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Dental regeneration via bioengineering: systematic review.

Presentado por: Daria Proshchenkova

Tutor: Santiago Peydró Tomás

Campus de Valencia
Paseo de la Alameda, 7
46010 Valencia
universidadeuropea.com

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1. RESUMEN

Introducción: Esta revisión sistemática explora las técnicas de bioingeniería que utilizan células madre (CM) para la regeneración dental, destacando metodologías actuales, aplicaciones clínicas potenciales y perspectivas futuras en la ingeniería de tejidos dentales, comparando la efectividad de diferentes materiales de andamiaje y CM de diversos orígenes.

Material y Métodos: Se realizó una búsqueda electrónica en las bases de datos PubMed, Scopus y Web of Science sobre la regeneración dental utilizando andamios naturales y sintéticos, y células madre de la pulpa dental (CMPD) u otras CM hasta enero de 2024.

Resultados: De los 251 artículos obtenidos en la búsqueda inicial, se eligieron 10 para incluir en la revisión sistemática, cumpliendo con los criterios de inclusión y exclusión. En el grupo de andamios naturales, los porcentajes promedio de esmalte, dentina, cemento y odontoblastos regenerados fueron 15%, 23.6%, 17.4% y 23.6%, respectivamente. En el grupo de andamios sintéticos, los valores fueron 43.4%, 61.8%, 38.7% y 61.8%. En términos de regeneración de estructuras de corona, raíz y pulpa, el grupo de andamios naturales mostró 20.5%, 8.7%, 23.6%; el grupo de andamios sintéticos: 53.2%, 18.8% y 71.9%. El grupo de CMPD regeneró 35.8% (esmalte), 36.3% (dentina), 32.8% (cemento) y 47% (odontoblastos), mientras que el grupo de CM de otro origen regeneró 34.9%, 53.3%, 18.7% y 53.3%. Para la regeneración de partes dentales, el grupo de CMPD presentó 38.9% (corona), 10.4% (raíz), 41% (pulpa), mientras que el grupo de otras CM mostró 17.4%, 11.8% y 36.1%.

Conclusiones: Todas las técnicas analizadas condujeron a la regeneración de los cuatro tipos de tejidos dentales, pero el uso de andamios sintéticos y CMPD proporcionó estructuras más similares a un diente natural. Todos los andamios apoyaron la viabilidad y diferenciación de las células madre, excepto el PLA y sus copolímeros en su forma pura, que dificultaron el potencial completo de diferenciación. Los CMPD demostraron el mayor potencial de regeneración de tejidos dentales. Los estudios que utilizaron agentes bioactivos lograron buenos resultados, pero se necesita más investigación para aclarar cómo afectan el proceso de regeneración a lo largo de períodos más prolongados.

2. ABSTRACT

Introduction: This review explores the bioengineering techniques that use stem cells (SC) for tooth regeneration, highlighting current methodologies, potential clinical applications, and future prospects in dental tissue engineering, while comparing the effectiveness of different scaffold materials and SC of various origins.

Material and Methods: An electronic search was performed in PubMed, Scopus, and Web of Science databases on tooth regeneration using natural and synthetic scaffolds, and dental pulp SC (DPSC) or other SC until January 2024.

Results: From 251 articles obtained from the initial search, 10 were chosen to be included, complying with inclusion and exclusion criteria. In the natural scaffold group, the average percentages of regenerated enamel, dentin, cementum, and odontoblast-like cells were 15%, 23.6%, 17.4%, 23.6%. In the synthetic scaffold group, the values were 43.4%, 61.8%, 38.7%, and 61.8%. For regeneration of distinguishable crown, root and pulp structures, the natural scaffold group showed the following results – 20.5%, 8.7%, 23.6%; the synthetic scaffold group – 53.2%, 18.8%, and 71.9%. The group of DPSC regenerated 35.8% (enamel), 36.3% (dentin), 32.8% (cementum), and 47% (odontoblasts), while the group of SC of other origin regenerated 34.9%, 53.3%, 18.7%, and 53.3%. For the regeneration of dental parts, the DPSC group presented 38.9% (crown), 10.4% (root), 41% (pulp), while the other SC group showed 17.4%, 11.8%, and 36.1%.

Conclusions: All analysed techniques led to the regeneration of all 4 types of dental tissues, but using synthetic scaffolds and DPSC provided structures more similar to a natural tooth. All scaffolds supported stem cell viability and differentiation, except for PLA and its copolymers in their pure form, which hindered the full differentiation potential. DPSC demonstrated the highest regeneration potential of dental tissues. The studies utilizing bioactive agents achieved good results but further research is necessary to clarify how they affect the process of regeneration over longer periods of time.

3. KEYWORDS

I. Bioengineering
II. Tissue regeneration
III. Tooth regeneration
IV. Scaffolds
V. Stem cells
VI. Bioactive agents
VII. Dental pulp stem cells
VIII. Natural scaffolds
IX. Synthetic scaffolds
X. Cell differentiation
XI. Cell viability
XII. Cell proliferation
XIII. Tissue engineering
XIV. Cell-tissue recombination
XV. Odontogenesis

4. INTRODUCTION

4.1. Function and importance of teeth

Teeth are a very important part of the stomatognathic system with many functions. One primary function of teeth is their pivotal role in effective chewing, aiding in the breakdown of food into digestible particles and facilitating optimal nutrient absorption during digestion. Moreover, teeth play a crucial role in speech articulation. Properly aligned teeth contribute to clear pronunciation, enabling individuals to communicate effectively and participate fully in social interactions. Beyond functionality, teeth contribute significantly to facial aesthetics, influencing an individual's appearance and self-esteem (1).

The loss of tooth structure partially or completely is a very serious issue among people of all ages and ethnicities. It can result in social anxiety, self-consciousness, and a diminished quality of life. The loss can be caused by various factors, such as traumatisms, incorrect occlusion, caries, genetic conditions, etc. Successful tooth reconstruction or replacement not only restores oral function but also enhances overall facial aesthetics, positively impacting self-confidence and social well-being (2).

The most common methods in dentistry today to achieve this goal are removable dental prostheses, fixed dental prostheses (bridges) and implants. However, these therapies have certain disadvantages. For example, denture therapy is frequently associated with denture-induced stomatitis, soft tissue hyperplasia, traumatic ulcers, altered taste perception and burning mouth syndrome. While implants have a direct connection with bone, they still lack periodontium and cementum tissues present in naturally formed teeth. Therefore, they are unable to cushion and modulate the mechanical stress of mastication. In order to find a new therapeutic approach that would allow us to avoid these issues while achieving the same outcomes, new methods of tooth regeneration via bioengineering are being explored (3).

4.2. Characteristics of human dentition

Unfortunately for our species, we only possess two sets of dentition (deciduous and permanent), labelling us diphyodonts, while some other species,

such as sharks and crocodiles, are polyphyodonts and continuously replace/regenerate their teeth throughout life (4). It remains unknown as to why the majority of mammals do not possess this quality.

Another characteristic of human dentition is the low crown/root ratio (brachydont), common to many omnivore species. Mice and rats present brachydont molars that do not exhibit extra crown growth upon eruption in the oral cavity (5). These similarities with humans and the availability of rodents as animals used in scientific research made them the prime animal models used for research on the molecular mechanisms responsible for tooth development (1).

4.3. Odontogenesis

Tooth development, also referred to as odontogenesis, is a complex process of interaction between dental epithelial and ectomesenchymal cells. A thorough understanding of this process is of utmost importance to achieve complete tooth regeneration using stem cells, as scientists will essentially be aiming to replicate the whole process (1).

Odontogenesis consists of different stages. In the beginning, oral epithelium thickens and forms a dental placode, which then, proliferates and invaginates into the ectomesenchyme layer derived from neural crest, forming dental lamina. This interaction induces mesenchyme to condense around the epithelium (5). Right before the transition from the bud to the cap stage of odontogenesis, a signalling center forms at the tip of the bud – primary enamel knot (EK). It releases various signalling molecules, such as Shh (sonic hedgehog), BMP (bone morphogenetic protein), FGF (fibroblast growth factor), and Wnt (wingless/integration 1) (6). These signalling pathways play an important role in the proliferation and folding of the dental epithelium into cusps. In monocuspid teeth (incisors and canines) there is only one EK formed, while in multicuspid molars, the formation of secondary EKs can be observed. Their numbers and positions correspond to those of future tooth cusps (7).

The following stage is called the bell stage, where continuous folding divides the dental epithelium into inner and outer enamel epithelium (IEE and OEE). The dental papilla is created by mesenchymal cells adjacent to the IEE, while the dental follicle is formed by cells surrounding the OEE. Additionally, distal

dental epithelial tissues penetrate into the underlying dental papilla, resulting in the development of cervical loops (CLs) on both sides of the secondary EKs. The cells surrounding the OEE and IEE in this context are referred to as the stellate reticulum (SR) (8).

These initial odontogenic stages play a crucial role in shaping the ultimate dental morphology, as this is when the shape and size of the teeth are established. In the later odontogenic stages, dental epithelial cells undergo differentiation, giving rise to ameloblasts responsible for enamel deposition, while dental mesenchymal cells differentiate into odontoblasts responsible for dentin formation. Concurrently, cementoblasts (cells that produce cementum on the outer surface of the dental root) and fibroblasts (cells producing periodontal ligament fibers that attach teeth to the alveolar bone around them), also undergo differentiation during these stages from the dental mesenchymal cells of the dental follicle (9).

The exact mechanisms and signaling molecules responsible for the patterning of the number and size of teeth and their cusps in the process of odontogenesis remain unknown. However, it has been suggested that the tooth size and cusp size are determined by the dental mesenchyme and dental epithelium respectively and co-regulate cusp number by the epithelial–mesenchymal interactions. The quantity of teeth and cusps can be conceptualised as a reaction-diffusion mechanism. Within this mechanism, pivotal molecules, including activators and inhibitors, play a crucial role in influencing the micro (cusp size and number) and macro (tooth size and number) patterns of teeth. Understanding these fundamental molecules is essential for fine-tuning the dimensions of both individual teeth and their cusps in the prospective development of bioengineered teeth (10).

4.4. Dental regeneration via bioengineering

As previously mentioned, several new approaches to regenerate an entire tooth have been proposed and are being investigated by the field of biological tissue engineering. These are dental tissue-engineering scaffolds, stimulation of third dentition formation, cell-tissue recombination, chimeric tooth tissue

engineering and gene-manipulated tooth regeneration. However, the two major ones are cell-tissue recombination and the use of scaffolds (2,11).

The dental cell-tissue recombination approach is based on using a tooth germ, preferably at the cap stage, where dental epithelial and mesenchymal cells are both present. The epithelial and mesenchymal tissues are isolated and completely dissociated into single cells. The bioengineered tooth germ then gets reconstituted using these dissociated cells. The newly recombined tooth germ is implanted into the defect site in the jaw, where it develops into a complete tooth, with enamel, dentin, pulp, and root (12).

In the tissue engineering approach based on the use of scaffolds, stem cells are seeded in/onto a scaffold, where they then proliferate and differentiate into other cell types (13). This structure is supplied with bio-active agents, e.g. growth factors, that control the spatial and temporal organization of dental progenitor cell proliferation, differentiation and function (2). Each of the main three elements involved in this method has many variations that inevitably affect the final result.

4.4.1. Scaffolds

In this way, the choice of scaffold is very important, as its physical aspects and composition must guarantee physical support for the development of new tissues in a manner that mimics the function of the natural extracellular matrix (ECM) (14). The design of the scaffolds must prioritize mechanical integrity and functionality, and the surface of the scaffold should possess suitable properties to facilitate proper cell adhesion, proliferation, and differentiation. Notably, the rigidity of the scaffold substrate significantly influences cell fate, potentially promoting enhanced cell spreading (15).

Nowadays there are various materials available for the production of scaffolds. They can be divided into biological polymer scaffolds (fibrin, collagen), scaffolds from decellularized tissues, ceramic scaffolds (hydroxyapatite), and artificial polymer scaffolds (poly(lactic-co-glycolic acid) or PLGA, polycaprolactone or PCL).

The fibrin hydrogel is formed through the polymerization of purified allogeneic fibrinogen using purified thrombin. It has found extensive applications

as scaffolds in the regeneration of various tissues, including cardiovascular tissue, bone, neural tissues, cartilage, and more (16). The fibrin hydrogel offers several benefits, including a controllable degradation rate, low immunogenicity, and the ability to facilitate relatively even cell distribution during cell seeding and polymerization. Despite these advantages, it does exhibit drawbacks such as shrinkage and low mechanical stiffness in its properties (17).

Collagen, a key extracellular matrix component found in various tissues, is commonly used in tissue engineering, particularly collagen type I. Allogeneic collagen, such as bovine collagen sponge or gel, shows excellent biocompatibility but has modest physical strength (18). To enhance strength, chemical cross-linking with agents like glutaraldehyde can be employed, although it may compromise the biocompatibility of the material. Collagen's mechanical properties can also be improved by creating hybrid scaffolds with materials like β-TCP/polyethylene and hydroxyapatite, similar to strategies used with fibrin (14,19).

Decellularization is a technique used to eliminate cellular components from tissues/organs, reducing foreign body reactions, inflammation, and potential immune rejection, while creating instructing extracellular matrix templates. The construction of natural tooth bud ECM scaffolds involves the use of decellularized post-natal tooth buds (dTB) to guide the formation of bioengineered teeth with specific size and shape (20). This strategy is based on prior research demonstrating the safe application of gentle decellularization processes, removing immunogenic components from whole organs and tissues while preserving the natural ECM and its signalling elements. The goal of these decellularized scaffolds is to maintain structure, shape compatibility, mechanical integrity, and gradients of bioactive molecules to facilitate cell interaction, adhesion, and ECM formation in the process of tooth development (2,21).

Hydroxyapatite is a calcium phosphate ceramic, found naturally in human body as part of bone, dentin, and enamel. It is bio-compatible, nontoxic, biodegradable and has the ability to form mineralized tissues. This bioceramic has several biomedical applications, but it has been more used in bone regeneration because it can be fabricated with a 3D structure similar to the trabecular bone. Unfortunately, due to its brittleness, it cannot be used as a replacement for highly load-bearing bone (22,23).

Poly(lactic-co-glycolic acid) and polycaprolactone are both synthetic polymers that have superior mechanical integrity and machinability in comparison with biological materials. PLGA can be obtained in different molecular weights and copolymer ratios, which allows for adjusting its final behavior for the required use. This material can be processed into any shape and size, has great water solubility and allows for a tunable drug release (24). A new method of creating PLGA scaffolds using CO_2 as a solvent has been reported to make a net-shaped porous scaffold in a few minutes. The obtained scaffolds had a high degree of porosity and interconnectivity, which is essential for teeth regeneration (25). PCL is another polymer frequently used as a scaffold material due to its biocompatibility, low immunogenicity and optimal degradation. In addition to that, it can adapt well with other synthetic and natural polymers, obtaining a scaffold with desirable characteristics (26).

4.4.2. Stem cells

Stem cells (SC) are cells that present two distinctive characteristics: continuous self-renewal, and possible differentiation into multiple specialised cell types (27). As previously explained, in the process of odontogenesis two different types of stem cells are involved: dental epithelial cells that later give rise to enamel, and ectomesenchymal cells responsible for the production of dentin, pulp, cementum and periodontal ligament. It must be emphasised once again that ameloblasts which differentiate from dental epithelial cells come from the ectoderm of the oral cavity, while all other dental cells are derived from the neural crest ectomesenchyme. Essentially, the cells derived from these two different embryological origins interact with each other and initiate the process of odontogenesis (28).

Dental epithelial cells can only be directly obtained from the embryo, as once they differentiate into ameloblasts to produce enamel, they undergo apoptosis (programmed cell death). Even though embryonic stem cells (ESC) have the ability to differentiate into hundreds of other cell types, research involving human embryos has been impeded due to bioethical considerations (29).

In order to overcome this issue, scientists have developed a new type of stem cells – induced pluripotent stem cells (iPS). They are essentially differentiated cells that have been experimentally reprogrammed to an embryonic stem cell-like state. It has been reported that iPS cells can differentiate into different cell types, such as neurons, cardiac myocytes, and renal lineage cells, under appropriate conditions (30). They can potentially be used to obtain dental epithelial cells. In the study published in 2010, the authors have proved that iPS can be obtained from mesenchymal dental stem cells, such as stem cells isolated from the remnant pulp of exfoliated deciduous teeth (SHED), stem cells isolated from the tissue at the apex of the root of developing teeth – apical papilla (SCAP), and dental pulp stem cells (DPSCs) (31).

Fortunately, dental mesenchymal stem cells are much easier to obtain. Furthermore, there are many different kinds available. In addition to the three previously mentioned ones, there are periodontal ligament SCs (PDLSCs, that present different degrees of commitment to either fibroblastic or cementoblastic/osteoblastic lineages), dental follicle progenitor cells (DFPCs, which form the 3 tissues of periodontium: cementum, periodontal ligament, and alveolar bone), and periodontal ligament of deciduous teeth SCs (DePDL) (32). DPSCs, SHED and SCAP were able to produce dentin or dentin-like tissue when transplanted *in vivo*. While DFPCs and PDLSCs could produce cementum-like tissue and periodontal ligament respectively. All these types of SC differ in their clonogenicity, proliferative ability, and differentiation potential *in vitro* and *in vivo* (27). Therefore, it is still unknown which one of them is a more suitable choice to use in tooth regeneration.

4.4.3. Bioactive agents

As previously mentioned, multiple bioactive agents, including growth and transcription factors from several signalling families, are involved in the process of regulating odontogenesis throughout all of its stages. There have been identified at least 12 transcription factors in odontogenic mesenchyme and more than 200 genes in the oral epithelium, dental epithelium and dental mesenchyme in the initial stage of odontogenesis (28,33). Therefore, it is believed that the

controlled release of selected bio-active agents from biodegradable scaffolds is capable of enhancing the efficacy of tooth regeneration via bioengineering.

The primary bioactive agents commonly employed are growth factors (GFs), crucial for the development, maturation, maintenance, and repair of craniofacial and dental tissues. They play a vital role in establishing communication between cells and tissues (28). GFs, recognised for their involvement in cell migration, differentiation, proliferation, gene expression, and the organization of functional tissues, function by binding to the extracellular domain of target GF receptors, thus activating intracellular signal transduction pathways (34).

Numerous GFs, including bone morphogenetic protein (BMP), transforming growth factors (TGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), are expressed during tooth formation and repair. Consequently, incorporating a diverse range of GFs into scaffolds to locally regulate fundamental cellular functions can potentially facilitate tooth regeneration. The main challenges in dental tissue engineering involve ensuring long-term stability, appropriate dosage, and the precise regulation of multiple GF gradients in a specific spatiotemporal pattern (35).

4.5. Challenges in tooth regeneration

One of the most important challenges faced in the area of bioengineering in their quest to achieve the regeneration of a whole tooth is the limited number of sources of human dental epithelial stem cells. One of the reasons for this is that dental epithelial cells undergo apoptosis after enamel formation is completed and therefore, they are absent in erupted teeth. In this way, the only available sources are unerupted wisdom or impacted teeth, which can be obtained only from children or young adults (3). The other reason is the difficulty of ex vivo dental epithelial cell expansion in culture in comparison to mesenchymal cells. The most promising solution to this challenge is autologous iPS cells, which could then be differentiated into DE cells (36).

Another important issue that still has not been resolved is the unpredictable final shape and size of a tooth obtained via bioengineering. It is essential to have teeth with the correct size, shape and cusp morphology in order

to achieve proper occlusion and function. Even though certain growth factors have been identified, the specific ways, in which they must be manipulated to achieve the precise shape and size of bioengineered teeth, remain unknown (10,36).

5. JUSTIFICATION AND HYPOTHESIS

JUSTIFICATION

As we know, one of the most important ways our body can stay healthy is through a healthy and well-balanced diet. This is the only way we obtain the necessary macro- and micro-nutrients required for proper growth, cognitive development, immune system support, disease prevention and overall life sustainability. Unfortunately, some people are unable to obtain this proper nutrition, either due to the lack of it, or the lack of means to consume it i.e. functional stomatognathic system. The part of the system that is most frequently compromised is the teeth. There are various ways nowadays that missing teeth can be restored: prosthetic restorations, such as removable prostheses, bridges, and implants (1).

However, these restorations occasionally lead to various complications. In the case of implants, that could be periimplantitis, nerve damage, and hypersensitivity to implant metal, which could lead to the body rejecting the implant completely (37). As for the removable prosthesis, some of the more common complications are denture stomatitis due to maladapted dentures, periodontal disease, and caries that could lead to the loss of the remaining teeth (3).

These complications would not be an issue if we use natural dental implants – teeth created via bioengineering using a person's own stem cells. There would be no problems with biocompatibility, nor with nerve/surrounding tissue damage upon implantation procedure. Granted that this method is still in development, we can already consider it as an alternative technique used for replacing missing dental pieces in the foreseeable future.

With the increased progress in the field of bioengineering, the number of systematic reviews discussing the use of stem cells in dentistry has increased significantly over the past decade. However, the majority of these reviews either talk about pulp or periodontal ligament regeneration, without touching upon whole tooth regeneration, or if they do discuss it, there is no comparison between specific techniques, only overall descriptions of different approaches.

This work will focus on identifying the most effective technique for the regeneration of a whole tooth, which could lead to eventually achieving the

ultimate goal of a natural implant restoration faster, leading to a healthy and functional stomatognathic system capable of proper mastication of all types of nutrition. Therefore, the objective most related to the current work is Sustainable Development Goal #3 – Good health and wellbeing.

HYPOTHESIS

The use of natural scaffolds and dental pulp stem cells in a complete tooth regeneration from a dental germ produced by tissue bioengineering will be more effective in providing a dental structure with distinguishable crown, root and pulp structures and presenting enamel-like tissues, dentin, cementum, odontoblast-like cells than synthetic scaffolds and stem cells from another origin.

6. OBJECTIVES

Principal objective

To identify the most effective technique in the regeneration of a complete tooth from a dental germ produced by tissue bioengineering that will provide enamel-like tissues, dentin, cementum, and odontoblast-like cells, and present distinguishable crown, root and pulp structures.

Secondary objectives:

- To compare the effect of different scaffolds on cell viability and differentiation.
- To investigate the type of stem cells that show the most proliferation and differentiation.
- To investigate which bioactive agents can improve differentiation and proliferation of stem cells.

7. MATERIAL AND METHODS

This systematic review was developed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Guidelines.

7.1. Identification of the PICO question

The following three databases (Medline-Pubmed, Web Of Science, Scopus) were used to find and analyse scientific articles published until January 2024 that focused on the use of stem cells in dentistry and answered the following investigation question:

To regenerate a complete tooth, with crown, root, and pulp structures, as well as enamel-like tissues, dentin, cementum, odontoblast-like cells, from a dental germ produced by tissue bioengineering, is it more effective to use natural scaffolds and dental pulp stem cells than synthetic scaffolds and stem cells from another origin?

This question followed the PICO structure and can be broken down in the following way:

- P (population): a dental germ produced by tissue bioengineering
- I (intervention): using natural scaffolds and dental pulp stem cells
- C (comparison): using synthetic scaffolds and stem cells from another origin
- O (outcomes):
    - O1 – the presence of enamel-like tissues, dentin, cementum, odontoblast-like cells
    - O2 – regeneration of the tooth with distinguishable crown, root and pulp structures
    - O3 – level of cell viability, differentiation and proliferation.

7.2. Eligibility criteria

The inclusion criteria were the following:

- Type of study – animal studies, *in vitro* experiments, clinical studies. Publication made in English, Spanish or Russian language, published until January 2024.
- Type of patient – studies in humans, animals, *in vitro, in vivo.*
- Type of intervention – dental regeneration using natural scaffolds and dental pulp stem cells.
- Type of control – dental regeneration using synthetic scaffolds and stem cells from another origin.
- Type of final measuring variables – studies that present distinguishable crown, root and pulp structures as the final result, or in their absence, enamel-like tissues, dentin, cementum, odontoblast-like cells. As secondary variables: level of cell viability, differentiation and proliferation overall and depending on the origin of scaffold used.

The exclusion criteria consisted of reviews, expert opinions, letters to the editor, studies where no histological analysis was performed, studies with the final goal of obtaining only dentin-pulp complex regeneration or differentiation into odontoblast-like cells, duplicate studies submitted to more than one journal.

There were no restrictions on the publication date.

7.3. Sources of information and data search strategy

The automatized search was performed in the 3 databases mentioned earlier: PubMed, Scopus, and Web Of Science. Keywords and MeSH (Medical Subject Headings) terms were combined with Boolean logical operators AND and OR. The keywords used were the following: “tooth germ”, “bioengineering”, “scaffold”, “natural scaffold”, “decellularized scaffold”, “fibrin scaffold”, “stem cell”, “human pulp stem cells”, “dental stem cells”, “synthetic scaffold”, “hydrogel

scaffold", "whole-tooth regeneration", "whole-tooth restoration", "tooth regeneration", "enamel", and "dentin".

PubMed search: (((tooth germ[MeSH Terms]) OR (tooth germ)) AND (bioengineering[MeSH Terms])) OR (bioengineering) AND (((((natural scaffold)) OR (decellularized scaffold)) OR (fibrin scaffold)) AND (stem cell[MeSH Terms])) OR (human pulp stem cells) OR ((((synthetic scaffold) OR (hydrogel scaffold)) AND (stem cell[MeSH Terms])) OR (stem cell)) OR (dental stem cell) AND ((((("whole-tooth regeneration")) OR (whole-tooth restoration)) OR (tooth regeneration) AND enamel AND dentin). Filters: English, Spanish, Russian.

Scopus search: ((ALL (tooth AND germ) AND ALL (bioengineering))) AND ((ALL (synthetic AND scaffold) OR ALL (hydrogel AND scaffold) AND ALL (stem AND cell) OR ALL (dental AND stem AND cell))) OR ((ALL (natural AND scaffold) OR ALL (decellularized AND scaffold) OR ALL (fibrin AND scaffold) AND ALL (stem AND cell) OR ALL (human AND pulp AND stem AND cell))) AND ((ALL ("whole tooth regeneration") OR ALL ("whole tooth restoration") OR ALL (tooth AND regeneration) AND ALL (enamel) AND ALL (dentin))).

Web Of Science search: (((ALL=(tooth germ AND bioengineering)) AND ALL=(natural scaffold OR decellularized scaffold OR fibrin scaffold AND stem cell OR human dental pulp stem cell)) OR ALL=(synthetic scaffold OR hydrogel scaffold AND stem cell OR dental stem cell)) AND ALL=("whole-tooth regeneration" OR "whole tooth restoration" OR tooth regeneration AND enamel AND dentin).

In Table 1 which can be found in the Annexes of this review, the summary of the searches is presented.

In order to make sure that no potentially suitable studies were missed in the automatized searches, a manual search was performed through references found in the bibliographies of the selected studies and certain reviews used in the Introduction section.

All duplicated studies were eliminated from the review.

7.4. Selection process of the studies

The selection of the studies was performed by the author of this review in 3 stages. The first stage consisted of filtering the studies by their title, excluding all irrelevant ones. In the second stage, the summaries/abstracts of the studies were analysed and selected based on the type of study, scaffold materials, type of stem cells used, and the final measuring variables. The final stage was completed by reading the articles completely.

7.5. Data extraction

The following data was extracted from the studies and presented in the tables later: name of the authors, year of publication, type of study (*in vitro, in vivo* studies), sample size (number of teeth, number of animals), bioengineering approach used, type of stem cells, type of scaffold, any bioactive agents used, presence of enamel-like tissues, presence of dentin, presence of cementum, presence of odontoblast-like cells, regeneration of dental crown, regeneration of dental root, regeneration of dental pulp, level of stem cell viability, stem cell differentiation, stem cell proliferation.

Principal variables:

- Presence of enamel-like tissues – assessed through histological analysis.
- Presence of dentin – assessed through histological analysis.
- Presence of cementum – assessed through histological analysis.
- Presence of odontoblast-like cells – assessed through histological analysis.
- Regeneration of dental crown – assessed clinically, through computed tomography scan, or radiograph.
- Regeneration of dental root – assessed clinically, through computed tomography scan, or radiograph.
- Regeneration of dental pulp – assessed clinically, through computed tomography scan, or radiograph.

Secondary variables:

- Level of stem cell viability – assessed via dye exclusion assays, cell proliferation assays, colorimetric assays, bioluminometric assays, or enzymatic activity.
- Stem cell differentiation – assessed through their capacity to produce matrix (enamel, dentin, cementum, etc.).
- Stem cell proliferation – measured via metabolic activity assays, cell proliferation marker assays, ATP concentration assays, and DNA synthesis assays.

7.6. Quality assessment

The selected studies were assessed for risk of bias by the author of this systematic review.

For the assessment of the quality of *in-vivo* animal studies ARRIVE guidelines 2.0 were used (https://arriveguidelines.org/arrive-guidelines). First assessing the Essential 10 items and then the Recommended Set.

7.7. Data synthesis

In order to analyse and compare the final results between the selected studies, averages were drawn from the sample size/number of implants/teeth used in the studies.

Due to different sources of dental stem cells, different animal species, various evaluation times, and different scaffolds employed in the selected articles, a meta-analysis was not possible to perform. Instead of it, a qualitative systematic review was developed.

8. RESULTS

8.1. Study selection. Flowchart

251 articles were obtained from the initial search: Medline - PubMed (n=62), SCOPUS (n=117) and the Web of Science (n=72). After screening by title and abstract, 13 articles were found eligible. Their full-text versions were obtained and analysed. Finally, 10 articles satisfied both inclusion and exclusion criteria and were included in this systematic review. The flowchart of the selection process performed can be found below (Fig. 1). The reasons for excluding the 3 studies upon their full-text review are listed in Table 2.

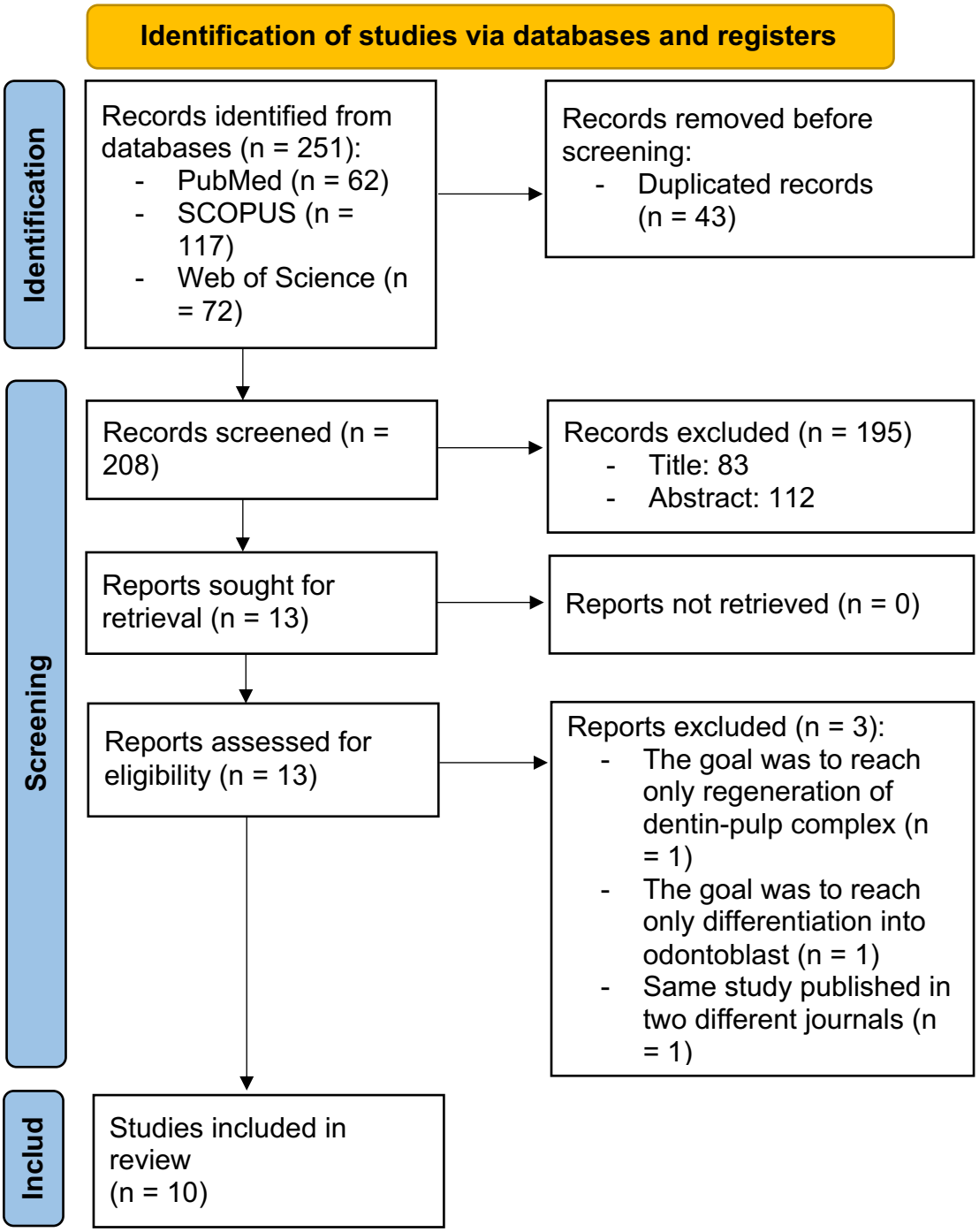

Fig. 1. Flowchart of the search and article selection process.

Table 2. Articles excluded from this systematic revision.

Author, year	Journal	Reason for exclusion
Zhang, 2020 (38)	Frontiers in Bioengineering and Biotechnology	The goal was to reach only regeneration of dentin-pulp complex

Chen, 2015 (39)	Stem Cells International	The goal was to reach only differentiation into odontoblast
Zhang, 2009 (40)	Methods	Same study published in two different journals under different names

8.2. Study characteristics

All 10 of the studies included in this systematic review were animal studies. The majority used the scaffold seeding bioengineering approach, apart from Ono et al. (41) and Zhang et al. (42) which employed a cell-tissue recombination approach.

A variety of animal species were used as models for implantation: mice, goats, dogs, and miniature pigs. Overall number of implants performed was 191: 25 were composed of natural scaffold (decellularized dentin matrix or fibrin glue with platelet-rich fibrin) (43,44), 57 of synthetic scaffold (45–49). Implants containing dental pulp stem cells, even if they were used in combination with other types of stem cells, were 113, while implants that only contained stem cells of an origin other than dental pulp were 78.

Only two studies used bioactive agents in the preparation of implants: Yang et al. (45) used TGF-β1, while Kuo et al. (47) preferred bone marrow fluid.

Table 3. Characteristics of the selected studies.

Author, Year	**Technique**	**Nº of implants**	**Origin of SC**	**Type of scaffold**	**Bioactive agents**	**Duration**
Chang et al., 2020 (50)	SS	unclear	hDPSC	Decellularized dentin matrix	--	3 months
Toriumi et al., 2018 (46)	SS	8	Human iPS, DE, DM	Hydroxyapatite/PLGA	--	16 weeks

Zhang et al., 2017 (43)	SS	16	hDPSC, pDE, HUVEC	Decellularized tooth bud	--	3 or 6 months
Ono et al., 2017 (41)	CTR	37	DE, DM	Collagen	--	4 or 8 weeks, 180 days
Zhang et al., 2017 (42)	CTR	72	Human, gingival epithelium, hDPSC, pDE, pDM	Collagen	--	1 or 3 months
Yang et al., 2016 (45)	SS	16	DPSC, gingival epithelium	Gelatin-chondroitin-hyaluronan	TGF-β1	13.5 months
Yang et al., 2011 (44)	SS	9	Dental bud	Fibrin glue + platelet-rich fibrin	--	36 weeks
Kuo et al., 2010 (47)	SS	8	Dental bud	Gelatin-chondroitin-hyaluronan	Bone marrow fluid	40 weeks
Abukawa et al., 2009 (48)	SS	9	Pulp organ, enamel organ	PGA/PLLA, Gelfoam strips	--	12 or 20 weeks
Duailibi et al., 2008 (49)	SS	16	Dental bud	PGA/PLLA, PLGA	--	12 weeks

SC, stem cells; SS, scaffold seeding; CTR, cell-tissue recombination; hDPSC, human dental pulp stem cells; DE, dental epithelial cells; DM, dental mesenchymal cells; HUVEC, human umbilical vein endothelial cells; pDE, porcine dental epithelium; pDM, porcine dental mesenchyme.

8.3. Risk of bias assessment of the selected studies

All of the studies were assessed for the risk of bias using ARRIVE 2.0 guidelines, which can be found in the Annex of the current review.

None of the selected articles mentioned how the sample sizes were determined, nor anything regarding the inclusion and exclusion criteria for the animals. In addition to that, none of the studies (except for Toriumi et al. which only had the experimental group) mentioned anything about randomisation and blinding methods.

Only 2 studies mentioned statistical analysis used, and only 4 reported housing and husbandry conditions of the animals. Finally, 5 studies have not mentioned anything about possible conflicts of interest, and 2 of these have neither mentioned any funding sources.

Table 4. Assessment of the risk of bias of the animal studies using ARRIVE 2.0 guidelines.

Item	Rec.	Chang et al.	Toriumi et al.	Zhang et al.	Ono et al.	Zhang et al.	Yang et al.	Yang et al.	Kuo et al.	Abukawa et al.	Duailibi et al.
1. Study design	a										
	b										
2. Sample size	a										
	b										
3. Inclusion and exclusion criteria	a										
	b										
	c										
4. Randomisation	a										
	b										
5. Blinding											
6. Outcome measures	a										
	b										
7. Statistical methods	a										
	b										
8. Experimental animals	a										
	b										
9. Experimental procedures	a										
	b										
	c										

	d										
10. Results	a										
	b										
11. Abstract											
12. Background	a										
	b										
13. Objectives											
14. Ethical statement											
15. Housing and husbandry											
16. Animal care and monitoring	a										
	b										
	c										
17. Interpretation/scientific implications	a										
	b										
18. Generalisibility/translation											
19. Protocol registration											
20. Data access											
21. Declaration of interests	a										
	b										

Green, yes; red, no; blank, not applicable.

8.4. Synthesis of results

8.4.1. Presence of enamel-like tissues, dentin, cementum, odontoblast-like cells

Descriptive results on the regeneration of enamel, dentin, cementum and the presence of odontoblast-like cells are summarised in Table 5. In all studies analysed in this systematic review, all 4 types of tissues were identified after the implantation, except for Duailibi et al. where no cementum was produced (49).

Table 5. Descriptive results on the presence/absence of enamel-like tissues, dentin, cementum, odontoblast-like cells

	Scaffold	**Results**
DPSC		
Chang et al. (50)	Decellularized dentin matrix	All 4 tissue types regenerated
Zhang et al. (43)	Decellularized tooth bud	All 4 tissue types regenerated

Zhang et al. (42)	Collagen	All 4 tissue types regenerated
Yang et al. (45)	Gelatin-chondroitin-hyaluronan	All 4 tissue types regenerated
Abukawa et al. (48)	PGA/PLLA, Gelfoam strips	All 4 tissue types regenerated
SC of other origin		
Toriumi et al. (46)	Hydroxyapatite/PLGA	All 4 tissue types regenerated
Ono et al. (41)	Collagen	All 4 tissue types regenerated
Yang et al. (44)	Fibrin glue + platelet-rich fibrin	All 4 tissue types regenerated
Kuo et al. (47)	Gelatin-chondroitin-hyaluronan	All 4 tissue types regenerated
Duailibi et al. (49)	PGA/PLLA, PLGA	Enamel, dentin, odontoblasts regenerated

In the natural scaffold group, only two studies provided quantitative results (43,44). The highest percentage of implants with regenerated enamel, dentin and odontoblasts – 18.8%, 25%, 25%, respectively, was presented by Zhang et al. (43). Meanwhile, Yang et al. presented a higher percentage of implants with regenerated cementum – 22.2% (44). The average percentage of implants with regenerated enamel, dentin, cementum and odontoblasts in the natural scaffold group was 15%, 23.6%, 17.4%, 23.6%, respectively.

In regards to the synthetic scaffold group, 4 studies provided data on the number of regenerated tissues (45,47–49). Yang et al. showed the highest percentage of implants with regenerated enamel, dentin, cementum and odontoblasts – 93.8% for every tissue type; 15 out of 16 implants (45). The average percentage of implants with regenerated enamel, dentin, cementum and odontoblasts in the synthetic scaffold group was 43.4%, 61.8%, 38.7%, and 61.8%.

When separating the studies based on the type of stem cells used, the group that utilised dental pulp stem cells consisted of 4 studies (42,43,45,48). The highest percentage of implants with regenerated tissues was once again corresponding to Yang et al. (45). The averages for this group were 35.8%, 36.3%, 32.8%, and 47%.

The final group compared in the review was the one utilizing stem cells originating from a source other than dental pulp. In this group, there were also 4 studies that provided the data (41,44,47,49). Ono et al. showed the highest percentage of implants that regenerated enamel – 59.5% (41). While the highest percentage of implants that regenerated dentin, cementum, and odontoblasts was shown by Kuo et al. – 75%, 50%, 75% (47). The average percentage values for this group were 34.9%, 53.3%, 18.7%, and 53.3%.

Table 8 represents the summarised contents of tables 6 and 7, which can be consulted in the Annex section showing detailed results of each study. Not all of the studies provided quantitative data, therefore they were not included in the calculation of the averages.

Table 8. Average results on the presence/absence of enamel-like tissues, dentin, cementum, odontoblast-like cells

	Presence of			
Group	**Enamel-like tissues**	**Dentin**	**Cementum**	**Odontoblast-like cells**
Natural scaffold	15%	23.6%	17.4%	23.6%
Synthetic scaffold	43.4%	61.8%	38.7%	61.8%
Dental pulp SC	35.8%	36.3%	32.8%	47%
SC of other origin	34.9%	53.3%	18.7%	53.3%

8.4.2. Regeneration of the tooth with distinguishable crown, root and pulp structures

In the natural scaffold group, only two studies provided quantitative results (43,44). The highest percentage of implants with regenerated crown, root, and pulp – 22.2%, 11.1%, and 22.2%, respectively, was presented by Yang et al. (44). The average percentage of implants with the 3 regenerated parts of the tooth in the natural scaffold group was 20.5% (crown), 8.7% (root), 23.6% (pulp).

In regards to the synthetic scaffold group, also only 2 studies provided data on this parameter (45,47). Yang et al. showed the highest percentage of implants with regenerated crown and pulp – 93.8%, as well as root – 25% (45). The average percentage of implants with regenerated crown, root, and pulp in the synthetic scaffold group was 53.2%, 18.8%, and 71.9%.

The group that utilised dental pulp stem cells consisted of 3 studies that provided information on the regenerated anatomical parts of the tooth (42,43,45,). The highest percentage was once again in the study by Yang et al. (45). The averages for this group were 38.9%, 10.4%, 41%.

The final group was the one utilizing stem cells of origin other than dental pulp. In this group, there were also 3 studies that provided the data (41,44,47). However, the result by Ono et al. was omitted from the analysis, even though it showed the highest percentage of implants that regenerated crown, root and pulp – 100%, they only tested one implant (41). This outlier was skewing the average of the whole group. The second highest percentage of implants that regenerated crown is 22.2% from Yang et al. (44), root and pulp – 12.5% and 50% from Kuo et al. (47). The average percentage values for this group were 17.4%, 11.8%, and 36.1%.

Table 11 represents the summarised contents of tables 9 and 10, which can be consulted in the Annex section showing detailed results of each study. Not all of the studies provided quantitative data, therefore they were not included in the calculation of the averages.

Table 11. Average results on the regeneration of distinguishable crown, root and pulp structures

	Presence of distinguishable		
Group	**Crown**	**Root**	**Pulp**
Natural scaffold	20.5%	8.7%	23.6%
Synthetic scaffold	53.2%	18.8%	71.9%
Dental pulp SC	38.9%	10.4%	41%
SC of other origin	17.4%	11.8%	36.1%

8.4.3. Effect of scaffold on the level of cell viability and differentiation

Only one study selected for this review provided information on the cell viability values (50). Chang et al. compared the viability of human dental pulp stem cells seeded on decellularized dentin matrix (DDM), autoclaved decellularized dentin matrix (a-DDM) and control group (without scaffold). The results showed that the stem cells with a-DDM had a significantly higher viability (260% on Day 5) than the control group (190% on Day 5) but showed no significant difference compared with the DDM group (230% on Day 5). All groups reached their peak values on day 5 and showed a slight regression on day 7.

Stem cell differentiation was assessed by the ability to produce a corresponding tissue: enamel for ameloblasts, dentin for odontoblasts, and cementum for cementoblasts. Even though the studies did not always explicitly mention that certain types of cells were observed on histological analysis, it can be assumed that in all the studies, except for Duailibi et al. (49) where no cementum was produced, the stem cells successfully differentiated into specialised cells. Table 5 can be consulted for further information.

8.4.4. Stem cell proliferation and differentiation

None of the studies have explicitly mentioned the values for the proliferation of stem cells. However, the fact that in all of them, new tissues have been regenerated implies that proliferation has happened.

In regards to stem cell differentiation, as mentioned in the previous section, in all studies but one (Duailibi et al. (49)) the cells have managed to differentiate into ameloblasts, odontoblasts and cementoblasts judging by the formation of new dental tissues.

8.4.5. Effect of bioactive agents on stem cell differentiation and proliferation

Two studies analysed in this systematic review utilised bioactive agents: Yang et al. (45) and Kuo et al. (47). The first one used TGF-β1 and demonstrated the highest percentage in the regeneration of enamel, dentin, cementum and odontoblasts, as well as the presence of distinguishable crown and pulp structures across all 10 studies compared in the review – 93.8%. Kuo et al. used bone marrow fluid and subsequently showed second-best results in regeneration of dentin (75%), cementum (50%), odontoblasts (75%), and presence of distinguishable pulp (50%) across all the studies.

9. DISCUSSION

The current systematic review provides information on whole tooth regeneration using natural scaffolds compared to synthetic scaffolds, and dental pulp stem cells compared to stem cells of other origin. The primary objective of this review was to compare these groups in their effectiveness in achieving regeneration of a complete tooth with enamel-like tissues, dentin, cementum, and odontoblast-like cells, and present distinguishable crown, root and pulp structures. On a secondary basis, the effect of different scaffolds on cell viability and differentiation, the type of stem cells with the highest differentiation and proliferation ability, as well as the effect of bioactive agents on cell differentiation and proliferation was investigated.

9.1. Presence of enamel-like tissues, dentin, cementum, odontoblast-like cells

The results of this systematic review, based on the comparison of 6 scientific investigations reveal the higher effectiveness of synthetic scaffolds in regeneration of enamel, dentin, cementum and odontoblasts compared to natural scaffolds. Furthermore, in the synthetic scaffold group, the study by Yang et al. stood out with exceptionally high percentages of regenerated tissues across the board (93.8%) (45). Such a high result could be partially explained by the use of TGF-β1, which demonstrated the ability to initiate an odontoblast-like differentiation of DPSC in vitro in a study by He et al. (51).

These findings suggest that while both natural and synthetic scaffolds have shown potential in promoting tissue regeneration, the choice of scaffold material may significantly impact the outcomes. Further investigation into the possible reasons for these differences is warranted to optimize regenerative strategies in dental tissue engineering. Regrettably, due to the absence of studies specifically investigating and comparing these two groups, it is impossible to provide additional sources for comparison. The current analysis is based solely on the available data provided within the context of the discussed studies.

In regards to the comparison of the group that utilised dental pulp stem cells with the group utilizing stem cells of other origin, the results are more

ambivalent. In this way, the first group showed higher effectiveness in regenerating cementum, while the second one demonstrated higher numbers in the regeneration of dentin and odontoblasts. A higher percentage of regeneration of enamel was reported by the group of DPSC, however, the difference can hardly be considered significant (35.8% VS. 34.9%).

It is surprising to see that the DPSC group has achieved a higher percentage in regeneration of cementum because it is thought that cementoblasts derive from the differentiation of dental follicle mesenchymal cells (52). However, recently Mata et al. (53) have conducted a study that demonstrated the potential of human dental pulp stem cells to differentiate into cells that secrete a cementoid-like matrix, which goes in accordance with the results of the current systematic review.

9.2. Regeneration of the tooth with distinguishable crown, root, and pulp structures

When comparing the effectiveness of natural and synthetic scaffolds for the regeneration of distinguishable dental structures, the results clearly favour the use of synthetic scaffolds. In particular, the synthetic group showed at least twice the percentage of regeneration of key dental components, such as the crown, root and pulp, compared to the natural scaffold group. This significant difference emphasizes the clear advantage of synthetic scaffolds in promoting dental tissue regeneration.

The two studies that formed the synthetic scaffold group employed gelatin-chondroitin-hyaluronan scaffolds, indicating that the observed advantage is likely attributable to this specific scaffold composition (45,47). This type of scaffold was first developed with the goal of achieving cartilage tissue engineering by Chang et al. (54). It showed to have good biocompatibility, being biodegradable, and producing nontoxic metabolites. In addition to that, this scaffold provided information for cell attachment to meet the requirement for dynamic reciprocity for cartilage regeneration. Throughout the years it has been used for skin tissue engineering as well (55). As can be noted this scaffold has a lot of potential in various areas of tissue engineering.

However, the material of the scaffold may not be the sole reason for the high rates of dental regeneration observed in these studies. It is also essential to consider the duration of the studies, as this could have influenced the results. The study conducted by Yang et al. (45) lasted approximately 54 weeks (13.5 months), while the study by Kuo et al. (47) spanned 40 weeks. These prolonged observation periods may have allowed for more extensive tooth development and regeneration within the synthetic scaffold group.

In contrast, the natural scaffold group in the study by Zhang et al. (43) underwent shorter durations of approximately 12 or 24 weeks (3 or 6 months), while the study conducted by Yang et al. (44) had a duration of 36 weeks. The disparity in duration between these studies raises the possibility that differences in regeneration rates may be influenced by the extended timeframe provided to the synthetic scaffold group for tooth development.

While the findings clearly demonstrate the superiority of synthetic scaffolds in promoting the regeneration of crown, root, and pulp structures, further research is needed to evaluate the long-term effectiveness and safety of these scaffolds in clinical applications.

When comparing the effectiveness of dental regeneration, the group using DPSCs demonstrated superior results in terms of regenerating both the dental crown and pulp when compared to the group utilizing stem cells from alternative sources. However, there was barely any difference in the percentage of regeneration of the dental root – a 1.4% advantage was observed for the group using stem cells from other origins.

It is worth noting that the regeneration of dental root structure presented the lowest percentage in both groups. This difference in regeneration rates may be attributed to the inherent complexity of dental root formation, which requires extended periods of time for complete growth and maturation. Therefore, it is possible that the studies were terminated before the full extent of dental root regeneration could be achieved.

Once again, it is worth mentioning the factor of the study duration, as the study with the highest rates of regeneration in the DPSC group was also the one with the longest duration – Yang et al., 54 weeks (45). In contrast to the studies

conducted within the group utilizing SC from other origins, which had durations of 36 and 40 weeks (44,47).

Therefore, while the superior performance of DPSCs in regenerating dental crown and pulp structures is evident, it is essential to acknowledge the potential influence of study duration as a confounding factor. Future research efforts should aim to standardize study durations and incorporate longitudinal assessments to determine the optimal conditions and timeframes required for achieving successful regeneration of all three parts of the tooth.

9.3. Effect of scaffold on the level of cell viability and differentiation

The assessment of cell viability is a crucial aspect in determining the biocompatibility and cytotoxicity of scaffolds used in tissue engineering. Although quantitative data on cell viability was limited, it is important to note that across all of the studies included in this systematic review, the scaffolds showed a significant tendency to support cell viability and promote proliferation. Despite the lack of numerical data, qualitative assessments from various investigations consistently confirmed the scaffolds' ability to support cellular growth and promote tissue regeneration without causing adverse effects on cells. This combined observation emphasizes the inherent biocompatibility and safety of scaffold materials used in the reviewed studies, highlighting their potential for clinical applications in tissue regeneration.

While the regeneration of tissues has been achieved in most studies, there have been some challenges in achieving specific cell differentiation outcomes. For example, the study conducted by Duailibi et al. reported difficulties in obtaining the differentiation of dental bud stem cells into cementoblasts (49). This limitation could be attributed to the properties of the scaffold material used in that particular study – PGA/PLLA, PLGA, considering that other studies, such as by Yang et al. (44), Kuo et al. (47), and Honda et al. (56), have shown successful differentiation of dental bud stem cells into various cell types, including ameloblasts, odontoblasts, and cementoblasts. One of the possible explanations is the possible toxicity of PLA and its copolymers due to the accumulation of acidic degradation products – lactic and glycolic acids. When the surrounding tissue fails to eliminate these by-products rapidly enough, an inflammatory

response may occur (57). This could have affected the differentiation capacity of dental bud SC. It was suggested that in order to overcome the formation of an acidic environment, a bioactive hydroxyapatite (HA) could be combined with PLA or its copolymers (58). As can be noted from the results of the study by Toriumi et al. (46), where scaffolds of HA/PLGA were used and all 4 kinds of tissues were regenerated, the suggested modification had a positive effect on stem cell differentiation.

This finding confirms that scaffold material characteristics play a significant role in determining cellular fate, and not all scaffolds can lead to successful cell differentiation.

9.4. Stem cell proliferation and differentiation

Despite the lack of direct measurements of cell proliferation in the studies analysed in this systematic review, it seems reasonable to conclude that stem cells underwent substantial division as newly regenerated tissues were observed in all of the studies.

Additionally, as discussed in the previous section, all of the investigations (with the exception of Duailibi et al. (49)) reported successful cell differentiation into ameloblasts, odontoblasts, and cementoblasts, confirming that all types of stem cells compared in this review are capable of differentiation.

The study by Yang et al. stands out among the rest for its high percentage of regenerated dental tissues, crown and pulp structures (45). The authors used a combination of dental pulp stem cells and cells derived from the gingival epithelium, which likely contributed to their enhanced regenerative potential. This dual-source approach emphasizes the importance of using multiple stem cell populations to optimize tissue regeneration outcomes, as it is known that in the natural process of odontogenesis, two cell types are involved: dental epithelial cells and ectomesenchymal cells (28).

In fact, all of the studies, except for one, used a combination of epithelial and mesenchymal stem cells. The one study that only used human DPSC was conducted by Chang et al. (50). The authors reported successful regeneration of enamel, dentin, cementum and odontoblasts, although they did not provide any quantitative results on the number of implants with regenerated tissues.

Nonetheless, their findings show the potential of DPSC to differentiate not only into odontoblasts and cementoblasts but also into ameloblasts. Unfortunately, the mechanisms of this differentiation remain unknown, and further studies are needed to confirm these results. If confirmed, one of the main challenges in whole tooth regeneration would be resolved – there will be no more need for dental epithelial cells that are only present during the tooth formation stage. Human DPSC, which can be obtained from mature permanent teeth, could be sufficient for regenerating a whole tooth.

9.5. Effect of bioactive agents on stem cell differentiation and proliferation

The exploration of bioactive agents for stem cell differentiation and proliferation is at the forefront of dental tissue engineering. This field aims to harness the natural processes of odontogenesis in order to enhance tissue regeneration. Odontogenesis, as mentioned in the introduction to this review, involves a complex interaction of various growth and transcription factors that regulate the development and maturation of dental tissues. The controlled release of specific bioactive agents from biodegradable scaffolds has the potential to significantly improve the effectiveness of tooth regeneration. These agents can be used to stimulate the growth and differentiation of stem cells, as well as to regulate the expression of genes involved in tooth formation and development.

Two studies analysed in this systematic review, conducted by Yang et al. and Kuo et al., highlight the significant impact of these agents on dental structure regeneration (45,47). The study by Yang et al., which explored the efficacy of TGF-β1, demonstrated the highest percentage of regeneration in various dental components, including enamel, dentin, cementum, and odontoblasts, as well as the development of distinguishable crown and pulp structures. He et al. (51) and Li et al. (59) confirm in their separate studies the capability of TGF-β1 to induce odontogenic differentiation and subsequent dentin formation by dental pulp SC. Meanwhile, research by Kuo et al., which utilised bone marrow fluid as a bioactive agent, aimed to develop a more cost-effective approach to obtaining GFs and morphogens from bone marrow stem cells. While their findings were not as

significant as those of Yang et al., they nevertheless demonstrated notable advancements in dental tissue regeneration.

Once again it is worth mentioning the increased duration of these studies, which could be partially responsible for such high results in dental regeneration. It must be taken into account that the bioactive agents were added to the implants only in the beginning, right before the implantation procedure. Like any other chemical substance, they lose their effect over time. In fact, this remains one of the main challenges in growth factor-based tissue regeneration approach – maintaining an optimum critical minimum therapeutic level over prolonged periods of time (60).

Nonetheless, these findings emphasize the significance of bioactive agents in tissue engineering and their potential for advancing regenerative therapies in dentistry. The incorporation of bioactive substances into scaffold-based techniques represents a promising approach for enhancing stem cell differentiation and proliferation in dental tissue regeneration.

9.6. Study limitations

Upon analysing the 10 studies included in the current systematic review, several limitations have been identified.

One of the most important ones is the variable duration of the studies. This difference could impact the observed outcomes, with a possibility that shorter-duration studies got terminated before their full regeneration potential has been reached, as odontogenesis is a process that takes a significant amount of time. Therefore, the inclusion of studies with diverse durations may introduce variability into the synthesised findings, potentially complicating the interpretation of results.

Another significant limitation is the absence of standardised protocols across the included studies. Each study presented different methodologies, biomaterials, and regenerative techniques, making direct comparisons challenging. This is partially due to the novelty of the research and the fact that the investigations are still at the stage of animal experiments. However, without standardised protocols, it is difficult to assess the reproducibility of findings and evaluate the exact effect of independent variables, such as type of scaffold or stem cells, on the dependent variable – dental tissue regeneration.

The variability in sample sizes across the studies adds to a more complicated comparison and potential confounding in the interpretation of findings. Studies with larger sample sizes typically provide more precise measurements than smaller studies, which are more susceptible to random variation. Unfortunately, the majority of the included studies had a sample size of 16 or fewer implants.

Some studies included in the review have provided only descriptive data, rather than quantitative results. This limitation complicated the statistical analyses, as these studies were excluded from the calculation and could not contribute to determining reliable averages.

Also, none of the studies mentioned any randomization techniques in allocating the animals to experimental or control groups, nor any blinding methods. As these are essential aspects of experimental design aimed at minimizing bias and increasing the reliability of the study's results, it is essential that in future research these flaws are corrected.

Finally, one of the limitations of this systematic review is the grouping of the studies. All studies that used dental pulp stem cells were sorted into one group even if there were other types of stem cells involved. The reason behind this decision is primarily the scarcity of studies exclusively utilizing DPSC. This leads to a certain degree of ambiguity regarding the attribution of the observed results solely to the DPSC.

9.7. Clinical implications and future research

The findings of this systematic review provide a foundation for advancing towards clinical trials in human subjects. In the past decade, the number of human clinical trials with promising outcomes in pulp and alveolar bone regeneration using stem cells has significantly increased (61,62). The transition to human trials in whole tooth regeneration becomes a logical next step. In the future, this achievement will revolutionize dental care by offering novel treatment options for patients with various dental conditions, including agenesis, periodontal diseases, and tooth loss.

One of the main challenges in whole tooth regeneration via bioengineering is obtaining suitable stem cell populations. A practical solution to this is the use

of stem cell banks (63). Extracted third molars or premolars, due to orthodontic reasons, are viable sources of dental pulp stem cells. These cells offer a readily available and ethically sound reservoir for autologous stem cell therapies. In addition, third molars can be extracted during childhood, providing viable dental SC sources, including dental epithelial SC. Leveraging stem cell banks to store and retrieve dental SC simplifies the process of obtaining patient-specific stem cell populations, facilitating personalised regenerative treatments tailored to each patient's needs (64).

As the field of stem cell research and regenerative medicine advances towards clinical trials in dentistry, it is essential to prioritize several key areas of future research in order to maximize its potential. One such area is further preclinical research aimed at optimizing and refining regenerative protocols. This includes the development of standardised procedures for isolating, culturing, and administering dental stem cells, as well as clarifying the mechanisms underlying tooth regeneration. These improvements will enhance the efficacy of regenerative therapies and ensure their long-term success.

10. CONCLUSIONS

Principal conclusions:

1. All analysed techniques led to the regeneration of all 4 types of dental tissues, but using synthetic scaffolds and dental pulp stem cells provided structures more similar to a natural tooth than natural scaffolds and stem cells of other origin.

Secondary conclusions:

2. All scaffolds supported stem cell viability and differentiation, except for PLA and its copolymers in their pure form, which hindered the full differentiation potential.
3. All types of stem cells compared in the review showed successful proliferation and differentiation capabilities, but dental pulp stem cells demonstrated the highest regeneration potential of dental tissues.
4. The studies that used bioactive agents like TGF-β1 or bone marrow fluid have achieved good results but further research is necessary to clarify how they affect the process of regeneration over longer periods of time.

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12. ANNEXES

Table 1. The summary of the searches in each of the databases used.

Database	Search	Number of articles	Date
PubMed	(((tooth germ[MeSH Terms]) OR (tooth germ)) AND (bioengineering[MeSH Terms])) OR (bioengineering) AND (((((natural scaffold)) OR (decellularized scaffold)) OR (fibrin scaffold)) AND (stem cell[MeSH Terms])) OR (human pulp stem cells) OR ((((synthetic scaffold) OR (hydrogel scaffold)) AND (stem cell[MeSH Terms])) OR (stem cell)) OR (dental stem cell) AND ((((("whole-tooth regeneration")) OR (whole-tooth restoration)) OR (tooth regeneration) AND enamel AND dentin). Filters: English, Spanish, Russian.	62	15.01.2024
Scopus	((ALL (tooth AND germ) AND ALL (bioengineering))) AND ((ALL (synthetic AND scaffold) OR ALL (hydrogel AND scaffold) AND ALL (stem AND cell) OR ALL (dental AND stem AND cell))) OR ((ALL (natural AND scaffold) OR ALL (decellularized AND scaffold) OR ALL (fibrin AND scaffold) AND ALL (stem AND cell) OR ALL (human AND pulp AND stem AND cell))) AND ((ALL ("whole tooth regeneration") OR ALL ("whole tooth restoration") OR ALL (tooth AND regeneration) AND ALL (enamel) AND ALL (dentin)))	117	15.01.2024
Web Of Science	(((ALL=(tooth germ AND bioengineering)) AND ALL=(natural scaffold OR decellularized scaffold OR fibrin scaffold AND stem cell OR human dental pulp stem	72	15.01.2024

	cell)) OR ALL=(synthetic scaffold OR hydrogel scaffold AND stem cell OR dental stem cell)) AND ALL=("whole-tooth regeneration" OR "whole tooth restoration" OR tooth regeneration AND enamel AND dentin)		

Table 6. Quantitative results on the presence/absence of enamel-like tissues, dentin, cementum, odontoblast-like cells depending on the type of scaffold used.

	Presence of							
	Enamel-like tissues		**Dentin**		**Cementum**		**Odontoblast-like cells**	
Natural scaffold	Implants	%	Implants	%	Implants	%	Implants	%
Zhang et al. (43)	3/16	18.8	4/16	25	2/16	12.5	4/16	25
Yang et al. (44)	1/9	11.1	2/9	22.2	2/9	22.2	2/9	22.2
Average %		**15**		**23.6**		**17.4**		**23.6**
Synthetic scaffold								
Yang et al. (45)	15/16	93.8	15/16	93.8	15/16	93.8	15/16	93.8
Kuo et al. (47)	1/8	12.5	6/8	75	4/8	50	6/8	75
Abukawa et al. (48)	1/9	11.1	2/9	22.2	1/9	11.1	2/9	22.2
Duailibi et al. (49)	9/16	56.3	9/16	56.3	0/16	0	9/16	56.3
Average %		**43.4**		**61.8**		**38.7**		**61.8**

Table 7. Quantitative results on the presence/absence of enamel-like tissues, dentin, cementum, odontoblast-like cells depending on the type of stem cells used.

	Presence of							
	Enamel-like tissues		**Dentin**		**Cementum**		**Odontoblast-like cells**	
Dental pulp SC	Implants	%	Implants	%	Implants	%	Implants	%
Zhang et al. (43)	3/16	18.8	4/16	25	2/16	12.5	4/16	25
Zhang et al. (42)	14/72	19.4	3/72	4.2	10/72	13.9	unclear	--
Yang et al. (45)	15/16	93.8	15/16	93.8	15/16	93.8	15/16	93.8
Abukawa et al. (48)	1/9	11.1	2/9	22.2	1/9	11.1	2/9	22.2
Average %		**35.8**		**36.3**		**32.8**		**47**
SC of other origin								
Ono et al. (41)	22/37	59.5	22/37	59.5	1/37	2.7	22/37	59.5
Yang et al. (44)	1/9	11.1	2/9	22.2	2/9	22.2	2/9	22.2
Kuo et al. (47)	1/8	12.5	6/8	75	4/8	50	6/8	75
Duailibi et al. (49)	9/16	56.3	9/16	56.3	0/16	0	9/16	56.3
Average %		**34.9**		**53.3**		**18.7**		**53.3**

Table 9. Quantitative results on the regeneration of distinguishable crown, root and pulp structures depending on the type of scaffold used.

	Presence of distinguishable					
	Crown		**Root**		**Pulp**	
Natural scaffold	Implants	%	Implants	%	Implants	%
Zhang et al. (43)	3/16	18.8	1/16	6.3	4/16	25
Yang et al. (44)	2/9	22.2	1/9	11.1	2/9	22.2
Average %		**20.5**		**8.7**		**23.6**
Synthetic scaffold						
Yang et al. (45)	15/16	93.8	4/16	25	15/16	93.8
Kuo et al. (47)	1/8	12.5	1/8	12.5	4/8	50
Average %		**53.2**		**18.8**		**71.9**

Table 10. Quantitative results on the regeneration of distinguishable crown, root and pulp structures depending on the type of stem cells used.

	Presence of distinguishable					
	Crown		**Root**		**Pulp**	
Dental pulp SC	Implants	%	Implants	%	Implants	%
Zhang et al. (43)	3/16	18.8	1/16	6.3	4/16	25
Zhang et al. (42)	3/72	4.2	0/72	0	3/72	4.2
Yang et al. (45)	15/16	93.8	4/16	25	15/16	93.8
Average %		**38.9**		**10.4**		**41**
SC of other origin						
Yang et al. (44)	2/9	22.2	1/9	11.1	2/9	22.2
Kuo et al. (47)	1/8	12.5	1/8	12.5	4/8	50
Average %		**17.4**		**11.8**		**36.1**

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Cover
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1, 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	13-14
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	15
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	17
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	17-18
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	17-18
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	19
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	19
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	19-20
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	19-20
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	20
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	20
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	19
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	19-20
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	20
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	21-22
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	22-23
Study characteristics	17	Cite each included study and present its characteristics.	23-24
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	25-26
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	51-53
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	26-31
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	32-38
	23b	Discuss any limitations of the evidence included in the review.	38-39
	23c	Discuss any limitations of the review processes used.	39
	23d	Discuss implications of the results for practice, policy, and future research.	39-40
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	
Data access	20	Provide a statement describing if and where study data are available.	
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	

DENTAL REGENERATION VIA BIOENGINEERING: SYSTEMATIC REVIEW.

Running title: Dental regeneration via bioengineering: systematic review.

Authors:

Daria Proshchenkova[1], Santiago Peydró Tomás[2]

[1] *5th year student of the Dentistry degree at the European University of Valencia, Valencia, Spain.*

[2] *Professor Faculty of Dentistry, European University of Valencia, Valencia, Spain.*

Corresponding and reprints author:

Santiago Peydró Tomás

Paseo Alameda 7, Valencia

46010, Valencia

santiago.peydro@universidadeuropea.es

Abstract

Introduction: This review explores the bioengineering techniques that use stem cells (SC) for tooth regeneration, highlighting current methodologies, potential clinical applications, and future prospects in dental tissue engineering, while comparing the effectiveness of different scaffold materials and SC of various origins.

Material and Methods: An electronic search was performed in PubMed, Scopus, and Web of Science databases on tooth regeneration using natural and synthetic scaffolds, and dental pulp SC (DPSC) or other SC until January 2024.

Results: From 251 articles obtained from the initial search, 10 were chosen to be included, complying with inclusion and exclusion criteria. In the natural scaffold group, the average percentages of regenerated enamel, dentin, cementum, and odontoblast-like cells were 15%, 23.6%, 17.4%, 23.6%. In the synthetic scaffold group, the values were 43.4%, 61.8%, 38.7%, and 61.8%. For regeneration of distinguishable crown, root and pulp structures, the natural scaffold group showed the following results – 20.5%, 8.7%, 23.6%; the synthetic scaffold group – 53.2%, 18.8%, and 71.9%. The group of DPSC regenerated 35.8% (enamel), 36.3% (dentin), 32.8% (cementum), and 47% (odontoblasts), while the group of SC of other origin regenerated 34.9%, 53.3%, 18.7%, and 53.3%. For the regeneration of dental parts, the DPSC group presented 38.9% (crown), 10.4% (root), 41% (pulp), while the other SC group showed 17.4%, 11.8%, and 36.1%.

Conclusions: All analyzed techniques led to regeneration of all 4 types of dental tissues, but using synthetic scaffolds and DPSC provided structures more similar to a natural tooth. All scaffolds supported stem cell viability and differentiation, except for PLA and its copolymers in their pure form, which hindered the full differentiation potential. DPSC demonstrated highest regeneration potential of dental tissues. The studies utilizing bioactive agents achieved good results but further research is necessary to clarify how they affect the process of regeneration over longer periods of time.

Keywords: *stem cell regeneration, dental regeneration, natural scaffolds, synthetic scaffolds, dental pulp stem cells, bioengineering*

Introduction

Several new approaches to regenerate an entire tooth have been proposed and are being investigated by the field of biological tissue engineering. The two major ones are cell-tissue recombination and the use of scaffolds (1,2). The dental cell-tissue recombination approach is based on using a tooth germ. The epithelial and mesenchymal tissues are isolated and completely dissociated into single cells, which are then used to reconstitute a bioengineered tooth germ. The newly recombined tooth germ is implanted into the defect site in the jaw, where it develops into a complete tooth (3).

In the tissue engineering approach based on the use of scaffolds, stem cells (SC) are seeded in/onto a scaffold, where they then proliferate and differentiate into other cell types (4). This structure is supplied with bio-active agents, e.g. growth factors (GF), that control the spatial and temporal organization of dental progenitor cell proliferation, differentiation and function (1). Each of the main three elements involved in this method has many variations that inevitably affect the final result.

The choice of scaffold is very important, as its physical aspects and composition must guarantee physical support for the development of new tissues in a manner that mimics the function of the natural extracellular matrix (ECM) (5). SC are cells that present continuous self-renewal and possible differentiation into multiple specialized cell types (6). In the process of odontogenesis, two different types of stem cells are involved: dental epithelial cells that later give rise to enamel, and ectomesenchymal cells responsible for the production of dentin, pulp, cementum and periodontal ligament.

The primary objective of this review is to identify the most effective technique in the regeneration of a complete tooth from a dental germ produced by tissue bioengineering that will provide enamel-like tissues, dentin, cementum, and odontoblast-like cells, and present distinguishable crown, root and pulp structures. On a secondary basis, the effect of different scaffolds on cell viability and differentiation, the type of stem cells with the highest differentiation and proliferation ability, as well as the effect of bioactive agents on cell differentiation and proliferation was investigated.

Material and Methods

This systematic review was developed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Guidelines.

Focus question:

To regenerate a complete tooth, with crown, root, and pulp structures, as well as enamel-like tissues, dentin, cementum, odontoblast-like cells, from a dental germ produced by tissue bioengineering, is it more effective to use natural scaffolds and dental pulp stem cells than synthetic scaffolds and stem cells from another origin?

This question followed the PICO structure and can be broken down in the following way:

- P (population): a dental germ produced by tissue bioengineering
- I (intervention): using natural scaffolds and dental pulp stem cells
- C (comparison): using synthetic scaffolds and stem cells from another origin
- O (outcomes):
    - O1 – the presence of enamel-like tissues, dentin, cementum, odontoblast-like cells
    - O2 – regeneration of the tooth with distinguishable crown, root and pulp structures
    - O3 – level of cell viability, differentiation and proliferation.

Eligibility criteria:

The inclusion criteria were the following:

- Type of study – animal studies, *in vitro* experiments, clinical studies. Publication made in English, Spanish or Russian language, published until January 2024.
- Type of patient – studies in humans, animals, *in vitro, in vivo.*
- Type of intervention – dental regeneration using natural scaffolds and dental pulp stem cells.

- Type of control – dental regeneration using synthetic scaffolds and stem cells from another origin.
- Type of final measuring variables – studies that present distinguishable crown, root and pulp structures as the final result, or in their absence, enamel-like tissues, dentin, cementum, odontoblast-like cells. As secondary variables: level of cell viability, differentiation and proliferation overall and depending on the origin of scaffold used.

The exclusion criteria consisted of reviews, expert opinions, letters to the editor, studies where no histological analysis was performed, studies with the final goal of obtaining only dentin-pulp complex regeneration or differentiation into odontoblast-like cells, duplicate studies submitted to more than one journal.

There were no restrictions on the publication date.

Information sources and data search:

The automatized search was performed in 3 databases mentioned earlier: PubMed, Scopus, and Web Of Science. Keywords and MeSH (Medical Subject Headings) terms were combined with boolean logical operators AND and OR. The keywords used were the following: “tooth germ”, “bioengineering”, “scaffold”, “natural scaffold”, “decellularized scaffold”, “fibrin scaffold”, “stem cell”, “human pulp stem cells”, “dental stem cells”, “synthetic scaffold”, “hydrogel scaffold”, “whole-tooth regeneration”, “whole-tooth restoration”, “tooth regeneration”, “enamel”, and “dentin”.

In order to make sure that no potentially suitable studies were missed in the automatized searches, a manual search was performed through references found in the bibliographies of the selected studies and certain reviews used in the Introduction section.

Search strategy:

The selection of the studies was performed by the author of this review in 3 stages. The first stage consisted of filtering the studies by their title,

excluding all irrelevant ones. In the second stage, the summaries/abstracts of the studies were analysed and selected based on the type of study, scaffold materials, type of stem cells used, and the final measuring variables. The final stage was completed by reading the articles completely.

Data extraction:

The following data was extracted from the studies and presented in the tables later: name of the authors, year of publication, type of study (*in vitro, in vivo* studies), sample size (number of teeth, number of animals), bioengineering approach used, type of stem cells, type of scaffold, any bioactive agents used, presence of enamel-like tissues, presence of dentin, presence of cementum, presence of odontoblast-like cells, regeneration of dental crown, regeneration of dental root, regeneration of dental pulp, level of stem cell viability, stem cell differentiation, stem cell proliferation.

Risk of bias assessment:

The selected studies were assessed for risk of bias by the author of this systematic review. For the assessment of the quality of *in-vivo* animal studies ARRIVE guidelines 2.0 were used (https://arriveguidelines.org/arrive-guidelines). First assessing the Essential 10 items and then the Recommended Set.

Data synthesis:

In order to analyse and compare the final results between the selected studies, averages were drawn from the sample size/number of implants/teeth used in the studies.

Due to different sources of dental stem cells, different animal species, various evaluation times, and different scaffolds employed in the selected articles, a meta-analysis was not possible to perform. Instead of it, a qualitative systematic review was developed.

Results

Study selection:

251 articles were obtained from the initial search: Medline - PubMed (n=62), SCOPUS (n=117) and the Web of Science (n=72). After screening by title and abstract, 13 articles were found eligible. Their full-text versions were obtained and analyzed. Finally, 10 articles satisfied both inclusion and exclusion criteria and were included in this systematic review. The flowchart of the selection process performed can be found below (Fig. 1).

Study characteristics:

All 10 of the studies included in this systematic review were animal studies. The majority used the scaffold seeding bioengineering approach, apart from Ono et al. (7) and Zhang et al. (8) which employed a cell-tissue recombination approach.

A variety of animal species were used as models for implantation: mice, goats, dogs, and miniature pigs. Overall number of implants performed was 191: 25 were composed of natural scaffold (decellularized dentin matrix or fibrin glue with platelet-rich fibrin) (9,10), 57 of synthetic scaffold (11–15). Implants containing dental pulp stem cells, even if they were used in combination with other types of stem cells, were 113, while implants that only contained stem cells of an origin other than dental pulp were 78.

Only two studies used bioactive agents in the preparation of implants: Yang et al. (11) used TGF-β1, while Kuo et al. (13) preferred bone marrow fluid (Table 1).

Risk of bias:

All of the studies were assessed for the risk of bias using ARRIVE 2.0 guidelines. They complied with all of the requirements except for the following: none of the selected articles mentioned how the sample sizes were determined, nor anything regarding the inclusion and exclusion criteria for the animals. In addition to that, none of the studies (except for Toriumi et al. which only had the experimental group) mentioned anything about randomisation and blinding methods.

Only 2 studies mentioned statistical analysis used, and only 4 reported housing and husbandry conditions of the animals. Finally, 5 studies have not mentioned anything about possible conflicts of interest, and 2 of these have neither mentioned any funding sources.

Synthesis of results:

Presence of enamel-like tissues, dentin, cementum, odontoblast-like cells

In all studies analysed in this systematic review, all 4 types of tissues were identified after the implantation, except for Duailibi et al. where no cementum was produced (15).

In the natural scaffold group, the average regeneration percentages were 15% for enamel, 23.6% for dentin, 17.4% for cementum, and 23.6% for odontoblasts.

In the synthetic scaffold group, four studies provided data. Yang et al. achieved the highest regeneration percentages for all tissue types (93.8%) (11). The average results were 43.4% for enamel, 61.8% for dentin, 38.7% for cementum, and 61.8% for odontoblasts.

When analyzing studies based on the type of stem cells used, the DPSC group demonstrated the following results: 35.8% for enamel regeneration, 36.3% for dentin, 32.8% for cementum, and 47% for odontoblasts.

The group using SC from other origin also had four studies. The average percentages for this group were 34.9% for enamel, 53.3% for dentin, 18.7% for cementum, and 53.3% for odontoblasts (Table 2).

Regeneration of the tooth with distinguishable crown, root and pulp structures

In the natural scaffold group, the average percentage of implants with the 3 regenerated parts of the tooth was 20.5% (crown), 8.7% (root), 23.6% (pulp). While in the synthetic scaffold group, it was 53.2%, 18.8%, and 71.9%.

The group that utilized DPSC consisted of 3 studies that provided the following results: 38.9% (crown), 10.4% (root), 41% (pulp).

The final group was the one utilizing stem cells of origin other than dental pulp. In this group, also 3 studies provided the data. However, the result by Ono et al. was omitted from the analysis, even though it showed the highest percentage of implants that regenerated crown, root and pulp – 100%, they only tested one implant (7). This outlier was skewing the average of the whole group. The average percentage values for this group were 17.4%, 11.8%, and 36.1% (Table 3).

Effect of scaffold on the level of cell viability and differentiation

One study in the review reported cell viability values: Chang et al. (16) compared human dental pulp stem cells on decellularized dentin matrix (DDM), autoclaved DDM (a-DDM), and a control group without a scaffold. Stem cells on a-DDM showed significantly higher viability (260% on Day 5) compared to the control group (190% on Day 5), but there was no significant difference between a-DDM and DDM groups (230% on Day 5).

Stem cell differentiation was evaluated based on tissue production: enamel for ameloblasts, dentin for odontoblasts, and cementum for cementoblasts. Most studies implied successful differentiation into specialized cells, except for Duailibi et al. (15), where no cementum was produced.

Stem cell proliferation and differentiation

None of the studies explicitly mentioned stem cell proliferation values, but the regeneration of new tissues in all studies implies that proliferation occurred. Regarding differentiation, as previously noted, all studies except Duailibi et al. (15) demonstrated that the cells differentiated into ameloblasts, odontoblasts, and cementoblasts, as evidenced by the formation of new dental tissues.

Effect of bioactive agents on stem cell differentiation and proliferation

Two studies analysed in this systematic review utilized bioactive agents: Yang et al. (11) and Kuo et al. (13). Yang et al. used TGF-β1 and demonstrated the highest percentage in the regeneration of enamel, dentin,

cementum and odontoblasts, as well as the presence of distinguishable crown and pulp structures – 93.8%. Kuo et al. used bone marrow fluid and showed second-best results in the regeneration of dentin (75%), cementum (50%), odontoblasts (75%), and the presence of distinguishable pulp (50%).

Discussion

Presence of enamel-like tissues, dentin, cementum, odontoblast-like cells

The systematic review compared six studies and found that synthetic scaffolds are more effective than natural scaffolds in regenerating enamel, dentin, cementum, and odontoblasts. Notably, the study by Yang et al. (11) reported exceptionally high tissue regeneration percentages (93.8%), likely due to the use of TGF-β1, which promotes odontoblast-like differentiation of DPSCs as shown by He et al. (17). These findings indicate that the choice of scaffold material significantly impacts tissue regeneration outcomes. Further research is needed to understand the reasons behind these differences and to optimize dental tissue engineering strategies.

In comparing DPSC with SC of other origins, the results are more ambivalent. The first group showed higher effectiveness in regenerating cementum, while the second one demonstrated higher numbers in the regeneration of dentin and odontoblasts. The regeneration of enamel was slightly higher in the DPSC group (35.8% vs. 34.9%), but this difference is hardly significant. The higher cementum regeneration by DPSC is notable since cementoblasts are thought to be derived from dental follicle mesenchymal cells (18). However, a study by Mata et al. (19) supports the ability of DPSC to differentiate into cells that secrete a cementoid-like matrix, aligning with the findings of this review.

Regeneration of the tooth with distinguishable crown, root and pulp structures

Synthetic scaffolds significantly outperformed natural scaffolds in regenerating dental structures, showing at least double the regeneration

percentages for crown, root, and pulp. This success can likely be attributed to the use of gelatin-chondroitin-hyaluronan scaffolds, known for their biocompatibility and biodegradability (20). However, longer study durations for synthetic scaffolds, Yang et al. (54 weeks) and Kuo et al. (40 weeks), could have also contributed to their higher regeneration rates compared to shorter studies in the natural scaffolds group.

DPSCs were more effective in regenerating dental crown and pulp compared to SC from other sources, although root regeneration rates were similarly low between the two groups. The low percentages of root regeneration suggest the complexity of root formation and the need for extended timeframes. The longest study for DPSCs (Yang et al., 54 weeks) showed the highest regeneration rates, indicating the importance of study duration.

In conclusion, synthetic scaffolds and DPSCs show superior performance in dental regeneration. Future research should standardize study durations to optimize conditions for regenerating all tooth parts.

Effect of scaffold on the level of cell viability and differentiation

Although quantitative data on cell viability was limited, it is important to note that across all of the studies included in this systematic review, the scaffolds showed a significant tendency to support cell viability and promote proliferation, judging by the qualitative assessments. There have been some challenges in achieving specific cell differentiation outcomes. For example, the study conducted by Duailibi et al. reported difficulties in obtaining differentiation of dental bud SC into cementoblasts (15). This limitation could be attributed to the properties of the scaffold material used in that particular study – PGA/PLLA, PLGA, considering that other studies, Yang et al. (10), Kuo et al. (13), and Honda et al. (21), have shown successful differentiation of the same type of SC.

Stem cell proliferation and differentiation

Despite the lack of direct measurements of cell proliferation in the studies analysed in this systematic review, it seems reasonable to conclude that stem cells underwent substantial division as newly regenerated tissues were observed in all of the studies.

Additionally, as discussed in the previous section, all of the investigations (with the exception of Duailibi et al. (15)) reported successful cell differentiation into ameloblasts, odontoblasts, and cementoblasts, confirming that all types of stem cells compared in this review are capable of differentiation.

Effect of bioactive agents on stem cell differentiation and proliferation

Two studies analyzed in this systematic review, conducted by Yang et al. and Kuo et al., highlight the significant impact of these agents on dental structure regeneration (11,13). The study by Yang et al., which explored the efficacy of TGF-β1, demonstrated the highest percentage of regeneration of enamel, dentin, cementum, and odontoblasts, as well as the development of distinguishable crown and pulp structures. He et al. (17) and Li et al. (22) confirm the capability of TGF-β1 to induce odontogenic differentiation and subsequent dentin formation by DPSC. Meanwhile, research by Kuo et al., which utilized bone marrow fluid, aimed to develop a more cost-effective approach to obtaining GFs and morphogens from bone marrow stem cells, and also demonstrated notable advancements in dental tissue regeneration.

It must be taken into account that the bioactive agents were added to the implants only in the beginning, right before the implantation procedure. Like any other chemical substance, they lose their effect over time. In fact, this remains one of the main challenges in growth factor-based tissue regeneration approach – maintaining an optimum critical minimum therapeutic level over prolonged periods of time (23).

In conclusion, all analyzed techniques led to the regeneration of all 4 types of dental tissues, but using synthetic scaffolds and DPSC provided structures more similar to a natural tooth. All scaffolds supported stem cell viability and differentiation, except for PLA and its copolymers in their pure

form, which hindered the full differentiation potential. DPSC demonstrated the highest regeneration potential of dental tissues. The studies utilizing bioactive agents achieved good results but further research is necessary to clarify how they affect the process of regeneration over longer periods of time.

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Table 1. Characteristics of the selected studies.

Author, Year	Technique	Nº of implants	Origin of SC	Type of scaffold	Bioactive agents	Duration
Chang et al., 2020 (16)	SS	unclear	hDPSC	Decellularized dentin matrix	--	3 months
Toriumi et al., 2018 (12)	SS	8	Human iPS, DE, DM	Hydroxyapatite/PLGA	--	16 weeks
Zhang et al., 2017 (9)	SS	16	hDPSC, pDE, HUVEC	Decellularized tooth bud	--	3 or 6 months
Ono et al., 2017 (7)	CTR	37	DE, DM	Collagen	--	4 or 8 weeks, 180 days
Zhang et al., 2017 (8)	CTR	72	Human, gingival epithelium, hDPSC, pDE, pDM	Collagen	--	1 or 3 months
Yang et al., 2016 (11)	SS	16	DPSC, gingival epithelium	Gelatin-chondroitin-hyaluronan	TGF-β1	13.5 months
Yang et al., 2011 (10)	SS	9	Dental bud	Fibrin glue + platelet-rich fibrin	--	36 weeks
Kuo et al., 2010 (13)	SS	8	Dental bud	Gelatin-chondroitin-hyaluronan	Bone marrow fluid	40 weeks

Abukawa et al., 2009 (14)	SS	9	Pulp organ, enamel organ	PGA/PLLA, Gelfoam strips	--	12 or 20 weeks
Duailibi et al., 2008 (15)	SS	16	Dental bud	PGA/PLLA, PLGA	--	12 weeks

SC, stem cells; SS, scaffold seeding; CTR, cell-tissue recombination; hDPSC, human dental pulp stem cells; DE, dental epithelial cells; DM, dental mesenchymal cells; HUVEC, human umbilical vein endothelial cells; pDE, porcine dental epithelium; pDM, porcine dental mesenchyme.

Table 2. Average results on the presence/absence of enamel-like tissues, dentin, cementum, odontoblast-like cells

	Presence of			
Group	**Enamel-like tissues**	**Dentin**	**Cementum**	**Odontoblast-like cells**
Natural scaffold	15%	23.6%	17.4%	23.6%
Synthetic scaffold	43.4%	61.8%	38.7%	61.8%
Dental pulp SC	35.8%	36.3%	32.8%	47%
SC of other origin	34.9%	53.3%	18.7%	53.3%

Table 3. Average results on the regeneration of distinguishable crown, root and pulp structures

	Presence of distinguishable		
Group	**Crown**	**Root**	**Pulp**
Natural scaffold	20.5%	8.7%	23.6%
Synthetic scaffold	53.2%	18.8%	71.9%
Dental pulp SC	38.9%	10.4%	41%
SC of other origin	17.4%	11.8%	36.1%

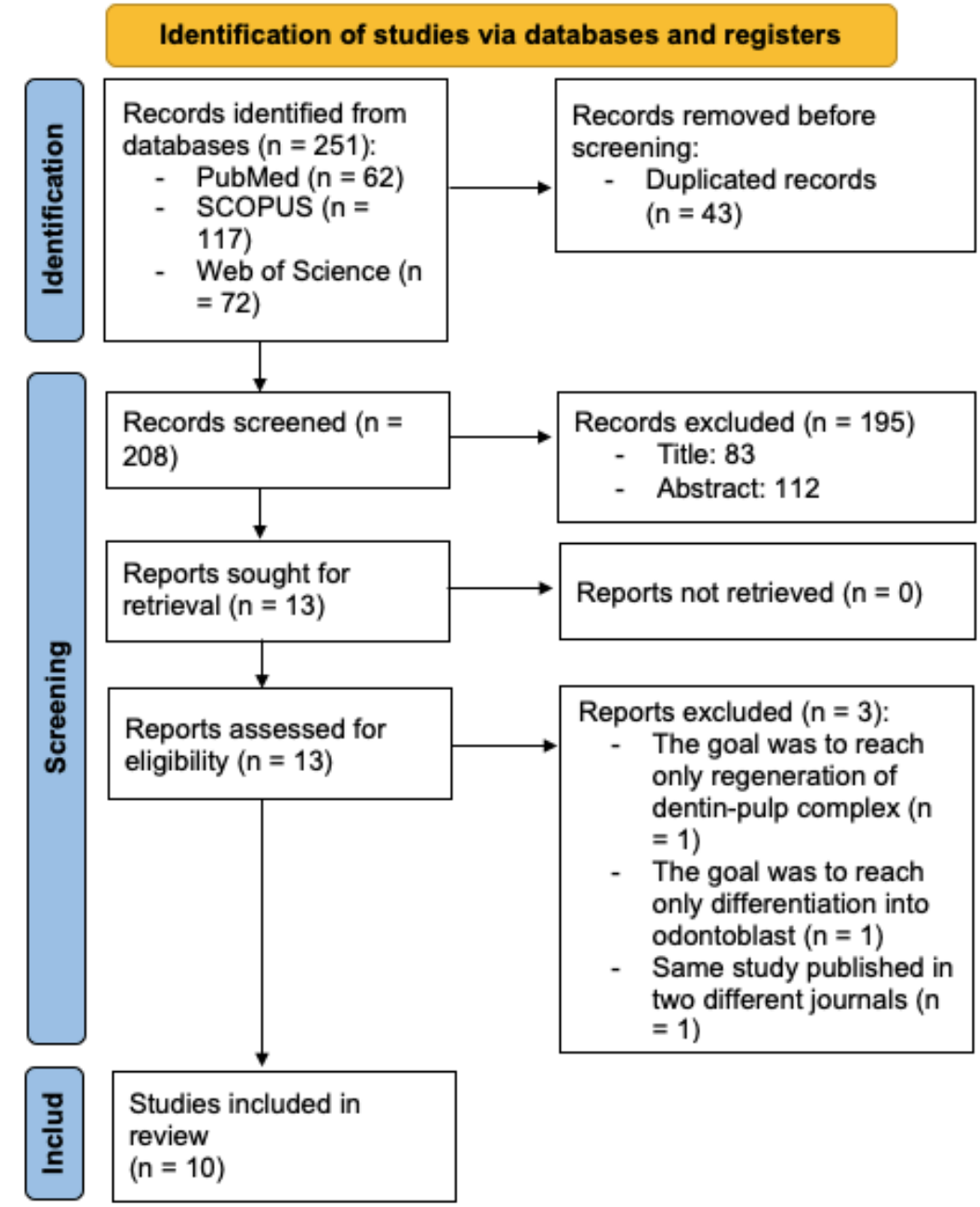

Fig. 1. Flowchart of the search and article selection process.

REGENERACIÓN DENTAL MEDIANTE BIOINGENIERÍA: REVISIÓN SISTEMÁTICA.

Título corto: Regeneración dental mediante bioingeniería: revisión sistemática.

Autores:

Daria Proshchenkova[1], Santiago Peydró Tomás[2]

[1] *Estudiante de 5º año de la carrera de Odontología en la Universidad Europea de Valencia, Valencia, España.*

[2] *Profesor de la Facultad de Odontología, Universidad Europea de Valencia, Valencia, España.*

Correspondencia:

Santiago Peydró Tomás

Paseo Alameda 7, Valencia

46010, Valencia

santiago.peydro@universidadeuropea.es

Resumen

Introducción: Esta revisión explora las técnicas de bioingeniería que utilizan células madre (CM) para la regeneración dental, destacando las metodologías actuales, las posibles aplicaciones clínicas y las perspectivas futuras en la ingeniería de tejidos dentales, comparando la efectividad de diferentes materiales de andamiaje y CM de diversos orígenes.

Materiales y métodos: Se realizó una búsqueda electrónica en las bases de datos PubMed, Scopus y Web of Science sobre la regeneración dental utilizando andamios naturales y sintéticos, y células madre de pulpa dental (CMPD) u otras CM hasta enero de 2024.

Resultados: De 251 artículos obtenidos en la búsqueda inicial, se eligieron 10 que cumplían con los criterios de inclusión y exclusión. En el grupo de andamios naturales, los porcentajes promedio de esmalte, dentina, cemento y células similares a odontoblastos regenerados fueron 15%, 23.6%, 17.4%, y 23.6%. En el grupo de andamios sintéticos, los valores fueron 43.4%, 61.8%, 38.7%, y 61.8%. Para la regeneración de estructuras diferenciables de corona, raíz y pulpa, el grupo de andamios naturales mostró los siguientes resultados: 20.5%, 8.7%, 23.6%; el grupo de andamios sintéticos: 53.2%, 18.8%, y 71.9%. El grupo de CMPD regeneró 35.8% (esmalte), 36.3% (dentina), 32.8% (cemento), y 47% (odontoblastos), mientras que el grupo de CM de otro origen regeneró 34.9%, 53.3%, 18.7%, y 53.3%. Para la regeneración de partes dentales, el grupo de CMPD presentó 38.9% (corona), 10.4% (raíz), 41% (pulpa), mientras que el otro grupo de CM mostró 17.4%, 11.8%, y 36.1%.

Conclusiones: Todas las técnicas analizadas llevaron a la regeneración de los cuatro tipos de tejidos dentales, pero el uso de andamios sintéticos y CMPD proporcionó estructuras más similares a un diente natural. Todos los andamios apoyaron la viabilidad y diferenciación de las células madre, excepto el PLA y sus copolímeros en su forma pura, que dificultaron el potencial de diferenciación completo. Las CMPD demostraron el mayor potencial de regeneración de tejidos dentales. Los estudios que utilizaron agentes bioactivos lograron buenos resultados, pero se necesita más investigación para aclarar cómo afectan el proceso de regeneración a lo largo del tiempo.

Palabras claves: *stem cell regeneration, dental regeneration, natural scaffolds, synthetic scaffolds, dental pulp stem cells, bioengineering*

Introducción

Se han propuesto e investigado varios enfoques nuevos para regenerar un diente completo en el campo de la ingeniería de tejidos biológicos. Los dos principales son la recombinación célula-tejido y el uso de andamios (1,2).

El enfoque de recombinación célula-tejido dental se basa en el uso de un germen dental. Los tejidos epiteliales y mesenquimales se aíslan y se disocian completamente en células individuales, que luego se utilizan para reconstituir un germen dental bioingenierizado. El nuevo germen dental recombinado se implanta en el sitio del defecto en la mandíbula, donde se desarrolla en un diente completo (3).

En el enfoque de ingeniería de tejidos basado en el uso de andamios, las células madre (CM) se siembran en/sobre un andamio, donde luego proliferan y se diferencian en otros tipos de células (4). Esta estructura se suministra con agentes bioactivos, como factores de crecimiento (FC), que controlan la organización espacial y temporal de la proliferación, diferenciación y función de las células progenitoras dentales (1). Cada uno de los tres elementos principales involucrados en este método tiene muchas variaciones que inevitablemente afectan el resultado final.

La elección del andamio es muy importante, ya que sus aspectos físicos y su composición deben garantizar un soporte físico para el desarrollo de nuevos tejidos de una manera que imite la función de la matriz extracelular (MEC) natural (5). CM son células que presentan una renovación continua y una posible diferenciación en múltiples tipos de células especializadas. (6). En el proceso de odontogénesis, están involucrados dos tipos diferentes de células madre: las células epiteliales dentales, que posteriormente dan lugar al esmalte, y las células ectomesenquimales, responsables de la producción de dentina, pulpa, cemento y ligamento periodontal.

El objetivo principal de esta revisión es identificar la técnica más efectiva en la regeneración de un diente completo a partir de un germen dental producido por bioingeniería de tejidos que proporcione tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos, y presente estructuras distinguibles de corona, raíz y pulpa. Como objetivo secundario, se investigó el efecto de

diferentes andamios sobre la viabilidad y diferenciación celular, el tipo de células madre con la mayor capacidad de diferenciación y proliferación, así como el efecto de los agentes bioactivos en la diferenciación y proliferación celular.

Material y métodos

Esta revisión sistemática se desarrolló siguiendo las directrices PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Pregunta PICO:

Para regenerar un diente completo, con estructuras de corona, raíz y pulpa, así como tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos, a partir de un germen dental producido por bioingeniería de tejidos, ¿es más efectivo usar andamios naturales y células madre de pulpa dental (CMPD) que andamios sintéticos y células madre de otro origen?

Esta pregunta siguió la estructura PICO y puede desglosarse de la siguiente manera:

- P (población): un germen dental producido por bioingeniería de tejidos.
- I (intervención): uso de andamios naturales y células madre de pulpa dental.
- C (comparación): uso de andamios sintéticos y células madre de otro origen.
- O (resultados):
    - O1: presencia de tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos.
    - O2: regeneración del diente con estructuras distinguibles de corona, raíz y pulpa.
    - O3: nivel de viabilidad celular, diferenciación y proliferación.

Criterios de elegibilidad:

Los criterios de inclusión fueron los siguientes:

- Tipo de estudio: estudios en animales, experimentos in vitro, estudios clínicos. Publicaciones en idioma inglés, español o ruso, publicadas hasta enero de 2024.
- Tipo de paciente: estudios en humanos, animales, in vitro, in vivo.
- Tipo de intervención: regeneración dental utilizando andamios naturales y células madre de pulpa dental.

- Tipo de control: regeneración dental utilizando andamios sintéticos y células madre de otro origen.
- Tipo de variables de medición final: estudios que presenten estructuras distinguibles de corona, raíz y pulpa como resultado final, o en su ausencia, tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos. Como variables secundarias: nivel de viabilidad celular, diferenciación y proliferación en general y según el origen del andamio utilizado.

Los criterios de exclusión consistieron en revisiones, opiniones de expertos, cartas al editor, estudios en los que no se realizó análisis histológico, estudios con el objetivo final de obtener solo regeneración del complejo dentino-pulpar o diferenciación en células similares a odontoblastos, estudios duplicados presentados en más de una revista.

No hubo restricciones en la fecha de publicación.

Fuentes de información y estrategia de búsqueda:

La búsqueda automatizada se realizó en 3 bases de datos mencionadas anteriormente: PubMed, Scopus y Web Of Science. Las palabras clave y los términos MeSH se combinaron con operadores lógicos booleanos AND y OR. Las palabras clave utilizadas fueron las siguientes: “tooth germ”, “bioengineering”, “scaffold”, “natural scaffold”, “decellularized scaffold”, “fibrin scaffold”, “stem cell”, “human pulp stem cells”, “dental stem cells”, “synthetic scaffold”, “hydrogel scaffold”, “whole-tooth regeneration”, “whole-tooth restoration”, “tooth regeneration”, “enamel”, y “dentin”.

Para asegurarse de que no se pasaran por alto estudios potencialmente adecuados en las búsquedas automatizadas, se realizó una búsqueda manual a través de las referencias encontradas en las bibliografías de los estudios seleccionados y ciertas revisiones utilizadas en la sección de Introducción.

Proceso de selección de los estudios:

La selección de los estudios se realizó por el autor de esta revisión en 3 etapas. La primera etapa consistió en filtrar los estudios por su título, excluyendo todos los irrelevantes. En la segunda etapa, se analizaron y seleccionaron los resúmenes de los estudios en función del tipo de estudio, los materiales de

andamiaje, el tipo de células madre utilizadas y las variables de medición finales. La etapa final se completó leyendo completamente los artículos.

Extracción de datos:

Los siguientes datos fueron extraídos de los estudios y presentados en las tablas más adelante: nombre de los autores, año de publicación, tipo de estudio (estudios in vitro, in vivo), tamaño de la muestra (número de dientes, número de animales), enfoque de bioingeniería utilizado, tipo de células madre, tipo de andamio, cualquier agente bioactivo utilizado, presencia de tejidos similares al esmalte, presencia de dentina, presencia de cemento, presencia de células similares a odontoblastos, regeneración de la corona dental, regeneración de la raíz dental, regeneración de la pulpa dental, nivel de viabilidad de células madre, diferenciación de células madre, proliferación de células madre.

Evaluación del riesgo de sesgo:

Los estudios seleccionados fueron evaluados por el autor de esta revisión sistemática para determinar el riesgo de sesgo. Para la evaluación de la calidad de los estudios in vivo en animales, se utilizaron las directrices ARRIVE 2.0 (https://arriveguidelines.org/arrive-guidelines). Primero se evaluaron los 10 ítems esenciales y luego el conjunto recomendado.

Síntesis de datos:

Para analizar y comparar los resultados finales entre los estudios seleccionados, se calcularon promedios a partir del tamaño de la muestra/número de implantes/dientes utilizados en los estudios.

Debido a las diferentes fuentes de células madre dentales, diferentes especies animales, diversos tiempos de evaluación y diferentes andamios empleados en los artículos seleccionados, no fue posible realizar un metaanálisis. En lugar de eso, se desarrolló una revisión sistemática cualitativa.

Resultados

Selección de estudios:

Se obtuvieron 251 artículos de la búsqueda inicial: Medline - PubMed (n=62), SCOPUS (n=117) y Web of Science (n=72). Después de revisar por título y resumen, se encontraron 13 artículos elegibles. Se obtuvieron y analizaron sus versiones completas. Finalmente, 10 artículos cumplieron tanto con los criterios

de inclusión como de exclusión y se incluyeron en esta revisión sistemática. El diagrama de flujo del proceso de selección realizado se puede encontrar al final de la revisión.

Características de los estudios:

Los 10 estudios incluidos en esta revisión sistemática fueron estudios en animales. La mayoría de los estudios utilizaron el enfoque de bioingeniería de siembra de andamios, aparte de Ono et al. (7) y Zhang et al. (8), que emplearon un enfoque de recombinación célula-tejido.

Una variedad de especies animales se utilizaron como modelos para la implantación: ratones, cabras, perros y cerdos en miniatura. El número total de implantes realizados fue de 191: 25 estaban compuestos de andamios naturales (matriz de dentina descelularizada o pegamento de fibrina con fibrina rica en plaquetas) (9,10), 57 de andamios sintéticos (11–15). Los implantes que contenían CMPD, incluso si se usaban en combinación con otros tipos de CM, fueron 113, mientras que los implantes que solo contenían CM de otro origen que no fuera la pulpa dental fueron 78.

Solo dos estudios utilizaron agentes bioactivos en la preparación de los implantes: Yang et al. (11) utilizaron TGF-β1, mientras que Kuo et al. (13) prefirieron el fluido de médula ósea (Tabla 1).

Riesgo de sesgo:

Todos los estudios fueron evaluados en cuanto al riesgo de sesgo utilizando las directrices ARRIVE 2.0. Cumplieron con todos los requisitos excepto los siguientes: ninguno de los artículos seleccionados mencionó cómo se determinaron los tamaños de muestra, ni nada sobre los criterios de inclusión y exclusión para los animales. Además de eso, ninguno de los estudios (excepto Toriumi et al., que solo tenía el grupo experimental) mencionó nada sobre métodos de aleatorización y enmascaramiento.

Solo 2 estudios mencionaron el análisis estadístico utilizado, y solo 4 informaron las condiciones de alojamiento y cría de los animales. Finalmente, 5 estudios no mencionaron nada sobre posibles conflictos de interés, y 2 de estos tampoco mencionaron ninguna fuente de financiación.

Síntesis de resultados:

Presencia de tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos:

En todos los estudios analizados en esta revisión sistemática, se identificaron los 4 tipos de tejidos después de la implantación, excepto en Duailibi et al., donde no se produjo cemento (15).

En el grupo de andamios naturales, los porcentajes promedio de regeneración fueron del 15% para el esmalte, 23.6% para la dentina, 17.4% para el cemento y 23.6% para las células similares a odontoblastos. En el grupo de andamios sintéticos, cuatro estudios proporcionaron datos. Yang et al. logró los porcentajes de regeneración más altos para todos los tipos de tejidos (93.8%) (11). Los resultados promedio fueron del 43.4% para el esmalte, 61.8% para la dentina, 38.7% para el cemento y 61.8% para los odontoblastos.

Al analizar los estudios según el tipo de células madre utilizadas, el grupo de CMPD demostró los siguientes resultados: 35.8% para la regeneración del esmalte, 36.3% para la dentina, 32.8% para el cemento y 47% para los odontoblastos. El grupo que utilizó CM de otro origen también contó con cuatro estudios. Los porcentajes promedio para este grupo fueron del 34.9% para el esmalte, 53.3% para la dentina, 18.7% para el cemento y 53.3% para los odontoblastos (Tabla 2).

Regeneración del diente con estructuras distinguibles de corona, raíz y pulpa:

En el grupo de andamios naturales, el porcentaje promedio de implantes con las 3 partes regeneradas del diente fue del 20.5% (corona), 8.7% (raíz), 23.6% (pulpa). Mientras que en el grupo de andamios sintéticos, fue del 53.2%, 18.8% y 71.9%.

El grupo que utilizó CMPD consistió en 3 estudios que proporcionaron los siguientes resultados: 38.9% (corona), 10.4% (raíz), 41% (pulpa). El último grupo fue el que utilizó CM de otro origen que no fuera la pulpa dental. En este grupo, también 3 estudios proporcionaron los datos. Sin embargo, el resultado de Ono et al. se omitió del análisis, a pesar de que mostró el porcentaje más alto de implantes que regeneraron corona, raíz y pulpa - 100%, solo probaron un implante (7). Este valor atípico estaba sesgando el promedio de todo el grupo.

Los valores de porcentaje promedio para este grupo fueron del 17.4%, 11.8% y 36.1% (Tabla 3).

Efecto del andamio en el nivel de viabilidad celular y diferenciación:

Un estudio en la revisión informó valores de viabilidad celular: Chang et al. (16) compararon CMPD humana en matriz de dentina descelularizada (DDM), DDM autoclavada (a-DDM) y un grupo de control sin andamio. Las células madre en a-DDM mostraron una viabilidad significativamente mayor (260% en el día 5) en comparación con el grupo de control (190% en el día 5), pero no hubo diferencia significativa entre los grupos a-DDM y DDM (230% en el día 5).

La diferenciación de las CM se evaluó según la producción de tejido: esmalte para ameloblastos, dentina para odontoblastos y cemento para cementoblastos. La mayoría de los estudios implicaron una diferenciación exitosa en células especializadas, excepto en Duailibi et al. (15), donde no se produjo cemento.

Proliferación y diferenciación de células madre

Ninguno de los estudios mencionó explícitamente los valores de proliferación de células madre, pero la regeneración de nuevos tejidos en todos los estudios implica que la proliferación ocurrió. En cuanto a la diferenciación, como se señaló anteriormente, todos los estudios excepto Duailibi et al. (15) demostraron que las células se diferenciaron en ameloblastos, odontoblastos y cementoblastos, como lo evidencia la formación de nuevos tejidos dentales.

Efecto de los agentes bioactivos en la diferenciación y proliferación de células madre

Dos estudios analizados en esta revisión sistemática utilizaron agentes bioactivos: Yang et al. (11) y Kuo et al. (13). Yang et al. utilizaron TGF-β1 y demostraron el mayor porcentaje en la regeneración de esmalte, dentina, cemento y células similares a odontoblastos, así como la presencia de estructuras de corona y pulpa distinguibles: 93.8%. Kuo et al. utilizaron fluido de médula ósea y mostraron los segundos mejores resultados en la regeneración de dentina (75%), cemento (50%), células similares a odontoblastos (75%) y la presencia de pulpa distinguible (50%).

Discusión

Presencia de tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos

La revisión sistemática comparó seis estudios y encontró que los andamios sintéticos son más efectivos que los andamios naturales en la regeneración de esmalte, dentina, cemento y células similares a odontoblastos. Notablemente, el estudio de Yang et al. (11) informó porcentajes excepcionalmente altos de regeneración de tejidos (93.8%), probablemente debido al uso de TGF-β1, que promueve la diferenciación similar a odontoblastos de las CMPD, como mostró He et al (17). Estos hallazgos indican que la elección del material del andamio impacta significativamente en los resultados de la regeneración de tejidos. Se necesita más investigación para comprender las razones detrás de estas diferencias y para optimizar las estrategias de ingeniería de tejidos dentales.

En la comparación entre CMPD y CM de otras procedencias, los resultados son más ambivalentes. El primer grupo mostró una mayor efectividad en la regeneración de cemento, mientras que el segundo demostró mayores números en la regeneración de dentina y odontoblastos. La regeneración de esmalte fue ligeramente mayor en el grupo CMPD (35.8% vs. 34.9%), pero esta diferencia es apenas significativa. La mayor regeneración de cemento por CMPD es notable, ya que se cree que los cementoblastos se derivan de células mesenquimatosas del folículo dental (18). Sin embargo, un estudio de Mata et al. (19) respalda la capacidad de las CMPD para diferenciarse en células que secretan una matriz similar a cemento, alineándose con los hallazgos de esta revisión.

Regeneración del diente con estructuras distinguibles de corona, raíz y pulpa

Los andamios sintéticos superaron significativamente a los andamios naturales en la regeneración de estructuras dentales, mostrando al menos el doble de los porcentajes de regeneración para corona, raíz y pulpa. Este éxito probablemente se deba al uso de andamios de gelatina-condroitina-hialuronano, conocidos por su biocompatibilidad y biodegradabilidad (20). Sin embargo, las duraciones de estudio más largas para los andamios sintéticos, Yang et al. (54 semanas) y Kuo et al. (40 semanas), también podrían haber contribuido a sus tasas de

regeneración más altas en comparación con estudios más cortos en el grupo de andamios naturales.

Las CMPD fueron más efectivas en la regeneración de la corona dental y la pulpa en comparación con las CM de otras fuentes, aunque las tasas de regeneración de la raíz fueron igualmente bajas entre los dos grupos. Los bajos porcentajes de regeneración de la raíz sugieren la complejidad de la formación de la raíz y la necesidad de marcos temporales extendidos. El estudio más largo para CMPD (Yang et al., 54 semanas) mostró las tasas de regeneración más altas, lo que indica la importancia de la duración del estudio.

En conclusión, los andamios sintéticos y las CMPD muestran un rendimiento superior en la regeneración dental. La investigación futura debería estandarizar las duraciones de estudio para optimizar las condiciones para regenerar todas las partes del diente.

Efecto del andamio en el nivel de viabilidad celular y diferenciación

Aunque los datos cuantitativos sobre la viabilidad celular fueron limitados, es importante destacar que en todos los estudios incluidos en esta revisión sistemática, los andamios mostraron una tendencia significativa a apoyar la viabilidad celular y promover la proliferación, según lo juzgado por las evaluaciones cualitativas. Ha habido algunos desafíos para lograr resultados específicos de diferenciación celular. Por ejemplo, el estudio realizado por Duailibi et al. informó dificultades para obtener la diferenciación de las CM del brote dental en cementoblastos (15). Esta limitación podría atribuirse a las propiedades del material del andamio utilizado en ese estudio en particular, PGA/PLLA, PLGA, considerando que otros estudios, Yang et al. (10), Kuo et al. (13), y Honda et al. (21), han mostrado diferenciación exitosa del mismo tipo de CM.

Proliferación y diferenciación de células madre

A pesar de la falta de medidas directas de proliferación celular en los estudios analizados en esta revisión sistemática, parece razonable concluir que las CM experimentaron una división sustancial ya que se observaron tejidos recién regenerados en todos los estudios.

Además, como se discutió en la sección anterior, todas las investigaciones (con la excepción de Duailibi et al. (15)) informaron una diferenciación celular exitosa en ameloblastos, odontoblastos y cementoblastos, confirmando que todos los tipos de células madre comparados en esta revisión son capaces de diferenciación.

Efecto de los agentes bioactivos en la diferenciación y proliferación de las células madre

Dos estudios analizados en esta revisión sistemática, realizados por Yang et al. y Kuo et al., resaltan el impacto significativo de estos agentes en la regeneración de la estructura dental (11,13). El estudio de Yang et al., que exploró la eficacia de TGF-β1, demostró el mayor porcentaje de regeneración de esmalte, dentina, cemento y odontoblastos, así como el desarrollo de estructuras de corona y pulpa distinguibles. He et al. (17) y Li et al. (22) confirman la capacidad de TGF-β1 para inducir la diferenciación odontogénica y la formación subsiguiente de dentina por CMPD. Mientras tanto, la investigación de Kuo et al., que utilizó fluido de médula ósea, tuvo como objetivo desarrollar un enfoque más rentable para obtener GFs y morfógenos de CM de médula ósea, y también demostró avances notables en la regeneración de tejido dental.

Debe tenerse en cuenta que los agentes bioactivos se agregaron a los implantes solo al principio, justo antes del procedimiento de implante. Como cualquier otra sustancia química, pierden su efecto con el tiempo. De hecho, este sigue siendo uno de los principales desafíos en el enfoque de regeneración de tejidos basado en factores de crecimiento: mantener un nivel terapéutico mínimo crítico óptimo durante períodos prolongados de tiempo (23).

En conclusión, todas las técnicas analizadas llevaron a la regeneración de los 4 tipos de tejidos dentales, pero el uso de andamios sintéticos y CMPD proporcionó estructuras más similares a un diente natural. Todos los andamios apoyaron la viabilidad y diferenciación de las células madre, excepto el PLA y sus copolímeros en su forma pura, que obstaculizaron el potencial completo de diferenciación. Las CMPD demostraron el mayor potencial de regeneración de tejidos dentales. Los estudios que utilizaron agentes bioactivos lograron buenos

resultados, pero se necesita más investigación para aclarar cómo afectan el proceso de regeneración durante períodos más largos.

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Tabla 1. Características de los estudios incluidos

Autor, Año	**Técnica**	**Nº de implantes**	**Origen de CM**	**Tipo de andamio**	**Agentes bioactivos**	**Duración**
Chang y cols., 2020 (16)	UA	No claro	*CMPDh*	Matriz de dentina descelularizada	--	3 meses
Toriumi y cols., 2018 (12)	UA	8	iPS humanas, DE, DM	Hidroxiapatita /PLGA	--	16 semanas
Zhang y cols., 2017 (9)	UA	16	*CMPDh*, pDE, HUVEC	Brote dental descelularizado	--	3 o 6 meses
Ono y cols., 2017 (7)	RCT	37	DE, DM	Colágeno	--	4 u 8 semanas, 180 días
Zhang y cols., 2017 (8)	RCT	72	Epitelio gingival humano, *CMPDh*, pDE, pDM	Colágeno	--	1 o 3 meses
Yang y cols., 2016 (11)	UA	16	*CMPD*, epitelio gingival	Gelatin-chondroitin-hyalurona n	TGF-β1	13.5 meses
Yang y cols., 2011 (10)	UA	9	Brote dental	Pegamento de fibrina + fibrina rica en plaquetas	--	36 semanas

Kuo y cols., 2010 (13)	UA	8	Brote dental	Gelatin-chondroitin-hyaluronan	Líquido de médula ósea	40 semanas
Abukawa y cols., 2009 (14)	UA	9	Órgano pulpar, órgano del esmalte	PGA/PLLA, tiras de Gelfoam	--	12 o 20 semanas
Duailibi y cols., 2008 (15)	UA	16	Brote dental	PGA/PLLA, PLGA	--	12 semanas

CM, células madre; UA, uso de andamios; RCT, recombinación célula-tejido; CMPDh, células madre de pulpa dental humana; DE, células epiteliales dentales; DM, células mesenquimales dentales; HUVEC, células endoteliales de vena umbilical humana; pDE, epitelio dental porcino; pDM, mesénquima dental porcino.

Tabla 2. Los resultados promedio sobre la presencia o ausencia de tejidos similares al esmalte, dentina, cemento y células similares a odontoblastos

	Presencia de			
Grupo	**Tejidos similares al esmalte**	**Dentina**	**Cemento**	**Células similares a odontoblastos**
Andamios naturales	15%	23.6%	17.4%	23.6%
Andamios sintéticos	43.4%	61.8%	38.7%	61.8%
CM de pulpa dental	35.8%	36.3%	32.8%	47%
CM de otro origen	34.9%	53.3%	18.7%	53.3%

Tabla 3. Los resultados promedio sobre la regeneración de las estructuras de corona, raíz y pulpa

	Presencia de distinguibles		
Grupo	**Corona**	**Raíz**	**Pulpa**
Andamios naturales	20.5%	8.7%	23.6%
Andamios sintéticos	53.2%	18.8%	71.9%
CM de pulpa dental	38.9%	10.4%	41%
CM de otro origen	17.4%	11.8%	36.1%

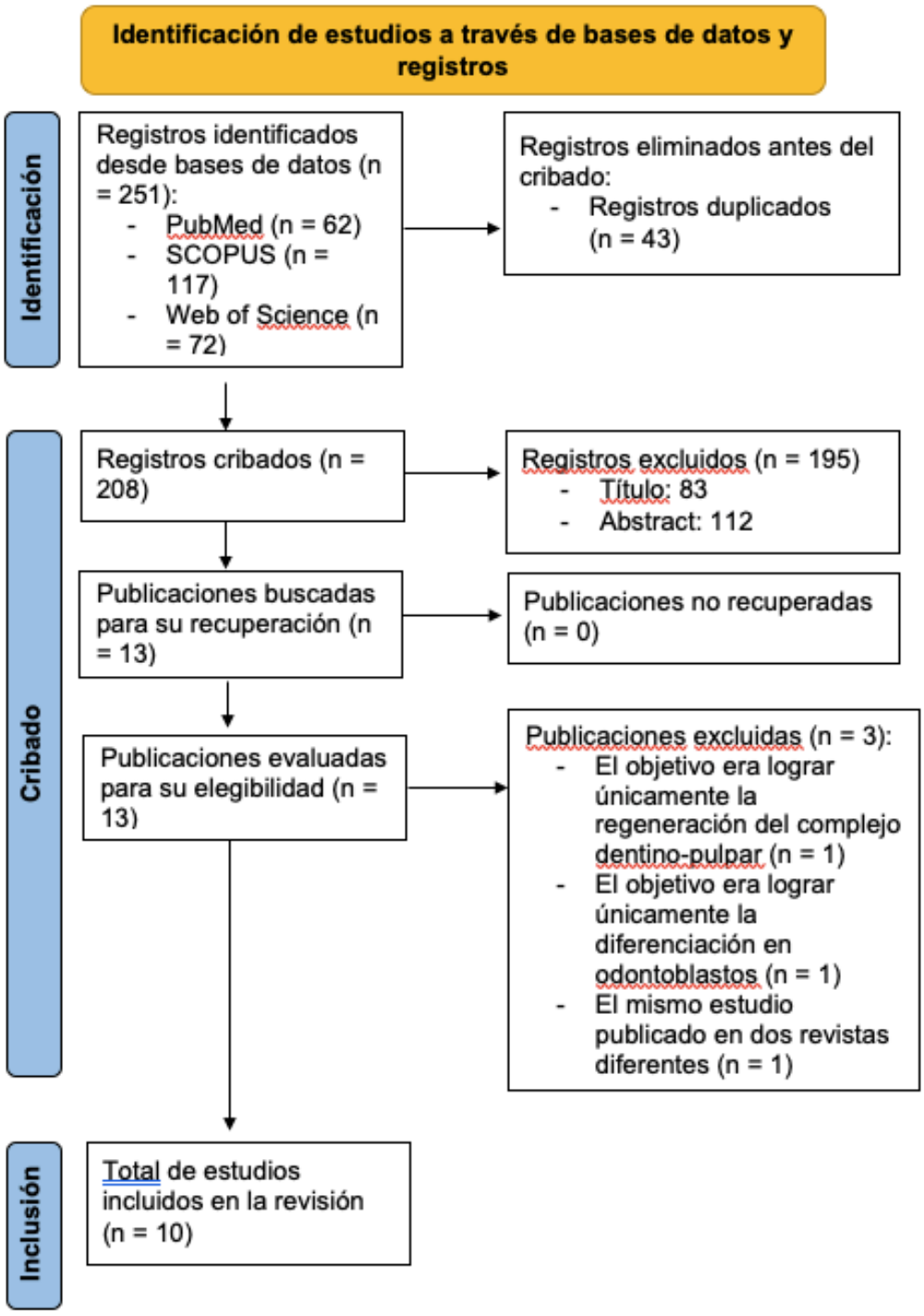

Fig. 1. Diagrama de flujo del proceso de búsqueda y selección de artículos.