organization's data bank which are based on the Community Periodontal Index of Treatment Needs (CPITN code: 0 = no disease; 1 = bleeding on probing; 2 = calculus; 3 = periodontal pocket (PD) 4-5 mm; 4 = PD (6+ mm). Age- and income-related periodontal disease inequalities were evaluated across the globe. *Results.* Compared with 9.3% of adults and 9.7% of older persons, 21.2% of adolescents had no periodontal disease ($P = 0.005$). Nearly 18.8% of adolescents compared with 8.9% of adults and 5% of older persons had bleeding on probing ($P \leq 0.001$). Similarly, 50.3% of adolescents, 44.6% of adults, and 31.9% older persons demonstrated the occurrence of calculus ($P = 0.01$). On the other hand, older persons had the highest prevalence of PD 4-5 mm and PD 6+ mm than adults and adolescents ($P \leq 0.001$). The distribution of periodontitis (CPITN code 3 + 4) in adults differed significantly in low- (28.7%), lower-middle- (10%), upper-middle- (42.5%), and high-income countries (43.7%) ($P = 0.04$). However, no significant differences in periodontitis (CPITN code 3 + 4) were observed in adolescents and older persons in low- to high-income countries. *Conclusions.* Within the limitations of data, this study found that the distribution of periodontal disease increases with age. Periodontitis was the most common in older persons and in population from high-income countries.

1. Introduction

Periodontal disease which comprises gingivitis and periodontitis is a common oral infection that affects the tissues that surround and support teeth [1]. The condition often presents as gingivitis which is characterized by bleeding, swollen gums, and pain, and if left untreated, it progresses to periodontitis which involves the loss of periodontal attachment and supporting bone [2]. According to the Global Burden of Disease Study (2016), severe periodontal disease was the 11th most prevalent condition in the world [3]. The prevalence of periodontal disease was reported to range from 20% to 50% around the world [4]. It is one of the major causes of tooth loss which can compromise mastication, esthetics, self-confidence, and quality of life [5, 6]. Globally, periodontal diseases accounted for 3.5 million years lived with disability (YLD) in 2016 [3]. During the period from 1990 to 2010, there was a 57.3% increase in the global burden of periodontal disease [7]. In 2010, worldwide loss of productivity due to severe periodontitis was estimated to be US$54 billion per year [8]. The global prevalence of periodontal disease is expected to increase in coming years due to growth in the aging population and increased retention of natural teeth due to a significant reduction in tooth loss in the older population [9].

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Stem Cell Research & Therapy

RESEARCH Open Access

Stem cell therapies for periodontal tissue regeneration: a network meta-analysis of preclinical studies

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Abstract

Background: Periodontal tissue regeneration (PTR) is the ultimate goal of periodontal therapy. Currently, stem cell therapy is considered a promising strategy for achieving PTR. However, there is still no conclusive comparison that distinguishes clear hierarchies among different kinds of stem cells.

Methods: A systematic review and network meta-analysis (NMA) was performed using MEDLINE (via PubMed), EMBASE, and Web of Science up to February 2020. Preclinical studies assessing five types of stem cells for PTR were included; the five types of stem cells included periodontal ligament-derived stem cells (PDLSCs), bone marrow-derived stem cells (BMSCs), adipose tissue-derived stem cells (ADSCs), dental pulp-derived stem cells (DPSCs), and gingival-derived stem cells (GMSCs). The primary outcomes were three histological indicators with continuous variables: newly formed alveolar bone (NB), newly formed cementum (NC), and newly formed periodontal ligament (NPDL). We performed pairwise meta-analyses using a random-effects model and then performed a random-effects NMA using a multivariate meta-analysis model.

Results: Sixty preclinical studies assessing five different stem cell-based therapies were identified. The NMA showed that in terms of NB, PDLSCs (standardized mean difference 1.87, 95% credible interval 1.24 to 2.51), BMSCs (1.88, 1.17 to 2.59), and DPSCs (1.69, 0.64 to 2.75) were statistically more efficacious than cell carriers (CCs). In addition, PDLSCs were superior to GMSCs (1.49, 0.04 to 2.94). For NC, PDLSCs (2.18, 1.48 to 2.87), BMSCs (2.11, 1.28 to 2.94), and ADSCs (1.55, 0.18 to 2.91) were superior to CCs. For NPDL, PDLSCs (1.69, 0.92 to 2.47) and BMSCs (1.41, 0.56 to 2.26) were more efficacious than CCs, and PDLSCs (1.26, 0.11 to 2.42) were superior to GMSCs. The results of treatment hierarchies also demonstrated that the two highest-ranked interventions were PDLSCs and BMSCs.

Conclusion: PDLSCs and BMSCs were the most effective and well-documented stem cells for PTR among the five kinds of stem cells evaluated in this study, and there was no statistical significance between them. To translate the stem cell therapies for PTR successfully in the clinic, future studies should utilize robust experimental designs and reports.

Keywords: Stem cell therapy, Periodontitis, Periodontal defects, Periodontal tissue regeneration, Tissue engineering, Network meta-analysis

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Review

Stem cell-delivery therapeutics for periodontal tissue regeneration

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ABSTRACT

Periodontitis, an inflammatory disease, is the most common cause of tooth loss in adults. Attempts to regenerate the complex system of tooth-supporting apparatus (i.e., the periodontal ligament, alveolar bone and root cementum) after loss/damage due to periodontitis have made some progress recently and provide a useful experimental model for the evaluation of future regenerative therapies. Concentrated efforts have now moved from the use of guided tissue/bone regeneration technology, a variety of growth factors and various bone grafts/substitutes toward the design and practice of endogenous regenerative technology by recruitment of host cells (cell homing) or stem cell-based therapeutics by transplantation of outside cells to enhance periodontal tissue regeneration and its biomechanical integration. This shift is driven by the general inability of conventional therapies to deliver satisfactory outcomes, particularly in cases where the disease has caused large tissue defects in the periodontium. Cell homing and cell transplantation are both scientifically meritorious approaches that show promise to completely and reliably reconstitute all tissue and connections damaged through periodontal disease, and hence research into both directions should continue. In view of periodontal regeneration by paradigms that unlock the body's innate regenerative potential has been reviewed elsewhere, this paper specifically explores and analyses the stem cell types and cell delivery strategies that have been or have the potential to be used as therapeutics in periodontal regenerative medicine, with particular emphasis placed on the efficacy and safety concerns of current stem cell-based periodontal therapies that may eventually enter into the clinic.

1. Introduction

Periodontitis is a common and widespread disease in the oral and maxillofacial region that causes the destruction of the tooth-supporting tissues including alveolar bone, the periodontal ligament (PDL) and root cementum. If left untreated, periodontitis will result in progressive periodontal attachment and bone loss that may eventually lead to early tooth loss [1]. As a consequence, periodontal disease is one of the most important concerns for dentists, patients and the public dental healthcare system. Of note, conventional treatment strategies, either non-surgical or surgical therapy, fail to restore true periodontal supporting structures damaged through periodontal disease [2]. The significant burden of periodontal disease and its negative impact on patient quality of life indicate the need for more effective management of this condition [3,4].

Following disease control interventions such as tooth cleaning/scaling, root planning and periodontal debridement, several procedures have thus far been attempted to achieve periodontal regeneration, including bone graft placement, guided tissue/bone regeneration (GTR/GBR) and the use of various growth factors and/or host modulating agents (e.g., Emdogain® and parathyroid hormone) [5,6]. These techniques have proven at least somewhat effective in promoting the reconstruction of the appendicular musculoskeletal system. However, periodontal regeneration is especially challenging, as it requires predictable regeneration of three quite diverse and unique tissues (e.g., cementum, PDL, and bone) and a triphasic interface between these different tissues to guarantee the restoration of their complex structure [7,8]. Unfortunately, current regenerative procedures that are used either alone or in combination have limited success in achieving this ambitious purpose, especially in advanced periodontal defects [3].

Recent insights into the reparative capability of the periodontium in conjunction with advances in stem cell biology and

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Article type : Randomized Clinical Trial

Human intrabony defect regeneration with micro-grafts containing dental pulp stem cells: a randomized controlled clinical trial.

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Running title:

Periodontal regeneration with DPSCs.

Keywords: dental pulp; periodontal pocket; periodontal regeneration; randomized controlled trial; stem cells; tissue engineering.

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REVIEW

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Stem cells and periodontal regeneration

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ABSTRACT

Periodontitis is an inflammatory disease which manifests clinically as loss of supporting periodontal tissues including periodontal ligament and alveolar bone. For decades periodontists have sought ways to repair the damage which occurs during periodontitis. This has included the use of a range of surgical procedures, the use of a variety of grafting materials and growth factors, and the use of barrier membranes. To date periodontal regeneration is considered to be biologically possible but clinically unpredictable. Recently, reports have begun to emerge demonstrating that populations of adult stem cells reside in the periodontal ligament of humans and other animals. This opens the way for new cell-based therapies for periodontal regeneration. For this to become a reality a thorough understanding of adult human stem cells is needed. This review provides an overview of adult human stem cells and their potential use in periodontal regeneration.

Key words: Mesenchymal stem cells, periodontal ligament stem cells, periodontal regeneration.

Abbreviations and acronyms: BMP = bone morphogenetic proteins; BMP-7 = bone morphogenetic protein-7; BMSSCs = bone marrow stromal stem cells; EGF = epidermal growth factor; ePTFE = expanded polytetrafluoroethylene; ES = embryonic stem; FGF = fibroblast growth factor; IGF = insulin-like growth factor; HA/TCP = hydroxyapatite/tricalcium phosphate ceramic; MHC = major histocompatibility; MSCs = mesenchymal stem cells; PDGF = platelet-derived growth factor; PDLSCs = periodontal ligament stem cells.

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INTRODUCTION

Periodontitis is a disease of the periodontium characterized by irreversible loss of connective tissue attachment and supporting alveolar bone.[1] These changes often lead to an aesthetically and functionally compromised dentition. For many decades, periodontists have been interested in regenerating tissues destroyed by periodontitis. Periodontal regeneration can be defined as the complete restoration of the lost tissues to their original architecture and function by recapitulating the crucial wound healing events associated with their development.[2] Conventional open flap debridement falls short of regenerating tissues destroyed by the disease,[3,4] and current regenerative procedures offer a limited potential towards attaining complete periodontal restoration.[5–9] Recently, the isolation of adult stem cells from human periodontal ligament has presented new opportunities for tissue engineering.[10,11] Clearly, in order for such therapies to be successful, a thorough understanding of stem cells and their role in regenerating periodontal tissues is required.

The aim of this review is to discuss the current state of our understanding of adult human stem cells in dental tissues and their potential application in regenerative periodontal therapy. Current regenerative procedures, in particular guided tissue regeneration, are critically assessed. Furthermore, potential clinical implications of dental stem cells as well as the challenges for further research are also highlighted.

Definition and types of stem cells

The term "stem cell" first appeared in the literature during the 19th century. Like many other terms in biology, the concept of a stem cell has expanded greatly with identification of novel sites and functions. A "stem cell" refers to a clonogenic, undifferentiated cell that is capable of self-renewal and multi-lineage differentiation.[12] In other words, a stem cell is capable of propagating and generating additional stem cells, while some of its progeny can differentiate and commit to maturation along multiple lineages giving rise to a range of specialized cell types. Depending on intrinsic signals modulated by extrinsic factors in the stem cell niche, these cells may either undergo prolonged self-renewal or differentiation.[13] A pluripotent stem cell can give rise to cell types from all three

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RESEARCH Open Access

Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial

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Abstract

Background: Periodontitis, which progressively destroys tooth-supporting structures, is one of the most widespread infectious diseases and the leading cause of tooth loss in adults. Evidence from preclinical trials and small-scale pilot clinical studies indicates that stem cells derived from periodontal ligament tissues are a promising therapy for the regeneration of lost/damaged periodontal tissue. This study assessed the safety and feasibility of using autologous periodontal ligament stem cells (PDLSCs) as an adjuvant to grafting materials in guided tissue regeneration (GTR) to treat periodontal intrabony defects. Our data provide primary clinical evidence for the efficacy of cell transplantation in regenerative dentistry.

Methods: We conducted a single-center, randomized trial that used autologous PDLSCs in combination with bovine-derived bone mineral materials to treat periodontal intrabony defects. Enrolled patients were randomly assigned to either the Cell group (treatment with GTR and PDLSC sheets in combination with Bio-oss®) or the Control group (treatment with GTR and Bio-oss® without stem cells). During a 12-month follow-up study, we evaluated the frequency and extent of adverse events. For the assessment of treatment efficacy, the primary outcome was based on the magnitude of alveolar bone regeneration following the surgical procedure.

Results: A total of 30 periodontitis patients aged 18 to 65 years (48 testing teeth with periodontal intrabony defects) who satisfied our inclusion and exclusion criteria were enrolled in the study and randomly assigned to the Cell group or the Control group. A total of 21 teeth were treated in the Control group and 20 teeth were treated in the Cell group. All patients received surgery and a clinical evaluation. No clinical safety problems that could be attributed to the investigational PDLSCs were identified. Each group showed a significant increase in the alveolar bone height (decrease in the bone-defect depth) over time ($p < 0.001$). However, no statistically significant differences were detected between the Cell group and the Control group ($p > 0.05$).

Conclusions: This study demonstrates that using autologous PDLSCs to treat periodontal intrabony defects is safe and does not produce significant adverse effects. The efficacy of cell-based periodontal therapy requires further validation by multicenter, randomized controlled studies with an increased sample size.

(Continued on next page)

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Review article

A systematic review of the effect of surgical debridement vs. non-surgical debridement for the treatment of chronic periodontitis

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Heitz-Mayfield L. J. A, Trombelli L, Heitz F, Needleman I, Moles D: A systematic review of the effect of surgical debridement vs. non-surgical debridement for the treatment of chronic periodontitis. J Clin Periodontol 2002; 29(Suppl. 3): 92–102. © Blackwell Munksgaard, 2002.

Abstract

Objective: To systematically review the evidence of effectiveness of surgical vs. non-surgical therapy for the treatment of chronic periodontal disease.

Methods: A search was conducted for randomized controlled trials of at least 12 months duration comparing surgical with non-surgical treatment of chronic periodontal disease. Data sources included the National Library of Medicine computerised bibliographic database MEDLINE, and the Cochrane Oral Health Group (COHG) Specialist Trials Register. Screening, data abstraction and quality assessment were conducted independently by multiple reviewers (L.H., F.H., L.T.). The primary outcome measures evaluated were gain in clinical attachment level (CAL) and reduction in probing pocket depth (PPD).

Results: The search provided 589 abstracts of which six randomized controlled trials were included. Meta-analysis evaluation of these studies indicated that 12 months following treatment, surgical therapy resulted in 0.6 mm more PPD reduction (WMD 0.58 mm; 95% CI 0.38, 0.79) and 0.2 mm more CAL gain (WMD 0.19 mm; 95% CI 0.04, 0.35) than non-surgical therapy in deep pockets (>6 mm). In 4–6 mm pockets scaling and root planing resulted in 0.4 mm more attachment gain (WMD −0.37 mm; 95% CI −0.49, −0.26) and 0.4 mm less probing depth reduction (WMD 0.35 mm; 95% CI 0.23, 0.47) than surgical therapy. In shallow pockets (1–3 mm) non-surgical therapy resulted in 0.5 mm less attachment loss (WMD −0.51 mm; 95% CI −0.74, −0.29) than surgical therapy.

Conclusions: Both scaling and root planing alone and scaling and root planing combined with flap procedure are effective methods for the treatment of chronic periodontitis in terms of attachment level gain and reduction in gingival inflammation. In the treatment of deep pockets open flap debridement results in greater PPD reduction and clinical attachment gain.

Key words: non-surgical therapy; periodontal diseases/ therapy; surgical therapy; systematic review

Chronic periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both (1999 International Workshop for a Classification of Periodontal Diseases). Chronic perio dontitis affects most of the adult population and may be further classified on the basis of extent and severity. Furthermore, chronic periodontitis may be associated with modifying factors such as systemic diseases, cigarette smoking, and local factors.

Cross-sectional epidemiological studies indicate that about 10–15% of the adult population have 'advanced periodontitis', while about 80% have 'moderate periodontitis', and 10% of the

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Clinical Study

Phase I/II Trial of Autologous Bone Marrow Stem Cell Transplantation with a Three-Dimensional Woven-Fabric Scaffold for Periodontitis

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Regenerative medicine is emerging as a promising option, but the potential of autologous stem cells has not been investigated well in clinical settings of periodontal treatment. In this clinical study, we evaluated the safety and efficacy of a new regenerative therapy based on the surgical implantation of autologous mesenchymal stem cells (MSCs) with a biodegradable three-dimensional (3D) woven-fabric composite scaffold and platelet-rich plasma (PRP). Ten patients with periodontitis, who required a surgical procedure for intrabony defects, were enrolled in phase I/II trial. Once MSCs were implanted in each periodontal intrabony defect, the patients were monitored during 36 months for a medical exam including laboratory tests of blood and urine samples, changes in clinical attachment level, pocket depth, and linear bone growth (LBG). All three parameters improved significantly during the entire follow-up period ($p < 0.0001$), leading to an average LBG of 4.7 mm after 36 months. Clinical mobility measured by Periotest showed a decreasing trend after the surgery. No clinical safety problems attributable to the investigational MSCs were identified. This clinical trial suggests that the stem cell therapy using MSCs-PRP/3D woven-fabric composite scaffold may constitute a novel safe and effective regenerative treatment option for periodontitis.

1. Introduction

Periodontitis is a highly prevalent disease well known to reduce the quality of life of middle-aged and older people [1]. This common chronic inflammatory disease is caused by the formation of a bacterial biofilm on the tissues supporting the mouth and teeth, leading to the progressive destruction of the tissues and the loss of the affected teeth. The formation of intrabony defects is a frequent complication of periodontitis, which is generally treated by functional periodontal regeneration involving restoration of the alveolar bone and new cementum [2]. Conventional nonsurgical periodontal treatment and/or open flap debridement can reduce pocket depth and inflammation. However, the functional regeneration of the lost periodontal tissue and the normal structure are insufficient [3].

Historically, β-tricalcium phosphate (β-TCP) and ceramics were used for regenerative approach, but these grafting procedures resulted in the formation of long junctional epithelium [4]. Systematic reviews revealed that bone replacement grafts (including autogenous bones and demineralized freeze-dried bone allografts) and guided tissue regeneration (GTR) [5] are effective methods for periodontal regeneration [4]. On the other hand, ongoing trials are exploring new treatments to accelerate the regeneration of periodontal tissue. The consensus is that biologics, such as

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Periodontal regeneration: a challenge for the tissue engineer?

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Abstract: Periodontitis affects around 15 per cent of human adult populations. While periodontal treatment aimed at removing the bacterial cause of the disease is generally very successful, the ability predictably to regenerate the damaged tissues remains a major unmet objective for new treatment strategies. Existing treatments include the use of space-maintaining barrier membranes (guided tissue regeneration), use of graft materials, and application of bioactive molecules to induce regeneration, but their overall effects are relatively modest and restricted in application. The periodontal ligament is rich in mesenchymal stem cells, and the understanding of the signalling molecules that may regulate their differentation has increased enormously in recent years. Applying these principles for the development of new tissue engineering strategies for periodontal regeneration will require further work to determine the efficacy of current experimental preclinical treatments, including pharmacological application of growth factors such as bone morphogenetic proteins (BMPs) or Wnts, use of autologous stem cell reimplantation strategies, and development of improved biomaterial scaffolds. This article describes the background to this problem, addresses the current status of periodontal regeneration, including the background biology, and discusses the potential for some of these experimental therapies to achieve the goal of clinically predictable periodontal regeneration.

Keywords: periodontal, regeneration, tissue engineering, stem cells, growth factors

1 INTRODUCTION

Periodontal disease is one of the most common afflictions of man. It is an inflammatory condition of the supporting tissues of the teeth that results in progressive destruction of the tooth attachment and its surrounding bone. Inflammation of the gingival tissues is initiated by accumulation of dental plaque on the tooth surface and is almost universally prevalent to some degree in human populations. The destructive form of periodontal disease, periodontitis, occurs when the superficial inflammation around the gingival tissues progresses to cause irreversible destruction of the periodontal tissues, which include the alveolar bone of the jaws, the periodontal ligament, and the superficial gingivae (gums). Progressive periodontitis is seen in most adult human populations, with a prevalence of around 15 per cent. In general, the rate of progression of the disease is slow, but it can vary considerably between different patients, which may be due to the action of a range of aetiologic factors, including smoking, genetic polymorphisms, diabetes, and the presence of some specific bacteria in the dental plaque. Current treatment strategies for periodontal disease revolve specifically around the removal and long-term control of dental plaque accumulation, both by the dental professional and through self-care measures (oral hygiene) by the affected patient. These treatment strategies are generally very successful in eliminating active disease and allowing tissue repair to occur, but the regeneration of the lost tissues remains an important goal and major challenge for the clinical periodontist.

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Biomaterials Research

RESEARCH ARTICLE Open Access

Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases

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Abstract

Background: Bio-products from stem/progenitor cells, such as extracellular vesicles, are likely a new promising approach for reprogramming resident cells in both acute and chronic kidney disease. Forty CKD patients stage III and IV (eGFR 15–60 mg/ml) have been divided into two groups; twenty patients as treatment group "A" and twenty patients as a matching placebo group "B". Two doses of MSC-derived extracellular vesicles had been administered to patients of group "A". Blood urea, serum creatinine, urinary albumin creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR) have been used to assess kidney functions and TNF-α, TGF-β1 and IL-10 have been used to assess the amelioration of the inflammatory immune activity.

Results: Participants in group A exhibited significant improvement of eGFR, serum creatinine level, blood urea and UACR. Patients of the treatment group "A" also exhibited significant increase in plasma levels of TGF-β1, and IL-10 and significant decrease in plasma levels of TNF-α. Participants of the control group B did not show significant improvement in any of the previously mentioned parameters at any time point of the study period.

Conclusion: Administration of cell-free cord-blood mesenchymal stem cells derived extracellular vesicles (CF-CB-MSCs-EVs) is safe and can ameliorate the inflammatory immune reaction and improve the overall kidney function in grade III-IV CKD patients.

Keywords: Chronic kidney disease, Extracellular vesicles, Mesenchymal stem cells, Microvesicles

Background

Mesenchymal stromal cells (MSCs) could reverse acute and chronic kidney injury [1]. The majority of the transplanted MSCs are trapped by the liver, lung and spleen whereas only less than 1 % is localized at targeted tissue supporting the notion that the efficacy of MSCs in treating diseases is independent of engraftment and differentiation [2]. Extracellular vesicles (EVs) derived from MSCs have recently exploited in regenerative medicine to repair damaged tissues. These membranous structures deliver bioactive molecules, such as proteins, mRNAs, micro-RNAs, bioactive lipids and signaling receptors that can horizontally transfer genetic information [3–5]. It has also been reported that MSCs-EVs can protect AKI in ischemia reperfusion injury (IRI) in animal models [6]. In addition to multipotent capabilities of MSC-derived EVs, it has been shown to modulate both innate and adaptive immune responses and mediate immune suppressive effects through suppression of T-cell proliferation and enhancement of proliferation of the regulatory T lymphocytes (CD4-CD25-FOXP3 T-reg.) [7, 8]. Few studies addressed the potential benefit of MSC in treatment of chronic kidney disease (CKD) [9, 10]. The effect of MSCs-EVs both in the acute and chronic models was attributed to their paracrine action rather than a trans-

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biomolecules

MDPI

Article

Effect of Adipose-Derived Stem Cells and Their Exo as Adjunctive Therapy to Nonsurgical Periodontal Treatment: A Histologic and Histomorphometric Study in Rats

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Abstract: Scaling and root planing (SRP) is of limited value in many cases, so adjunctive treatment was applied to augment its outcome. Adipose-derived stem/stromal cells (ADSCs) were investigated in periodontal regeneration with promising results. However, they have safety concerns. The exosomes (Exo.), which are extracellular vesicles mediating the action of stem/stromal cells, represent a new approach to overcome these concerns. Ligature-induced periodontitis was induced in 50 rats for 14 days, and they were divided into control (5 healthy rats for histologic comparison), SRP group, ADSCs group, and Exo. group, with evaluation intervals at 2 days, and 2 and 4 weeks, including 5 rats in each interval for each group. The specimens were evaluated for histologic description (H&E), histochemical study (Masson trichrome), and histomorphometric study, to evaluate the area % of newly formed tissues. The Exo. group revealed the best results in all intervals with significantly higher area % of newly formed tissues, followed by ADSCs and, finally, SRP. Both Exo. and ADSCs showed organized newly formed tissues with the Exo. group obtaining comparable histologic results to the normal, healthy tissues by 4 weeks. Adipose-derived stem/stromal cells and their Exo. represent a promising adjunctive treatment to SRP.

Keywords: nonsurgical periodontal treatment; adipose-derived stem cells; Exo.; adjunctive periodontal therapy; rats; histologic study

1. Introduction

Periodontitis is a chronic immune inflammatory disease of supporting tooth structures initiated by dysbiotic polymicrobial dental biofilm in a susceptible host [1]. The disease progression leads to attachment loss with the destruction of periodontal tissues and, finally, tooth loss [2]. Also, the periodontal diseases may impact many systemic conditions, including diabetes mellitus, cardiovascular disorders, and autoimmune diseases, e.g., rheumatoid arthritis [3].

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 lost or injured tissues are completely restored. However, few techniques can be regarded as regenerative methods in periodontics, including biologics [4]. To achieve periodontal regeneration, a complex process of migration, proliferation, and function of different cells, in coordinated manner, should be enhanced [5].

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REVIEW

Outcome of primary root canal treatment: systematic review of the literature – Part 1. Effects of study characteristics on probability of success

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Abstract

Ng Y-L, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature – Part 1. Effects of study characteristics on probability of success. *International Endodontic Journal*, **40**, 921–939, 2007.

Aims The aims of this study were (i) to conduct a comprehensive systematic review of the literature on the outcome of primary (initial or first time) root canal treatment; (ii) to investigate the influence of some study characteristics on the estimated pooled success rates.

Methodology Longitudinal clinical studies investigating outcome of primary root canal treatment, published up to the end of 2002, were identified electronically (MEDLINE and Cochrane database 1966–2002 December, week 4). Four journals (International Endodontic Journal, Journal of Endodontics, Oral Surgery Oral Medicine Oral Pathology Endodontics Radiology and Dental Traumatology & Endodontics), bibliographies of all relevant papers and review articles were hand-searched. Three reviewers (Y-LN, SR and KG) independently assessed, selected the studies based on specified inclusion criteria, and extracted the data onto a pre-designed proforma. The study inclusion criteria were: longitudinal clinical studies investigating root canal treatment outcome; only primary root canal treatment carried out on the teeth studied; sample size given; at least 6-month postoperative review; success based on clinical and/or radiographic criteria (strict, absence of apical radiolucency; loose, reduction in size of radiolucency); overall success rate given or could be calculated from the raw data. The findings by individual study were summarized and the pooled success rates by each potential influencing factor were calculated for this part of the study.

Results Of the 119 articles identified, 63 studies published from 1922 to 2002, fulfilling the inclusion criteria were selected for the review: six were randomized trials, seven were cohort studies and 48 were retrospective studies. The reported mean success rates ranged from 31% to 96% based on strict criteria or from 60% to 100% based on loose criteria, with substantial heterogeneity in the estimates of pooled success rates. Apart from the radiographic criteria of success, none of the other study characteristics could explain this heterogeneity. Twenty-four factors (patient and operative) had been investigated in various combinations in the studies reviewed. The influence of preoperative pulpal and periapical status of the teeth on treatment outcome were most frequently explored, but the influence of treatment technique was poorly investigated.

Conclusions The estimated weighted pooled success rates of treatments completed at least 1 year prior to review, ranged between 68% and 85% when strict criteria were used. The reported success rates had not improved over the last four (or five) decades. The quality of evidence for treatment factors affecting primary root canal treatment outcome is sub-optimal; there was substantial variation in the study–designs. It

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Research Reports: Clinical

Cell-Based Regenerative Endodontics for Treatment of Periapical Lesions: A Randomized, Controlled Phase I/II Clinical Trial

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Abstract

A randomized controlled phase I/II clinical trial was designed to evaluate the safety and efficacy of encapsulated human umbilical cord mesenchymal stem cells in a plasma-derived biomaterial for regenerative endodontic procedures (REPs) in mature permanent teeth with apical lesions. The trial included 36 patients with mature incisors, canines, or mandibular premolars showing pulp necrosis and apical periodontitis. Patients were randomly and equally allocated between experimental (REP) or conventional root canal treatment (ENDO) groups. On the first visit, cavity access and mechanical preparation of the root canal were performed. Calcium hydroxide medication was used, and the cavity was sealed. Three weeks later, patients were treated following their assigned protocol of ENDO or REP. Clinical follow-up examinations were performed at 6 and 12 mo. Categorical variables were evaluated by Fisher's exact test. Quantitative variables were compared using the Mann-Whitney test. The evolution over time of the percentage of perfusion units and the dimensions of lesion and cortical compromise were explored. After the 12-mo follow-up, no adverse events were reported, and the patients showed 100% clinical efficacy in both groups. Interestingly, in the REP group, the perfusion unit percentage measured by laser Doppler flowmetry revealed an increase from 60.6% to 78.1% between baseline and 12-mo follow-up. Sensitivity tests revealed an increase of the positive pulp response in the REP group at 12-mo follow-up (from 6% to 56% on the cold test, from 0% to 28% on the hot test, and from 17% to 50% on the electrical test). We present the first clinical safety and efficacy evidence of the endodontic use of allogenic umbilical cord mesenchymal stem cells encapsulated in a plasma-derived biomaterial. The innovative approach, based on biological principles that promote dentin-pulp regeneration, presents a promising alternative for the treatment of periapical pathology (ClinicalTrials.gov NCT03102879).

Keywords: cell therapy, bioengineering, clinical outcomes, regenerative medicine, stem cells, tissue scaffolds

Introduction

The operating philosophy of endodontic treatment is to instrument, disinfect, and obturate the tooth by filling the root canals with inert materials and decreasing the space available for microbial reinfection (He et al. 2017). A systematic review of 63 articles published between 1922 and 2002 alarmingly concluded that success rates of primary root canal treatment have not improved over the past 5 decades, ranging between 68% and 85% (Ng et al. 2007). Therefore, current efforts are focused on different approaches to endodontic treatment, for example, revitalization of dental pulp to restore health inside the canal and promote the resolution of apical infection (Prescott et al. 2008; Sun et al. 2011; Huang and Garcia-Godoy 2014; Del Fabbro et al. 2016; Altaii et al. 2017).

Regenerative endodontic procedures (REPs) have been defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex (Diogenes et al. 2016). REPs are based on 3 principles of tissue engineering: cellular component including mesenchymal stem cells (MSCs), scaffolds, and growth factors (Langer and Vacanti 1993).

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A supplemental appendix to this article is available online.

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Review Article

Pulp Revascularization of Immature Permanent Teeth: A Review of the Literature and a Proposal of a New Clinical Protocol

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Tissue engineering is a growing field. In the near future, it will probably be possible to generate a complete vital tooth from a single stem cell. Pulp revascularization is dependent on the ability of residual pulp and apical and periodontal stem cells to differentiate. These cells have the ability to generate a highly vascularized and a conjunctive rich living tissue. This one is able to colonize the available pulp space. Revascularization is a new treatment method for immature necrotic permanent teeth. Up to now, apexification procedures were applied for these teeth, using calcium dihydroxide or MTA to produce an artificial apical barrier. However, the pulp revascularization allows the stimulation of the apical development and the root maturation of immature teeth. Two pulp revascularization techniques are used in the literature, one using calcium dihydroxide and the second using a triple antibiotic paste. Based on these two different pulp revascularization protocols, which obtain the desired therapeutic success, the literature will be reviewed and analyzed according to the relevance of their choice of materials. Based on the literature, we propose a new relevant protocol and a new mixture of antibiotics.

1. Introduction

Tissue engineering is a growing field. In the near future, it will probably be possible to generate a complete vital tooth from a single stem cell. Stem cells are in fact totipotent cells, which have the capacity to proliferate and to produce cells, which are capable of differentiating into specialized cells.

Two types of stem cells exist: embryonic stem cells and adult stem cells (or postnatal cells) [1]. Concerning pulp revascularization, mature stem cells are rather of interest. These cells are found in many sites of the dental element: in the pulp, in the apical papilla, and in the periodontal ligament [1, 2]. These clonogenic cells, rapidly differentiating, have the capacity of inducing dentin-pulp regeneration if differentiating into appropriate cells. In addition, the pulp, which is a product from migration of the neural crest, would probably be a very good candidate to allow nerve regeneration [1]. Regarding the daily practice, it is imperative to find ways to save as much as possible the vitality of stem cells from the dental element and induce their differentiation.

Pulp revascularization is dependent on the ability of residual pulp and apical and periodontal stem cells to differentiate [3–5]. These cells have the ability to generate a highly vascularized and a conjunctive rich living tissue. This one is able to colonize the available pulp space. Subsequently, these stem cells will differentiate into newly formed odontoblasts that will induce an apposition of hard tissue. The nature of this latter is unknown yet [1].

Revascularization is a new treatment method for immature necrotic permanent teeth. Indeed, it would provide, after treatment, a vital tooth that would be able to complete its root maturation. Up to now, apexification procedures were applied for these teeth:

(i) using calcium dihydroxide to induce the formation of an apical calcified barrier;

(ii) using mineral trioxide aggregate (MTA) to produce an artificial apical barrier.

Both methods have shown to be effective regarding the narrowing of the apical foramen of an immature tooth. However,

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The Application of Tissue Engineering to Regeneration of Pulp and Dentin in Endodontics

Misako Nakashima, PhD, DDS, and Akifumi Akamine, PhD, DDS

Abstract

Caries, pulpitis, and apical periodontitis increase health care costs and attendant loss of economic productivity. They ultimately result in premature tooth loss and therefore diminishing the quality of life. Advances in vital pulp therapy with pulp stem/progenitor cells might give impetus to regenerate dentin-pulp complex without the removal of the whole pulp. Tissue engineering is the science of design and manufacture of new tissues to replace lost parts because of diseases including cancer and trauma. The three key ingredients for tissue engineering are signals for morphogenesis, stem cells for responding to morphogens and the scaffold of extracellular matrix. In preclinical studies cell therapy and gene therapy have been developed for many tissues and organs such as bone, heart, liver, and kidney as a means of delivering growth factors, cytokines, or morphogens with stem/progenitor cells in a scaffold to the sites of tissue injury to accelerate and/or induce a natural biological regeneration. The pulp tissue contains stem/progenitor cells that potentially differentiate into odontoblasts in response to bone morphogenetic proteins (BMPs). There are two strategies to regenerate dentin. First, is in vivo therapy, where BMP proteins or *BMP* genes are directly applied to the exposed or amputated pulp. Second is ex vivo therapy and consists of isolation of stem/progenitor cells from pulp tissue, differentiation into odontoblasts with recombinant BMPs or *BMP* genes and finally transplanted autogenously to regenerate dentin. This review is focused on the recent progress in this area and discusses the barriers and challenges for clinical utility in endodontics.

Key Words

Odontoblasts, gene therapy, dental pulp capping, reparative dentin, bone morphogenetic proteins (BMPs), pulp stem cells, tubular dentin

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There is a high rate of success in retention of teeth by endodontic therapy. A recent study of more than 1.4 million cases indicate that about 97% of treated teeth remain functional over an 8-yr follow-up period (1). However, many teeth are not restorable because of apical resorption and fracture, incompletely formed roots, or carious destruction of coronal structures. In addition, vital pulp therapy is not always predictable. One novel approach to restore tooth structure is based on biology: regenerative endodontic procedures by application of tissue engineering. Over the last two decades, tissue engineering has evolved from science fiction to science. Indeed, isolated clinical case reports are consistent with the concept that certain clinical treatments might evolve into regenerative endodontic procedures (2). However, additional translational research is needed to develop predictable clinical regenerative procedures. The purpose of this article is to review the biological principles of tissue engineering and the hurdles that must be overcome to develop regenerative endodontic procedures.

Tissue Engineering

Tissue engineering is the field of functional restoration of tissue structure and physiology for impaired or damaged tissues because of cancer, diseases, and trauma (3, 4). The key elements of tissue engineering are stem cells, morphogens, and a scaffold of extracellular matrix (3, 4) (Table 1).

Adult Stem/Progenitor Cells

Adult stem/progenitor cells reside in a variety of tissues. There is an explosion of interest in the utility of cell and gene therapy for regeneration (5, 6). Adult stem cells have unique characteristics (7); (a) they exist as undifferentiated cells and maintain this phenotype by the environment and/or the adjacent cell populations until they are exposed to and respond to the appropriate signals, (b) they have an ability to self-replicate for prolonged periods, (c) they maintain their multiple differentiation potential throughout the life of the organism (7). Progenitor cells retain the differentiation potential and high proliferation capability, but have lost the self-replication property unlike stem cells. Recent data suggests that the capacity and potential for adult stem cells to differentiate into a wider spectrum of phenotypes, 'stem cell plasticity', is caused by fusion of stem cells with endogenous tissue-specific cells (5, 8, 9).

Scaffold

The scaffold provides a physicochemical and biological three-dimensional microenvironment for cell growth and differentiation, promoting cell adhesion, and migration. The scaffold serves as a carrier for morphogen in protein therapy and for cells in cell therapy. Scaffold should be effective for transport of nutrients, oxygen, and waste. It should be gradually degraded and replaced by regenerative tissue, retaining the feature of the final tissue structure. They should have biocompatibility, nontoxicity, and proper physical and mechanical strength (10). Natural polymers such as collagen and glycosaminoglycan offer good biocompatibility and bioactivity, and synthetic polymers can elaborate physicochemical features such as degradation rate, microstructure, and mechanical strength. Commonly used synthetic materials are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers, poly(lactic-co-glycolic acid) (PLGA). Synthetic hydrogels include poly(ethylene glycol) (PEG) based polymers, and those modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acid (RGD), can improve cell adhesion and matrix synthesis within the three-dimensional network (11). Scaffolds containing inorganic compounds such as hydroxyapatite and calcium phosphate are used to enhance bone conductivity (12).

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

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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.

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The use of granulocyte-colony stimulating factor induced mobilization for isolation of dental pulp stem cells with high regenerative potential☆

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ABSTRACT

Human dental pulp stem cells (DPSCs) contain subsets of progenitor/stem cells with high angiogenic, neurogenic and regenerative potential useful for cell therapy. It is essential to develop a safe and efficacious method to isolate the clinical-grade DPSCs subsets from a small amount of pulp tissue without using conventional flow cytometry. Thus, a method for isolation of DPSCs subsets based on their migratory response to optimized concentration of 100 ng/ml of granulocyte-colony stimulating factor (G-CSF) was determined in this study. The DPSCs mobilized by G-CSF (MDPSCs) were enriched for CD105, C-X-C chemokine receptor type 4 (CXCR-4) and G-CSF receptor (G-CSFR) positive cells, demonstrating stem cell properties including high proliferation rate and stability. The absence of abnormalities/aberrations in karyotype and lack of tumor formation after transplantation in an immunodeficient mouse were demonstrated. The conditioned medium of MDPSCs exhibited anti-apoptotic activity, enhanced migration and immunomodulatory properties. Furthermore, transplantation of MDPSCs accelerated vasculogenesis in an ischemic hindlimb model and augmented regenerated pulp tissue in an ectopic tooth root model compared to that of colony-derived DPSCs, indicating higher regenerative potential of MDPSCs. In conclusion, this isolation method for DPSCs subsets is safe and efficacious, having utility for potential clinical applications to autologous cell transplantation.

1. Introduction

Dental pulp stem cells have many advantages for clinical applications in dentin/pulp regeneration compared with other mesenchymal stem cells (MSCs) derived from bone marrow and adipose tissue [1]. The dental pulp stem cells are easily isolated from discarded teeth following extraction with very low morbidity and no ethical issues. A higher immunosuppressivity of T-cell alloreactivity has been demonstrated in dental pulp stem cells than in bone marrow stem cells [2]. Some subfractions of adult dental pulp stem cells, such as the $CD31^-$ side population (SP), have greater migration and higher expression of many angiogenic/neurotrophic factors than bone marrow and adipose tissue-derived stem cells of the same individual, leading to enhanced pulp regeneration [1]. On the other hand, pulp $CD31^-$ SP cells and $CD105^+$ cells have a greater effect on angiogenesis/vasculogenesis and neurogenesis after transplantation both in mouse hindlimb ischemia and rat brain ischemia compared with colony-derived dental pulp stem cells (DPSCs) [3,4]. Regenerated pulp

Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction; G-CSF, granulocyte-colony stimulating factor; DPSCs, dental pulp stem cells.

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Stem Cell Research & Therapy

RESEARCH Open Access

Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study

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Abstract

Background: Experiments have previously demonstrated the therapeutic potential of mobilized dental pulp stem cells (MDPSCs) for complete pulp regeneration. The aim of the present pilot clinical study is to assess the safety, potential efficacy, and feasibility of autologous transplantation of MDPSCs in pulpectomized teeth.

Methods: Five patients with irreversible pulpitis were enrolled and monitored for up to 24 weeks following MDPSC transplantation. The MDPSCs were isolated from discarded teeth and expanded based on good manufacturing practice (GMP). The quality of the MDPSCs at passages 9 or 10 was ascertained by karyotype analyses. The MDPSCs were transplanted with granulocyte colony-stimulating factor (G-CSF) in atelocollagen into pulpectomized teeth.

Results: The clinical and laboratory evaluations demonstrated no adverse events or toxicity. The electric pulp test (EPT) of the pulp at 4 weeks demonstrated a robust positive response. The signal intensity of magnetic resonance imaging (MRI) of the regenerated tissue in the root canal after 24 weeks was similar to that of normal dental pulp in the untreated control. Finally, cone beam computed tomography demonstrated functional dentin formation in three of the five patients.

Conclusions: Human MDPSCs are safe and efficacious for complete pulp regeneration in humans in this pilot clinical study.

Keywords: Clinical study, Pulp regeneration, Mobilized dental pulp stem cells (Mobilized DPSCs), Autologous cell transplantation, Granulocyte colony-stimulating factor (G-CSF), Pulpectomy, Good manufacturing practice (GMP)

Background

Dental caries is a common health problem in humans. When dental caries is deep, reaching the dental pulp, the treatment of choice is generally pulpectomy. The dental pulp has several vital functions such as protection from infections by immunological surveillance, rapid reparative dentin formation to guard against noxious external stimuli, and maintenance of tensile strength to prevent tooth fractures [1]. Following pulpectomy and root canal filling, postoperative pain [2], apical periodontal lesions caused by microleakage from the tooth crown [3, 4], and vertical root fracture [5] may occur, leading to a higher incidence of extraction of the affected tooth. Recent advances in stem cell biology have aided stem cell therapy to regenerate the pulp/dentin complex for conservation and complete structural and functional restoration of the tooth by the triad of tissue engineering: 1) mesenchymal stem cells (MSCs), 2) growth/differentiation factors or cytokines, and migration/homing factors, and 3) the microenvironment (scaffold, extracellular matrix) [6]. We have demonstrated complete pulp regeneration by harnessing autologous dental pulp stem cell (DPSCs) subsets transplanted with stromal cell-derived factor 1 (SDF1) in a collagen scaffold into a canine pulpitis model [7, 8]. Next, a novel isolation method was developed employing an optimal granulocyte colony-stimulating factor (G-CSF)-induced mobilization of DPSCs for clinical-grade mesenchymal stem cells from a

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STEM CELLS

CONCISE REVIEWS

Salivary Gland Regeneration: Therapeutic Approaches from Stem Cells to Tissue Organoids

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Key Words. Salivary gland • Radiation therapy • Salivary hypofunction • Xerostomia • Regeneration • Transplantation • Stem cells • Organoids

ABSTRACT

The human salivary gland (SG) has an elegant architecture of epithelial acini, connecting ductal branching structures, vascular and neuronal networks that together function to produce and secrete saliva. This review focuses on the translation of cell- and tissue-based research toward therapies for patients suffering from SG hypofunction and related dry mouth syndrome (xerostomia), as a consequence of radiation therapy or systemic disease. We will broadly review the recent literature and discuss the clinical prospects of stem/progenitor cell and tissue-based therapies for SG repair and/or regeneration. Thus far, several strategies have been proposed for the purpose of restoring SG function: (1) transplanting autologous SG-derived epithelial stem/progenitor cells; (2) exploiting non-epithelial cells and/or their bioactive lysates; and (3) tissue engineering approaches using 3D (three-dimensional) biomaterials loaded with SG cells and/or bioactive cues to mimic in vivo SGs. We predict that further scientific improvement in each of these areas will translate to effective therapies toward the repair of damaged glands and the development of miniature SG organoids for the fundamental restoration of saliva secretion.
STEM CELLS *2016; 00:000–000*

SIGNIFICANCE STATEMENT

This review covers recent advances in translating cell-based research toward pre-clinical therapies. We focus on salivary gland (SG) loss-of-function and subsequent dry mouth syndrome as caused by radiation therapy or systemic disease, although the described concepts can be translated to other injured somatic tissues. Proposed therapies include implantation of autologous tissue-specific stem/progenitor cells, non-tissue specific cells and/or their bioactive lysates (secretome); and organoid-like constructs created by cells in the presence or not of bioactive cues and three-dimensional biomaterials. These emerging approaches to repair damaged SGs are discussed herein, and evaluated on their success to restore native tissue architecture, epithelial cell polarization, ductal branching, lumen formation, directionality of secretory flow, and clinically relevant tissue functionality.

INTRODUCTION

A Place for Cell-Based Therapies

Irreversible SG hypofunction and its associated symptoms, termed xerostomia, are a hallmark of several systemic diseases, such as Sjögren's syndrome, granulomatous diseases, graft-versus-host disease, cystic fibrosis, uncontrolled diabetes, human immunodeficiency virus infection, thyroid disease, and late-stage liver disease [1]. Hyposalivation is also the most significant long-term complication for more than 550,000 patients that are annually diagnosed with head and neck cancer (HNC) globally and for whom radiation therapy (RT) is the main treatment [2–4]. Saliva is required for digestion, lubrication, oral homeostasis and protection against a variety of microbial and environmental hazards. Thus, a lack in saliva production can cause various life-disrupting pathological events. Rampant caries, painful mucositis, oral fungal infections, taste loss, speech deficits, and difficulty in swallowing are just a few examples of events that greatly impair patients' oral and systemic health [3].

Current preventative therapies, such as surgical SG relocation outside the radiation field [5] or use of free radical scavengers [6] are challenging or not always effective. Using advanced SG-sparing intensity-modulated radiation therapy (IMRT) can still result in xerostomia, even though partial improvement of salivary secretion may occur [2, 3, 7]. This

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Saliva and dental erosion

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ABSTRACT

Dental erosion is a multifactorial condition. The consideration of chemical, biological and behavioral factors is fundamental for its prevention and therapy. Among the biological factors, saliva is one of the most important parameters in the protection against erosive wear. Objective: This review discusses the role of salivary factors on the development of dental erosion. Material and Methods: A search was undertaken on MEDLINE website for papers from 1969 to 2010. The keywords used in the research were "saliva", "acquired pellicle", "salivary flow", "salivary buffering capacity" and "dental erosion". Inclusion of studies, data extraction and quality assessment were undertaken independently and in duplicate by two members of the review team. Disagreements were solved by discussion and consensus or by a third party. Results: Several characteristics and properties of saliva play an important role in dental erosion. Salivary clearance gradually eliminates the acids through swallowing and saliva presents buffering capacity causing neutralization and buffering of dietary acids. Salivary flow allows dilution of the acids. In addition, saliva is supersaturated with respect to tooth mineral, providing calcium, phosphate and fluoride necessary for remineralization after an erosive challenge. Furthermore, many proteins present in saliva and acquired pellicle play an important role in dental erosion. Conclusions: Saliva is the most important biological factor affecting the progression of dental erosion. Knowledge of its components and properties involved in this protective role can drive the development of preventive measures targeting to enhance its known beneficial effects.

Key words: Dental erosion. Enamel. Dentin. Saliva.

INTRODUCTION

Dental erosion is defined as the loss of dental hard tissue by a chemical process that does not involve bacteria[67]. The continuous erosion process occurs in different stages. Initially, softening of enamel surface occurs and this process can vary according to the immersion time and the type of acids involved. If the erosive challenge persists, dissolution of consecutive layers of enamel crystals takes place, leading to a permanent loss of volume with a softened layer on top of the remaining tissue[59]. Dental erosion can have extrinsic or intrinsic causes. The intrinsic causes comprise recurrent vomiting as in patients suffering from anorexia and bulimia, cytostatic drug treatment or propulsion of gastric contents into the mouth due to gastroesophageal reflux. Extrinsic causes comprise frequent consumption of acidic foods or drinks, the use of acidic hygiene products and acidic medicines. such as effervescent vitamin C or aspirin. Alcohol has been also associated with erosion. Gaseous acids or chemicals breathed during work may also cause erosion[78].

In enamel, the lesion primarily develops in the prism sheath areas, followed by dissolution of prism cores. Eventually, the interprismatic areas are also affected. Bulk mineral is centripetally etched away in enamel erosion leaving a partly demineralized softened surface layer, which is prone to mineral deposits after topical fluoride application[18]. In dentin, erosive demineralization results in the exposure of an outer layer of fully demineralized organic matrix followed by a partly demineralized

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On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development

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Abstract

Radiation therapy for cancer of the head and neck can devastate the salivary glands and partially devitalize the mandible and maxilla. As a result, saliva production is drastically reduced and its quality adversely altered. Without diligent home and professional care, the teeth are subject to rapid destruction by caries, necessitating extractions with attendant high risk of necrosis of the supporting bone. Innovative techniques in delivery of radiation therapy and administration of drugs that selectively protect normal tissues can reduce significantly the radiation effects on salivary glands. Nonetheless, many patients still suffer severe oral dryness. I review here the functional morphology and development of salivary glands as these relate to approaches to preventing and restoring radiation-induced loss of salivary function. The acinar cells are responsible for most of the fluid and organic material in saliva, while the larger ducts influence the inorganic content. A central theme of this review is the extent to which the several types of epithelial cells in salivary glands may be pluripotential and the circumstances that may influence their ability to replace cells that have been lost or functionally inactivated due to the effects of radiation. The evidence suggests that the highly differentiated cells of the acini and large ducts of mature glands can replace themselves except when the respective pools of available cells are greatly diminished via apoptosis or necrosis owing to severely stressful events. Under the latter circumstances, relatively undifferentiated cells in the intercalated ducts proliferate and redifferentiate as may be required to replenish the depleted pools. It is likely that some, if not many, acinar cells may de-differentiate into intercalated duct-like cells and thus add to the pool of progenitor cells in such situations. If the stress is heavy doses of radiation, however, the result is not only the death of acinar cells, but also a marked decline in functional differentiation and proliferative capacity of all of the surviving cells, including those with progenitor capability. Restoration of gland function, therefore, seems to require increasing the secretory capacity of the surviving cells, or replacing the acinar cells and their progenitors either in the existing gland remnants or with artificial glands.

Key words: apoptosis, atrophy, differentiation, proliferation, radiation, regeneration, salivary glands, stem cells

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67.

Accepted Manuscript

Safety and Efficacy of Autologous Tissue-derived Mesenchymal Stem Cells for Radiation-Induced Xerostomia: A Randomized, Placebo-Controlled Phase I/II Trial (MESRIX)

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PHYSIOLOGY & BEHAVIOR

Integration of salivary biomarkers into developmental and behaviorally-oriented research: Problems and solutions for collecting specimens

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Abstract

Saliva has been championed as a diagnostic fluid of the future. Much of the attention that saliva receives as a biological specimen is due to the perception that the nature of sample collection is quick, uncomplicated, and non-invasive. In most cases, this perception matches reality; however, in some special circumstances and populations collecting saliva can be unexpectedly difficult, time consuming, and may not yield sufficient sample volume for assay. In this report, we review the nature and circumstances surrounding some of these problems in the context of developmental science and then present alternatives that can be used by investigators to improve the next generation of studies. We expect our findings will ease the burden on research participants and assistants, reduce the rate of missing values in salivary data sets, and increase the probability that salivary biomarkers will continue to be successfully integrated into developmental and behaviorally-oriented research.

Keywords: Salivary cortisol; Testosterone; DHEA; Alpha-amylase; Saliva collection; Microsponge; Filter paper; Passive drool

1. Introduction

Saliva has been championed as a diagnostic fluid of the future [1]. Much of the attention saliva receives as a biological specimen is due to the perception that the nature of sample collection is quick, uncomplicated, and non-invasive [2]. The literature suggests that in most cases this perception matches reality. Yet, in specific circumstances and populations, collecting saliva can be unexpectedly difficult, time consuming, and may require considerable creativity (e.g., [3–5]) to gather sufficient sample volumes for assay. When sample collection is inadequate and assay protocols or laboratory technicians cannot accommodate a partial sample, the missing data problems created can seriously compromise research, and hinder screening and potential diagnostic agendas.

In this report, we review the nature of some of the unique problems specific to the application of salivary biomarkers in developmental science and rigorously evaluate approaches to saliva collection that have been used in an attempt to resolve some of the difficulties with more traditional approaches. We present alternatives that can be used by investigators, especially in special circumstances and with unique populations of research participants, to improve the next generation of studies. We expect our findings will ease the burden on research participants and research assistants, reduce the rates of missing values in salivary data, and consequentially increase the probability of the successful integration of salivary biomarkers into behaviorally-oriented research.

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69.

Development of a Visual Analogue Scale questionnaire for subjective assessment of salivary dysfunction

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UNIVERSITY OF MICHIGAN, UNIVERSITY OF NORTH CAROLINA, AND NEW YORK UNIVERSITY

Objective. This study's objective was to develop a Visual Analogue Scale (VAS) xerostomia questionnaire and to evaluate the validity and reliability for the clinical diagnosis of salivary gland dysfunction.
Study design. Thirty-six healthy adults participated in this double-blind, crossover study. Each subject received an antisialagogue (glycopyrrolate) or placebo. Unstimulated and stimulated parotid and submandibular saliva samples were collected 16 times over a period of 6 hours. An 8-item VAS xerostomia questionnaire was administered after each saliva collection.
Results. The results demonstrated significant reliability for 7 of the 8 VAS items, whereas validity was significant for unstimulated submandibular saliva. Moving averages were calculated for VAS and salivary flow rate values, and significant correlations were observed between these factors, indicating that changes in VAS responses were predictive of changes in salivary flow.
Conclusions. These findings suggest that this VAS xerostomia questionnaire may be helpful in the diagnosis of salivary dysfunction and for detecting changes in salivary flow rate values over time.
(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:311-6)

Saliva is essential for the maintenance of oral health, and salivary dysfunction has been associated with numerous oral and pharyngeal disorders.[1] Previously, it was believed that salivary dysfunction was attributed to the aging process, but it is now accepted that most salivary disorders are caused by local obstructive and microbial conditions, systemic diseases, medications, chemotherapy, and head and neck radiotherapy.[1-3]

The diagnosis of salivary gland dysfunction requires a comprehensive dental and medical evaluation, including the assessment of salivary output.[4,5] However, there is approximately 50% variability in salivary flow rates in healthy unmedicated adults,[6] and it is unclear how much saliva is needed to maintain "normal" oral function and what constitutes "normal" salivary output.[7,8] Therefore, it is difficult to set a priori individual or population standards for an abnormal salivary flow rate.[7,8]

The use of a questionnaire in conjunction with saliva collection has been useful in determining subjective measures of salivary gland dysfunction. Eight xerostomia questions were used in one study,[9] and 4 items were correlated with diminished parotid and submandibular output. However, a recent study[5] found poor concurrence between objective and subjective measures of salivary function. Finally, other oral signs and symptoms have been used in an attempt to diagnose salivary dysfunction. One study reported 4 objective measures (lip dryness; buccal mucosa dryness; decayed, missing, filled teeth [DMFT]; and absence of salivary flow on glandular palpation) that were significant predictors of salivary dysfunction.[4]

In summary, research-based diagnostic tools are infrequently used in clinical practices, and few reliable and valid subjective measures exist for identification of salivary dysfunction. The aim of this study was to improve the diagnosis of salivary dysfunction by developing a Visual Analogue Scale (VAS) xerostomia questionnaire and to evaluate its reliability and validity for the diagnosis of salivary gland dysfunction.

MATERIAL AND METHODS

Subjects

Thirty six healthy volunteers (18 men, 18 women) were divided into 2 age groups: young (n = 18; 20-38 years) and older (n = 18; 60-77 years). Inclusion criteria included absence of systemic and salivary diseases and no use of prescription or antihistamine

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Article

Mesenchymal Stem Cells Extract (MSCsE)-Based Therapy Alleviates Xerostomia and Keratoconjunctivitis Sicca in Sjogren's Syndrome-Like Disease

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Abstract: Sjogren's syndrome (SS) is an autoimmune disease that manifests primarily in salivary and lacrimal glands leading to dry mouth and eyes. Unfortunately, there is no cure for SS due to its complex etiopathogenesis. Mesenchymal stem cells (MSCs) were successfully tested for SS, but some risks and limitations remained for their clinical use. This study combined cell- and biologic-based therapies by utilizing the MSCs extract (MSCsE) to treat SS-like disease in NOD mice. We found that MSCsE and MSCs therapies were successful and comparable in preserving salivary and lacrimal glands function in NOD mice when compared to control group. Cells positive for AQP5, AQP4, α-SMA, CK5, and c-Kit were preserved. Gene expression of AQP5, EGF, FGF2, BMP7, LYZ1 and IL-10 were upregulated, and downregulated for TNF-α, TGF-β1, MMP2, CASP3, and IL-1β. The proliferation rate of the glands and serum levels of EGF were also higher. Cornea integrity and epithelial thickness were maintained due to tear flow rate preservation. Peripheral tolerance was re-established, as indicated by lower lymphocytic infiltration and anti-SS-A antibodies, less BAFF secretion, higher serum IL-10 levels and FoxP3$^+$ T_{reg} cells, and selective inhibition of B220$^+$ B cells. These promising results opened new venues for a safer and more convenient combined biologic- and cell-based therapy.

Keywords: Sjogren's syndrome (ss); autoimmune diseases; biologic therapy; bone marrow; cell extract; lacrimal gland; mesenchymal stem cells (MSCs); non-obese diabetic mice (NOD); salivary glands; submandibular glands

1. Introduction

Sjogren's syndrome (SS) is a common progressive autoimmune disease that affects females predominantly [1–3]. The prevalence of SS is variable worldwide; ranging from 0.1% to 0.72% of the population [4–11]. SS progresses slowly and patients exhibit clinical symptoms years after the disease onset [12]. The immune system targets epithelial tissues, infiltrates it with lymphocytes, and later forms autoantibodies against glands antigens [3,13–16]. The aberrant immune dysregulation leads to the destruction of epithelial tissues, especially salivary and lacrimal glands, and to several extra-glandular manifestations. The secretory function of the glands diminishes gradually resulting in dryness of the mouth (xerostomia), eyes (keratoconjunctivitis sicca), and organs containing exocrine glands, such as the nose and vagina [17–20]. The current SS management is symptomatic-based to alleviate the dryness severity and complications [21,22]. However, patients with systemic involvement

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Quantitative Analysis of Protein and Gene Expression in Salivary Glands of Sjogren's-Like Disease NOD Mice Treated by Bone Marrow Soup

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Abstract

Background: Bone marrow cell extract (termed as BM Soup) has been demonstrated to repair irradiated salivary glands (SGs) and restore saliva secretion in our previous study. In the present study, we aim to investigate if the function of damaged SGs in non-obese diabetic (NOD) mice can be restored by BM Soup treatment and the molecular alterations associated with the treatment.

Methods: Whole BM cells were lysed and soluble intracellular contents ("BM Soup") were injected I.V. into NOD mice. Tandem mass tagging with 2-D liquid chromatography-mass spectrometry was used to quantify proteins in the submandibular glands (SMGs) between untreated and BM Soup-treated mice. Quantitative PCR was used to identify genes with altered expression in the treated mice.

Results BM Soup: restored salivary flow rates to normal levels and significantly reduced the focus scores of SMGs in NOD mice. More than 1800 proteins in SMG cells were quantified by the proteomic approach. Many SMG proteins involved in inflammation and apoptosis were found to be down-regulated whereas those involved in salivary gland biology and development/regeneration were up-regulated in the BM Soup-treated mice. qPCR analysis also revealed expression changes of growth factors and cytokines in the SMGs of the treated NOD mice.

Conclusion: BM Soup treatment is effective to restore the function of damaged SGs in NOD mice. Through gene/protein expression analysis, we have found that BM Soup treatment might effectuate via inhibiting apoptosis, focal adhesion and inflammation whereas promoting development, regeneration and differentiation of the SG cells in NOD mice. These findings provide important insights on the potential mechanisms underlying the BM Soup treatment for functional restoration of damaged SGs in NOD mice. Additional studies are needed to further confirm the identified target genes and their related signaling pathways that are responsible for the BM Soup treatment.

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

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Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease, with an estimated prevalence of ~4 million in the US [1,2]. Patients with SS suffer from dry mouth (xerostomia) and eyes (xeropthalmia) caused by lymphocytic infiltration of salivary and lachrymal glands. SS primarily affects women, with a ratio of 9:1 over the occurrence in men. The disease is classified as primary SS when it exists by itself or secondary SS when it is associated with another autoimmune disease such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or systemic sclerosis. In addition, patients with SS have a significant higher risk of developing lymphoma than both healthy population and patients with other autoimmune diseases [3]. The most common form is mucosa-associated lymphoid tissue (MALT) lymphoma that remains localized in the affected salivary glands.

A typical pathological feature of SS is the presence of progressive lymphocytic infiltrates in the exocrine glands, such as salivary and lachrymal glands, where lymphocytes are not normally found [4]. Histopathological examination of the affected major salivary glands in patients with SS reveals a benign lymphoepithelial lesion, which is characterized by lymphocytic replacement of the salivary epithelium and accompanied by the formation of epimyoepithelial islands mainly composed of keratin-containing epithelial cells [5,6]. The predominant cells in the salivary gland infiltrates are T cells, with a ratio of 3–5:1 between CD4+ and CD8+ phenotypes. SS is also associated with B cell hyperactivity as manifested by the production of autoantibodies,

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Gautham Sivamurthy

Current Applications and Future Prospects of Stem Cells in Dentistry

Abstract: Stem cells are defined as clonogenic, unspecialized cells capable of both self-renewal and multi-lineage differentiation, contributing to regenerating specific tissues. For years, restorative treatments have exploited the lifelong regenerative potential of dental pulp stem cells to give rise to tertiary dentine, which is therapeutically employed for direct and indirect pulp capping. Current applications of stem cells in endodontic research have revealed their potential to continue root development in necrotic immature teeth and transplanted/replanted teeth. Successful application of pulp revascularization is highlighted here with support of a clinical case report. This article also discusses the role of dental stem cells as a promising tool for regeneration of individual tissue types like dentine, pulp and even an entire functional tooth.

CPD/Clinical Relevance: This article will help practising dental surgeons understand the significance of stem cells in dentistry. Clinicians can harness the potential of stem cells using procedures like pulp regeneration/revascularization in endodontics and improve their knowledge on the recent advances in tissue engineering and future applications of dental-derived stem cells.

Dental Update 2015; 42: 556–561

Stem cells are a unique type of cell that have a specialized capacity for self-renewal and potency, which can give rise to one and sometimes many different cell types. They aid in the replacement of cells that are lost through normal wear, injury or disease.[1] Stem cells can be broadly divided into:

- Embryonic stem cells; and
- Post-natal stem cells.

Post-natal stem cells are multipotent stem cells and have been harvested from different kinds of tissues like bone marrow, umbilical cord, amniotic fluid, brain tissue, liver, pancreas, cornea, dental pulp, and adipose tissue. These stem cells are comparatively easier to isolate, do not have any ethical issues and are commonly used in current day practice.[2]

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Source of stem cells

The oral and maxillofacial region can be treated with stem cells from the following sources:

- Bone marrow stem cells (BMSCs);
- Stem cells from the oral and maxillofacial region.

Bone marrow stem cells (BMSCs)

Bone marrow stem cells (BMSCs) can be harvested from sternum or iliac crest and are composed of both hematopoietic stem cells and mesenchymal stem cells (MSCs). The majority of oro-maxillofacial oral structures are formed from mesenchymal cells. BMSCs exhibit the ability to generate osteoid and odontoid structures and have demonstrated good ability to form tooth-supporting periodontal structures like cementum, periodontal ligament (PDL) and alveolar bone, suggesting their potential use for treating periodontal diseases.[3]

Stem cells from oral and maxillofacial region

In the oral and maxillofacial area, different types of dental stem cells have been isolated and characterized and they include:

- Dental pulp stem cells (DPSCs);
- Stem cells from exfoliated deciduous teeth (SHEDs);
- Stem cells from apical papilla (SCAPs);

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REVIEW

Stem cells and tooth tissue engineering

Amanda H.-H. Yen · Paul T. Sharpe

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Abstract The notion that teeth contain stem cells is based on the well-known repairing ability of dentin after injury. Dental stem cells have been isolated according to their anatomical locations, colony-forming ability, expression of stem cell markers, and regeneration of pulp/dentin structures in vivo. These dental-derived stem cells are currently under increasing investigation as sources for tooth regeneration and repair. Further attempts with bone marrow mesenchymal stem cells and embryonic stem cells have demonstrated the possibility of creating teeth from non-dental stem cells by imitating embryonic development mechanisms. Although, as in tissue engineering of other organs, many challenges remain, stem-cell-based tissue engineering of teeth could be a choice for the replacement of missing teeth in the future.

Keywords Teeth · Stem cells · Tissue engineering · Odontogenesis · Epithelial-mesenchymal interaction

Introduction

Teeth are structures that develop on the maxilla and mandible of mammals and serve eating, defence and phonetic purposes. Although the morphology of teeth varies depending on species and location, they are similar in structure, being composed of enamel, dentin, pulp and periodontium. Tooth development is characterized by a series of reciprocal epithelial-mesenchymal interactions that result in differentiation and the spatial organization of cells to form organs (Fleischmajer 1967; Thesleff 2003). Since gene expression comparisons during teeth development have shown only slight differences between human and mouse teeth, mice have been used as the major animal model for studying tooth development. Human genetic diseases that encompass loss of teeth also contribute to our understanding of tooth formation (Lin et al. 2007; Tucker and Sharpe 2004).

Modern dentistry for replacing missing teeth utilizes metal implants capped with a ceramic crown (Crubezy et al. 1998). Although these prostheses serve the purpose, factors that interfere with ossointegration may cause surgery failure (Esposito et al. 1998). With advances in stem cell biology and emerging concepts of tissue engineering (Langer and Vacanti 1993), biological teeth (Sharpe and Young 2005) may become an alternative for replacing missing teeth. The idea is to cultivate stem cells with odontogenic induction signals through epithelial-mesenchymal interactions, thereby programming the stem cells to adopt dental lineages and, with the help of scaffold/extracellular matrix, to become part of a tooth.

Inspiration from tissue regeneration

The current concept of tooth tissue engineering is inherent in the belief that naturally occurring tissue regeneration can be reproduced in vitro. Experiments over 100 years ago by H. V. Wilson (Wilson 1907), showed that isolated sponge cells could reaggreagate to reform a functional sponge. Even in vertebrates, embryonic cells dissociated from animal tissues can autonomously aggregate and reassemble to reconstitute organs (Weiss and Taylor 1960). When tissues that normally form a particular structure are combined, they can rearrange to form a topographically correct resemblance

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Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine

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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.

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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 vivo* [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

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STEM-CELL BIOLOGY FOR TOOTH AND PERIODONTAL REGENERATION (M BARTOLD, SECTION EDITOR)

Dental Stem Cells: Sources and Potential Applications

Su-Min Lee · Qunzhou Zhang · Anh D. Le

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Abstract In recent years, stem cell research in dentistry has grown rapidly with the potential application for oral and maxillofacial tissue regeneration. Mesenchymal stem cells (MSCs) from the oral and maxillofacial region are easy to access, have a high proliferation rate, multipotency, and potent immunomodulatory functions. They are excellent cell sources not only for stem cell-based therapy of dental and craniofacial diseases, but also with the potential for the treatment of other inflammatory diseases. In this review, we provide an overview of different types of MSCs that have been isolated and characterized from several origins such as dental pulp, exfoliated deciduous teeth, the periodontal ligament, the dental follicle, the dental papilla, oral mucosa, and gingiva, with the focus on the potential clinical applications for each type of dental stem cell.

Keywords MSCs Mesenchymal Stem Cells · DSCs Dental Stem Cells · DPSCs Dental Pulp Stem Cells · SHED Stem Cells From Human Exfoliated Deciduous Teeth · SCAP Stem Cells From Root Apical Papilla · PDLSCs Periodontal Ligament Stem Cells · DFSCs Dental Follicle Stem Cells · GMSCs Gingiva-Derived Mesenchymal Stem/Stromal Cells · Multipotency · Immunomodulation · Inflammatory Disease

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Introduction

Stem cells are defined as a special group of clonogenic cells that are characterized by the ability for self-renewal and multilineage differentiation. These cells are responsible for normal tissue renewal as well as for healing and regeneration after injuries [1]. Stem cells can be divided into three main types, embryonic stem cells (ESCs), adult or postnatal stem cells (ASCs), and induced pluripotent stem cells (iPSCs). ESCs are found in the inner cell mass of mammalian blastocysts during the early stages of embryo development and have unlimited expansion and pluripotency to differentiate into all types of somatic cells [2]. A new source of pluripotent stem cells, iPSCs, have been innovatively generated from human somatic cells and potentially can provide tremendous opportunities for tissue regeneration and patient-specific therapies, while avoiding ethical issues surrounding the use of human embryos and tumorigenic potential [3, 4]. ASCs have been identified in almost all the postnatal tissues. Because of their easy accessibility and great ability to generate a tissue different to the site from their origin, ASCs have increasing potential to be used for treatment of degenerative diseases [5]. Mesenchymal stem cells (MSCs) are multipotent progenitor cells present in many tissues throughout the body and have the capacity to differentiate into bone, cartilage, tendon, fat, and muscle [6]. Furthermore, MSCs are important immunoregulatory cells because they can sense and control inflammation in injured tissues by expressing a variety of chemokines and cytokines [7]. To date, different types of MSCs have also been isolated and characterized from oral and maxillofacial regions. The objective of this review is to describe new findings in the field of dental stem cell research and their potential applications in tissue regeneration and therapy of inflammation-related diseases.

Dental Stem Cells (DSCs)

Sources of Stem Cells from the Oral-Maxillofacial Regions

Several types of dental MSCs have been identified in different dental tissues, including: 1) the dental pulp of permanent teeth

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STEM CELLS
TISSUE-SPECIFIC STEM CELLS

Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E_2-Dependent Mechanisms

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Key Words. Human gingival • Stromal cells • Immunomodulation • Contact hypersensitivity • Mast cells

ABSTRACT

The immunomodulatory and anti-inflammatory functions of mesenchymal stromal cells (MSCs) have been demonstrated in several autoimmune/inflammatory disease models, but their contribution to the mitigation of contact hypersensitivity (CHS) remains unclear. Here, we report a new immunological approach using human gingiva-derived MSCs (GMSCs) to desensitize and suppress CHS and the underlying mechanisms. Our results showed that systemic infusion of GMSCs before the sensitization and challenge phase dramatically suppress CHS, manifested as a decreased infiltration of dendritic cells (DCs), $CD8^+$ T cells, T_H-17 and mast cells (MCs), a suppression of a variety of inflammatory cytokines, and a reciprocal increased infiltration of regulatory T cells and expression of IL-10 at the regional lymph nodes and the allergic contact areas. The GMSC-mediated immunosuppressive effects and mitigation of CHS were significantly abrogated on pretreatment with indomethacin, an inhibitor of cyclooxygenases. Under coculture condition of direct cell-cell contact or via transwell system, GMSCs were capable of direct suppression of differentiation of DCs and phorbol 12-myristate 13-acetate-stimulated activation of MCs, whereas the inhibitory effects were attenuated by indomethacin. Mechanistically, GMSC-induced blockage of de novo synthesis of proinflammatory cytokines by MCs is mediated partly by the tumor necrosis factor-alpha/prostaglandin E_2 (PGE_2) feedback axis. These results demonstrate that GMSCs are capable of desensitizing allergic contact dermatitis via PGE_2-dependent mechanisms. STEM CELLS *2011;29:1849–1860*

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

INTRODUCTION

Mesenchymal stromal cells (MSCs), previously referred to as mesenchymal stem cells, possess multifunctional properties from tissue repair/regeneration to immunomodulatory functions [1–3]. An increasing body of evidence indicates that MSCs can home and engraft at the injured site and promote tissue repair through a combined downregulation of proinflammatory cytokines and increased production of growth factors, antioxidants, and soluble factors with anti-inflammatory functions [4, 5]. Studies have shown that MSCs exert their immunosuppressive functions via inhibiting the proliferation and activation of different subtypes of effector T cells, natural killer (NK) cells, dendritic cells (DCs), and macrophages or via promoting the differentiation of regulatory T cells (Tregs) [6–10]. These unique properties render MSC a potential novel immunotherapeutic tool for a variety of autoimmune and inflammation-related diseases [3, 4]. Recent studies have reported that MSCs can also suppress allergic responses and chronic inflammation in experimental mouse models of ragweed- and ovalbumin-induced asthma [11, 12] and allergic rhinitis [13]. However, the underlying mechanism of MSC-mediated attenuation of allergic response still remains elusive.

The hapten-induced murine contact hypersensitivity (CHS) is an experimental model for human allergic contact dermatitis (ACD), one of the prevalent skin diseases worldwide with significant economic burden [14, 15]. Comparable to the pathophysiology of human ACD, the murine CHS model comprises three phases: the sensitization phase (also termed as the afferent or induction phase), the elicitation or challenge phase, and the resolution/regulation phase [14]. The sensitization phase is initiated immediately after the first exposure of skin to haptens, during which dermal DCs uptake and process antigens and then migrate to regional draining lymph nodes (dLNs), where they stimulate the differentiation and activation of allergen-specific T lymphocytes, including $CD8^+$ cytotoxic and $CD4^+$ T helper cells. In the elicitation phase, allergen-specific effector $CD8^+$ and $CD4^+$ T cells and various types of innate immune cells, particularly mast cells

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Characterization of Mesenchymal Stem Cells From Human Normal and Hyperplastic Gingiva

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Human gingiva plays an important role in the maintenance of oral health and shows unique fetal-like scarless healing process after wounding. Here we isolate and characterize mesenchymal stem cells from human normal and hyperplastic gingival tissues (N-GMSC and H-GMSC, respectively). Immunocytochemical staining indicated that gingival lamina propria contained Stro-1 and SSEA-4 positive cells, implying existence of putative gingival MSC. Under attachment-based isolating and culturing condition, gingival MSC displayed highly clonogenic and long-term proliferative capability. By using single colony isolation and expansion approaches, we found both N-GMSC and H-GMSC possessed self-renewal and multipotent differentiation properties. N-GMSC and H-GMSC showed distinct immunoregulatory functions in a murine skin allograft setting via up-regulation of putative systemic regulatory T cells (Tregs). N-GMSC and H-GMSC were capable of regenerating collagenous tissue following in vivo transplantation, in which H-GMSC exhibited more robust regenerative capability. These findings suggest that gingival tissue contains tissue-specific mesenchymal stem cell population and is an ideal resource for immunoregulatory therapy due to its substantial availability and accessibility. In addition, gingival MSC over-activation may contribute to gingival hyperplastic phenotype.

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Gingiva surrounds the teeth and acts as mucosal barrier separating periodontium and outer space. Histologically and functionally, gingiva belongs to masticatory mucosa, which is defined by its ability to resist the friction of food during chewing and is distinguishable from lining mucosa of cheeks and inner lips. Gingival tissue is composed of an epithelial layer and underlying lamina propria, with the latter developing from the cranial neural crest as other craniofacial tissues of mesenchymal origin including dental papilla and periodontium (Palmer and Lubbock, 1995). To date, multiple types of cranial neural crest-derived mesenchymal stem cells (MSC) have been identified and proved not only to play essential role in local homeostasis but also to hold promise for regenerative therapy (Gronthos et al., 2000; Miura et al., 2003; Seo et al., 2004; Sonoyama et al., 2006; Fujii et al., 2008). However, despite several indications for identification of gingival epithelial stem/progenitor cells (Kirschner et al., 2006), little attention was directed towards demonstrating the MSC identity within human gingival propria.

Several lines of evidence have provided cues for imagination of presence of gingival mesenchymal stem cells, which in almost all the current studies are generally indistinguishable from gingival fibroblasts. Human gingival fibroblasts were demonstrated to express stem cell factor (SCF) and its receptor c-kit (Gagari et al., 2006), which were reported to be expressed by multiple stem cells (Dooner et al., 2008; Di Felice et al., 2009). While embryo-like gingival healing is characterized by the absence of fibrosis or irreversible elastin/collagen degradation, the paracrine effect of gingival fibroblasts results in the preservation of elastic fiber and decrease in MMP-1, -3, and -9 production contributing to diminish the degradation of artery when cocultured with human aortic smooth muscle cells (Naveau et al., 2007). A graft composed of fibronectin matrix-based multilayered cell sheets of human gingival fibroblasts modified to express alkaline phosphatase (ALP) supported alveolar bone regeneration and exhibited potential for periodontal tissue engineering applications (Nakajima et al., 2008). Mechanical stimulation up-regulates bone-related protein expression and accelerates osteoprotegerin production by gingival fibroblasts to suppress osteoclastogenesis (Kook et al., 2009). These findings hint that human gingival fibroblasts have unique features and ability of undergoing induction-directed differentiation and regeneration.

Recently, the isolation of mesenchymal stem cell population from human gingival tissue (GMSC) has been reported (Zhang et al., 2009). These GMSC showed characteristics with of self-renewal, multipotent differentiation, and immunomodulatory abilities both in vitro and in vivo. Similarly, a new population of gingival fibroblasts was reported to display the characteristics of true progenitor, including forming CFU-F at low cell density and undergoing multilineage differentiation in vitro and in vivo (Fournier et al., 2010). Today various types of adult MSC have been characterized and exploited for therapeutic utility. Previous findings suggested that by using attachment-based isolating and culturing protocol, MSC could be acquired from nearly all adult organs and tissues irrespective or their embryonic origin (da Silva Meirelles et al., 2006; Valtieri and

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