UNIVERSIDADE FERNANDO PESSOA

Hydrogel-based systems used in Dentistry to promote Tissue Regeneration

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Dissertação de Mestrado

Mestrado Integrado em Medicina Dentária

Adam Daniel Sellam

Orientador:

Doutora Carla Patrícia Fernandes Pereira

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À mes parents Brigitte et Hubert,

À ma grand-mère Simone,

À mon grand frère Jérémie et ma grande sœur Samantha,

Je dédie ce travail à vous, qui m'aimez, avez cru en moi et accompagné dans cette aventure.

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RESUMO

As doenças orais representam um grave e crescente problema de saúde pública, a nível mundial, tornando-se um desafio acentuado nos países desenvolvidos e em desenvolvimento.

Na medicina dentária, inúmeros tratamentos encontram-se já bem estabelecidos para o tratamento de uma vasta gama de patologias. Porém, a utilização de materiais eficazes que permitam uma rápida e melhor previsibilidade da regeneração dos tecidos dentários, com uma minimização dos erros cirúrgicos/efeitos secundários e um melhor fluxo operacional, é ainda restrita. De modo a ultrapassar estas limitações, é imperativo o desenvolvimento de novos materiais capazes de serem utilizados como matrizes extracelulares sintéticas na regeneração de tecidos, os quais exibam boa biocompatibilidade, biodegradabilidade e propriedades mecânicas flexíveis. Neste contexto, os sistemas baseados em hidrogéis têm-se afirmado como materiais promissores que exibem as caraterísticas mencionadas, sendo eficazes na mimetização do microambiente celular.

Esta revisão narrativa possui como foco a sistematização/análise das capacidades dos sistemas à base de hidrogéis em medicina dentária para promover a regeneração de tecidos – regeneração periodontal, da polpa dentária e remineralização do esmalte, sendo também descritas as caraterísticas gerais desta classe de materiais - classificação, estratégias de preparação, sistema de caraterização, propriedades peculiares e aplicações gerais.

A pesquisa bibliográfica foi realizada nas bases de dados PubMed, Scopus e Web of Science, utilizando as palavras-chave: "*Hydrogel*", '*Oral tissue*', '*Dental tissue*', '*Regeneration*', '*Tissue regenerative engineering*' e '*Dentistry*' combinadas entre si com o operador booleano *AND*, e *OR*. Nesta revisão, foram utilizadas as diretrizes PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*), tendo sido incluídas um total de noventa e uma publicações após o processo de análise de adequação dos artigos ao assunto aqui abordado.

O desenvolvimento de materiais à base de hidrogel que atuam como transportadores na entrega de células estaminais, fármacos e agentes terapêuticos constitui um importante campo de investigação, o qual se encontra ainda numa fase inicial na medicina dentária. Estes materiais apresentam um enorme potencial na regeneração dos tecidos dentários em medicina dentária moderna, devido às suas caraterísticas peculiares e às suas caraterísticas físicas e químicas passíveis de serem moldadas, o que lhes confere a capacidade de mimetizar a matriz extracelular, a libertação de moléculas sinalizadoras e a regulação do comportamento das células estaminais. No entanto, o desenvolvimento de processos de preparação mais fáceis e mais apelativos do ponto de vista económico e a falta de estudos de segurança robustos constituem, em certa medida, os principais problemas na translação da investigação do meio académico para a indústria.

Palavras-chave: Hidrogel, Medicina Dentária, Regeneração, Doença Periodontal, Polpa Dentária, Remineralização de Esmalte

ABSTRACT

Oral diseases represent a serious and growing worldwide public health, becoming a pronounced challenge in developed and developing countries.

Numerous treatments are already well-established in dentistry in order to treat a vast range of pathologies. Still, the use of effective materials prompting the fast and improved predictability of dental tissue regeneration with a minimization of surgical errors/side effects and an enhanced operational flow is still restricted. To overcome these limitations, the development of new materials capable of being used as synthetic extracellular matrices in tissue regeneration with good biocompatibility, biodegradability, and flexible mechanical properties is mandatory. In this context, the hydrogel-based systems have been established as promising materials that exhibit the mentioned features, being effective in mimetizing the native cell microenvironment.

This narrative review focuses on the systematization/analysis of the hydrogel-based systems capabilities in dentistry to promote tissue regeneration – periodontal, dental pulp regeneration, and enamel remineralization, being also described the general features of this class of materials – classification, preparation strategies, characterization system, peculiar properties, and general applications.

The literature survey was carried out in the PubMed, Scopus, and Web of Science databases using the keywords: "Hydrogel", "Oral tissue", "Dental tissue", "Regeneration", "Tissue regenerative engineering" and "Dentistry" combined with each other with the Boolean operator AND, and OR. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were used, being included in this review a total of ninety one publications after the screening process.

The development of hydrogel-based materials acting as carriers in the delivery of stem cells, drugs, and therapeutic agents is an exciting research field, that is still in an early stage in dentistry. These materials exhibit a huge potential in the dental tissue regeneration in modern dental medicine due to their peculiar features and tunable physical and chemical characteristics that allow them to mimetize the extracellular matrix, the release of signal molecules and the regulation of the stem cell behavior. However, the development of easier and more appealing preparation processes from an economic point of view and the lack of robust safety studies to some extent constitute the main issues in translation research from academia to industry.

Keywords: Hydrogel, Dentistry, Regeneration, Periodontal disease, Dental pulp; Enamel remineralization

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LIST OF ACRONYMS AND ABBREVIATIONS

ASA	Acetylsalicylic acid
ALP	Alkaline phosphatase
β-GP	*β*-glycerol phosphate
BMP	Bone morphogenetic protein
BMSCs	Bone marrow-derived stem cells
DPSC	Dental pulp stem cells
ECM	Extracellular matrix
EPO	Erythropoietin
FGF	Fibroblast growth factor
GelMA	Gelatin Methacryloyl
GMSCs	Gingival mesenchymal stem cells
HA	Hyaluronic Acid
HAP	Hydroxyapatite
hDPSCs	Human dental pulp stem cells
hMSCs	Human marrow-derived stem cells
IPN	Interpenetrating polymer networks
iPSC	Induced pluripotent stem cells
MMP	Matrix metalloproteinase
PAA	Polyacrylic acid
PAs	Peptide amphiphiles
PDLSCs	Periodontal ligament stem cells
PECs	Polyelectrolyte complexes
PEG	Polyethylene glycol
PLA	Polylactic Acid
PLGA	Poly(Lactic-co-Glycolic Acid)

PVA	Polyvinyl alcohol
REP	Regenerative endodontic procedure
SAP	Self-assembling peptide
SCAP	Stem cells from the apical dental papilla
SHED	Stem cells from human exfoliated deciduous teeth
TGF	Transforming growth factor
UV	Ultraviolet
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

Oral diseases represent a significant and growing public health concern worldwide, affecting individuals in developed and developing countries. The oral cavity and maxillofacial region are constituted by different anatomical structures, and the pathologies associated with this region generally conduce to structural defects that can be irreversible, which not only affect function and quality of life, but also have a profound effect on patients' overall physical and mental well-being (Huang et al., 2022).

When a defect concerns a part of tissue or organ, transplant surgery can be performed. In this scenario, to avoid immune rejection and other complications, it is possible in some cases to opt for autologous transplants, where the patient's tissue is taken from another part of the body, increasing the risk of donor-site morbidity (Moro et al., 2018).

The tissue engineering - an interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain or enhance tissue function - offers a way to overcome these difficulties, enabling damaged or defective tissue to be replaced by tissue regenerated from the patient's cells. There are three main strategies for its application: i) the use of isolated cells or cell substitutes; ii) the use of tissue-inducing substances, and finally, iii) the use of cells placed inside or on matrices (made from natural materials or synthetic polymers) (Mantha et al., 2019; Chen et al., 2023).

Hydrogels have received increased attention over the years, being established as excellent materials to be applied in various biomedical applications, namely as scaffolding systems in tissue engineering and regenerative medicine due to their peculiar properties. They are three-dimensional, insoluble polymer networks made from natural or synthetic materials that exhibit unique characteristics, including biocompatibility, biodegradability, high water content, and flexible mechanical properties. Because of these attractive features, they can effectively mimic the native cell microenvironment, which renders the capability to be used as synthetic extracellular matrices in tissue engineering and regenerative medicine. (Ahmed, 2015; Chen et al., 2023).

The hydrogel-based systems closely resemble natural soft tissues and exhibit excellent biological properties. Their ability to interact intrinsically with cells makes them excellent materials to be used in the human body (Huang et al., 2022; Sun et al., 2023; Chen et al.,

2024). The preparation of hydrogel-based materials with living cells and growth factors allows the stimulation of the healing or regeneration of damaged or missing tissue. In line with this latter approach, hydrogels offer a range of possibilities in dentistry. Indeed, oral diseases represent a major public health problem worldwide (Gomez-Florit et al., 2020).

Dentistry has come a long way and is now able to treat a wide range of pathologies, but some treatments remain limited, such as the use of composite resins, which can cause secondary caries after aging, root canal fillings during root canal treatment, which do not improve tooth fragility, and periodontal techniques which can cause side effects such as tooth sensitivity, among others. This is why the use of innovative biomaterials meets the major challenges of tissue regeneration in dentistry (Chen et al., 2024).

Hydrogels can, therefore, be used in a wide range of applications in the oral sphere, such as: periodontitis, pulpal necrosis, initial dental caries and control of antimicrobial activity (Zhang et al., 2019; Chen et al., 2024), but also, future prospects are just as promising for salivary gland tissue regeneration (Rose et al., 2023).

This narrative review intends to overview the current knowledge on hydrogel-based systems capabilities in dentistry to promote tissue regeneration. The classification of hydrogels based on source, as well as their preparation, characterization, unique properties, and general applications, are described. The studies on the application of hydrogel materials for the periodontal and dental pulp regeneration, as well as for the enamel remineralization, were collected and discussed herein.

2. DEVELOPMENT

2.1. Study Selection

This review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021). A bibliographic search was conducted using PubMed, Scopus, and Web of Science. The article search was performed from each database with the keywords “Hydrogel”, “Oral Tissue”, “Dental tissue”, “Regeneration”, “Tissue regenerative engineering” and “Dentistry” combined with the booleans operators “AND” and “OR”. The search covered articles published between 2014 and August 2024. In the search filters, the "free full text" and English language were selected.

2.1.1. Literature survey and screening process

In the preliminary bibliographic survey in the above-mentioned databases, 706 articles were obtained. The literature survey is shown in table 1.

Table 1

Literature survey of published research articles, using a search query with the following keywords: “Hydrogel”, “Oral Tissue”, “Dental tissue”, “Regeneration”, “Tissue regenerative engineering” and “Dentistry” combined with the booleans operators “AND” and “OR”, from 2014 to August 2024 via PubMed, Web of Science and Scopus.

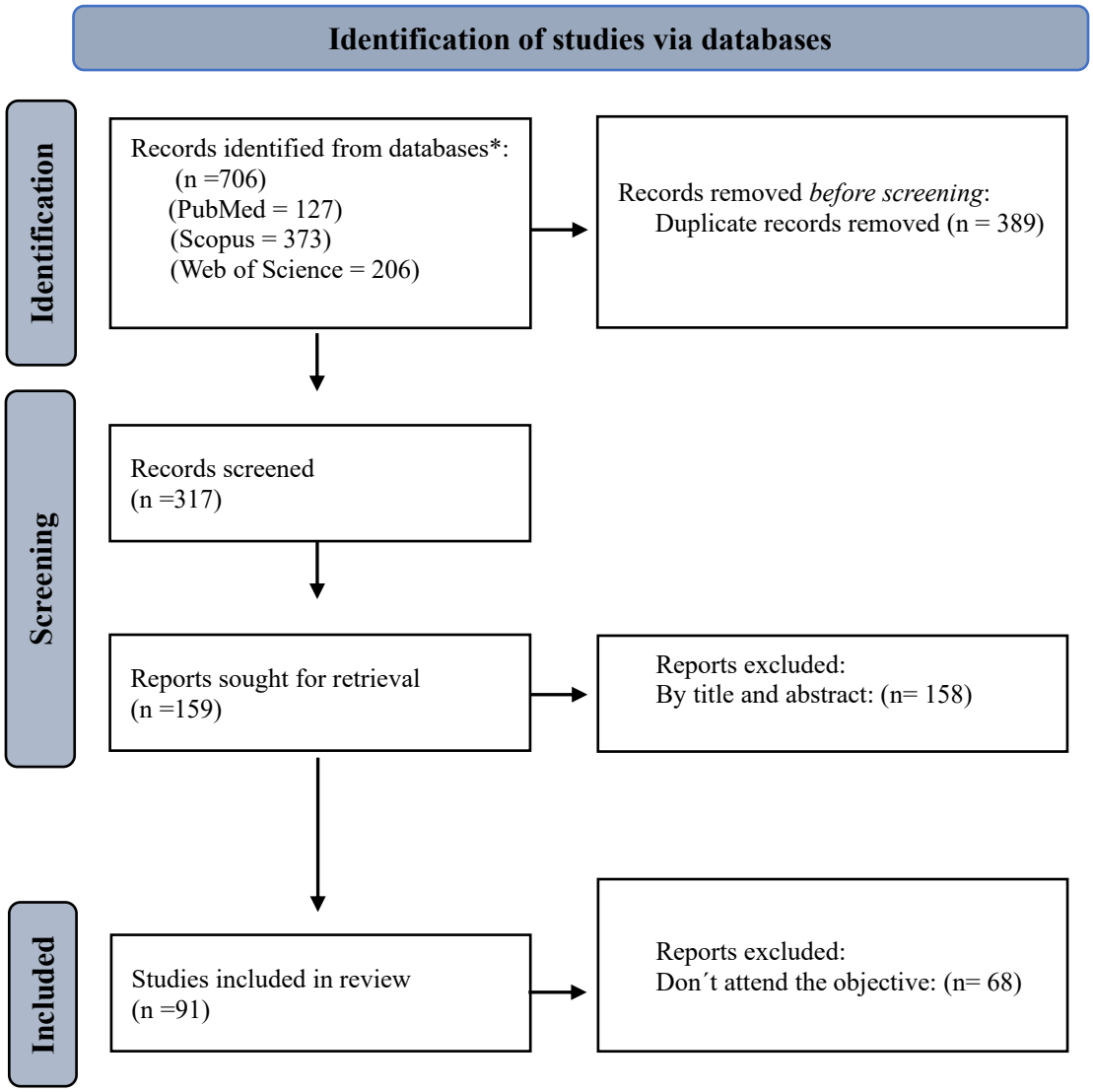
Databases	Search Terms	Results
PubMed	(Hydrogel AND (oral tissue) AND (tissue regenerative engineering) AND dentistry AND (periodontal OR (dental pulp) OR remineralization))	35
	(Hydrogel AND (dental tissue) AND (regeneration) AND (dentistry) AND (periodontal OR (dental pulp) OR remineralization))	92
Scopus	(Hydrogel AND (oral tissue) AND (tissue regenerative engineering) AND dentistry AND (periodontal OR (dental pulp) OR remineralization))	158
	(Hydrogel AND (dental tissue) AND (regeneration) AND (dentistry) AND (periodontal OR (dental pulp) OR remineralization))	215

Web of Science	((((TS=(Hydrogel)) AND TS=(dental tissue)) AND TS=(regeneration)) AND ALL=(dentistry)) AND TS=(periodontal)	39
	((((TS=(Hydrogel)) AND TS=(dental tissue)) AND TS=(regeneration)) AND ALL=(dentistry)) AND TS=(dental pulp)	151
	((((TS=(Hydrogel)) AND TS=(dental tissue)) AND TS=(regeneration)) AND ALL=(dentistry)) AND TS=(remineralization)	4
	((((TS=(Hydrogel)) AND TS=(oral tissue)) AND TS=(tissue regenerative engineering)) AND ALL=(dentistry)) AND TS=(periodontal)	6
	((((TS=(Hydrogel)) AND TS=(oral tissue)) AND TS=(tissue regenerative engineering)) AND ALL=(dentistry)) AND TS=(dental pulp)	5
	((((TS=(Hydrogel)) AND TS=(oral tissue)) AND TS=(tissue regenerative engineering)) AND ALL=(dentistry)) AND TS=(remineralization)	1

After the literature survey (Table 1), the screening process was performed according to the PRISMA instructions, as follows: after duplicates removal (n=389), 317 publications remained. By the lecture of title and abstract,158 articles were excluded, and 68 revealed that they did not attend to the purpose, which led to the selection of 91 publications that are included in this review.

Figure 1

PRISMA diagram (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).



Legend: n (Number)**.**

2.2. Hydrogel-based materials: Classification, Preparation, Characterization and Properties

This section encompasses the main characteristics of hydrogels, namely their classification, preparation mode, characterization system, and properties. This data is essential to understand the various hydrogel-based systems developed for dental tissue regeneration.

2.2.1. Classification of Hydrogels based on source

Hydrogels can be classified according to their origins as natural, synthetic or hybrid (Ur Rehman et al., 2020). Naturally derived hydrogels are obtained from animals, plants, and microorganisms and possess exceptional biocompatibility and biodegradability. These materials mainly include hydrogels of collagen, cellulose, sodium alginate, and hyaluronic acid, gelatin and chitosan (Sun et al., 2021). On the other hand, synthetic hydrogels are predominantly derived from synthetic polymers, which not only provide good biocompatibility but also enhance mechanical properties. Polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyacrylic acid (PAA) are commonly used in the preparation of synthetic hydrogels (Chen et al., 2023; Sun et al., 2024). Hybrid hydrogels were classified as a combination of natural and synthetic hydrogels (Liao et al., 2024).

2.2.1.1. Natural Hydrogels

Collagen-Based Hydrogels

The primary element of the extracellular matrix (ECM) in various mammalian tissues, collagen offers vital support and protection to the organism and its organs because of its remarkable coagulation properties. Researchers have shown considerable interest in collagen-based hydrogels, thanks to their low immunogenicity and favorable biocompatibility (Mahmood et al., 2022; Chen et al., 2023).

Cellulose

Cellulose is the most abundant natural polymer, which exhibits excellent biocompatibility, biodegradability, and mechanical properties. It is a linear polymer composed of β-1,4-glycosidic bonds that can be obtained from natural sources like wood and cotton (Ye et al., 2018). Cellulose and its derivatives have applications in dentistry (Zheng et al., 2020), including carboxymethylcellulose, hydroxy-ethylcellulose, methylcellulose, and bacterial cellulose (Tasza et al., 2020; Raut et al., 2023).

Sodium alginate

Sodium alginate is a polyanionic polysaccharide primarily derived from brown seaweed and bacteria. The sodium alginate-based hydrogels have important applications in wound healing, drug delivery, and cell transplantation because of their high-water content and favorable biocompatibility (Li et al., 2022).

Hyaluronic Acid (HA) Hydrogels

Hyaluronic acid (HA), a non-sulfated glycosaminoglycan consisting of two repeating units of saccharides, *N*-acetyl-D-glucosamine and D-glucuronic acid is present in all connective tissue ECM, ranks among the most widely utilized natural polymers today. Research indicates that HA is vital in various biological processes, including angiogenesis, inflammation, ECM structure, and wound healing (Lam et al., 2014).

Gelatin Hydrogels

Gelatin, a hydrophilic polymer, exhibits remarkable sol–gel transition characteristics and biocompatibility, rendering a highly adaptable substance in hydrogels. When utilized as a matrix, gelatin-based hydrogels can replicate diverse tissue properties and enable the customization of hydrogel attributes, including mechanical strength and degradation rates, to meet the needs of numerous biomedical applications (Mahmood et al., 2022).

Agarose Hydrogel

Agarose is a natural polysaccharide obtained from red algae, consisting of D-galactose and 3,6-anhydro-L-galactose repeating units. This material is used in the biomedical industry as a natural source of gel-forming polysaccharides that can form antimicrobial surfaces. Agarose, aside from acting as an excellent vehicle for drug-delivery systems, has an excellent biocompatibility and can be used as a hydrogel for tissue engineering applications (Jiang et al., 2023).

Chitosan-based hydrogel

Chitosan is the only naturally occurring alkaline polysaccharide which contains free amino groups. Chitosan-based materials can be cross-linked into chemical and physical hydrogels through methods such as ultraviolet irradiation, pH change, and temperature variation. The main feature here results from the tuning of its properties by the introduction of functional reactive groups with specific properties offering a wide range of chitosan derivatives *via* chemical modification. Chitosan-based hydrogel systems have

good biocompatibility, degradability, anti-inflammatory activity, broad-spectrum antibacterial action and also allow for adhesion growth and osteogenic differentiation (Ma et al., 2022; Guo et al., 2024).

2.2.1.2. Synthetic Hydrogels

Polylactic Acid (PLA)

Polylactic acid (PLA), a synthetic polymer that is biodegradable and sourced from plentiful materials, finds extensive application in the biomedical field thanks to its remarkable biocompatibility, minimal toxicity, biodegradability, straightforward processing, and eco-friendliness. It is possible to blend PLA with both natural and synthetic polymers to develop biodegradable PLA hydrogels. Additionally, PLA can undergo copolymerization with synthetic materials like polyethylene glycol to create PLA–PEG hydrogels, which have demonstrated the ability to enhance the differentiation of dental pulp stem cells *in vitro*. Furthermore, natural organic polymers, including polysaccharides and peptides, have been integrated into PLA to form hydrogels aimed at applications in tissue engineering, drug delivery, and wound healing (Munim et al., 2019; Chen et al., 2023).

Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) is a widely used synthetic polymer in biology due to its non-immunogenic, non-toxic, biodegradable, and highly hydrophilic nature. PEG-based hydrogels offer unique advantages in drug delivery and controlled release. However, they do not provide optimal conditions for cell survival, adhesion, and development because they are inherently biologically inert (Li et al., 2022; Guo et al., 2024).

Gelatin Methacryloyl (GelMA)

Gelatin methacryloyl (GelMA), a methacryloyl-derivatized copolymer of gelatin, can be dissolved in water and many organic solvents, allowing it to be used in three-dimensional bioprinting. It is lightly cross-linkable using ultraviolet light or redox chemistry at low concentrations while also having tunable mechanical properties. The ability to photo pattern cells within the material after encapsulation was also shown with a high-resolution technique, making it suitable for tissue engineering (Klotz et al., 2016; Li et al., 2024).

2.2.1.3. Hybrid Hydrogels

Hybrid hydrogels are created by cross-linking two or more polymers, thus merging each component's merits and obtaining the hydrogels' final superior properties. Multinetwork systems have polymers that include double to quintuple-network hydrogels (Liao et al., 2024).

2.2.2. Preparation

There are two main classifications for hydrogels based on the mechanisms that drive their formation: cross-linking and self-assembly. Cross-linking involves the creation of bonds between polymer chains, resulting in a network with viscoelastic properties. These bonds can be either covalent or non-covalent, leading to different physical and mechanical characteristics. On the other hand, self-assembly is a process where molecular units arrange themselves into organized structures of different shapes and sizes (Chen et al., 2024).

2.2.2.1. Covalently Cross-linked Hydrogels

Chemically cross-linked hydrogels, also known as hydrogels cross-linked by covalent bonds, consist of two types of covalent bonds: conventional covalent bonds and dynamic covalent bonds. These bonds create strong and irreversible junctions between polymer chains, resulting in hydrogels with exceptional mechanical properties and resistance to degradation (Hu et al., 2019). Various chemical cross-linking techniques, such as photopolymerization, enzymatic-induced cross-linking, oxime cross-linking, Michael-addition, and Schiff base formation, are employed to fabricate these polymeric networks. Once formed, these permanent cross-links are challenging to break down and remodel, limiting their applications in tissue engineering and cell culture. Additionally, thorough washing is necessary to eliminate toxic cross-linking agents from chemically cross-linked hydrogels (Chen et al., 2024).

A novel form of cross-link known as dynamic covalent interactions has emerged in the realm of hydrogels. By incorporating dynamic covalent bonds, hydrogels gain a level of flexibility and adaptability that sets them apart from static hydrogels (Webber & Tibbit, 2022). This means that they can degrade at a faster rate and undergo remodeling with greater ease. These dynamic covalent bonds can be broken and reformed under specific stimuli, allowing for reversibility. There are three main categories of dynamic covalent interactions based on the types of chemical reactions involved: exchange reactions

involving disulfides, diselenides, and thioesters; reversible addition/condensation reactions like Diels-Alder reactions involving imines, hydrazones, oximes, and boronic esters; and enzymatic covalent reactions utilizing enzymes such as monoamine oxidase B, catalase, plasma amine oxidase, and urease (Han & Cao, 2022). The presence of these dynamic bonds imparts new properties to materials, including controlled degradation, adjustable stiffness, responsiveness to environmental cues, moldability, and self-healing capabilities. As a result, the range of biomedical applications for these materials expands significantly (Perera & Ayres, 2022).

2.2.2.2. Noncovalently Cross-linked Hydrogels

Physical hydrogels, also known as noncovalent hydrogels, are formed by cross-linking polymer chains through noncovalent bonds. These bonds are a result of various physical interactions, including electrostatic interactions, hydrophobic interactions, metal-ligand coordination, hydrogen bonds, and host-guest interactions (Bertsch et al., 2022). These interactions play a crucial role in preventing the dissolution of hydrophilic polymers. One key advantage of noncovalent hydrogels is their lack of potential toxicity, as they do not require the use of chemical cross-linking agents during synthesis. Additionally, noncovalent hydrogels possess the unique ability to self-heal. This means that when subjected to high shear stress, they can temporarily fluidize and then reform their original structures once the stress is removed. However, it is important to note that the mechanical properties of these hydrogels are often suboptimal due to their reliance on relatively weak noncovalent interactions (Chen et al., 2024).

Polyelectrolyte complexes (PECs) can be formed by utilizing electrostatic interactions between oppositely charged polymers (Hu et al.,2019). One example of this is the combination of chitosan, a natural cationic polymer, with various anionic polyelectrolytes like alginate, pectin, and chondroitin sulfate. The properties of hydrogels made from PECs can be controlled by adjusting parameters such as charge density, mixing ratio, and solubility environment of the polymers. If the net charge of the compositional polymers in a PEC is zero, it may lead to decreased solubility and precipitation (Ullah et al., 2015). The equilibrium swelling ratio of PECs, which determines the amount of solvent within the hydrogel at equilibrium, is crucial for their applications. Even minor changes in solvent composition can induce reversible volume phase transitions, impacting permeability and molecular transport rates. In contrast to PECs, synthetic zwitterionic polymers can form physical hydrogels through intra/inter-molecular interactions of

copolymerized anionic and cationic building blocks, resulting in exceptional toughness and viscoelasticity (Bertsch et al., 2023).

The formation of hydrogen bonds occurs when a hydrogen atom, which is bound to an electronegative atom, interacts with another electronegative atom that possesses lone pairs of electrons. Hydrogels can be created through the process of hydrogen bonding, which involves the interaction between different functional groups such as amides, carboxylic acids, carbazole, and hydroxyl groups. Additionally, these functional groups can also interact with electron donors like pyridine and imidazole (Lu et al., 2018). In a study conducted by Sharma et al., a hydrogel was developed by cross-linking the network using flexible pendant sidechains of chitosan (a hydrogen donor) and acryloyl phenylalanine (a hydrogen acceptor). This particular hydrogel demonstrates impressive self-healing properties (Sharma et al., 2018).

Typically, metal-ligand coordination involves the sequestration of metallic ions by organic ligands through a process known as chelation. This interaction is classified as a Lewis acid-base interaction, which falls between noncovalent bonds and covalent interactions in terms of strength (Li et al., 2019).

Interactions between a host molecule and one or more guest molecules, known as host-guest interactions, are a unique type of hydrophobic interaction. Host molecules, such as cucurbiturils, cyclodextrins, crown ethers, and calixarenes, possess a spacious cavity volume that allows for interaction with guest molecules that match their form (Zou et al., 2019a, 2019b; Zou & Webber, 2019). Zhu et al. conducted a study on a thermosensitive hydrogel formed through orthogonal self-assembly, where poly(*N*-isopropylcrylamide) served as the guest and cyclodextrin acted as the host. Their findings revealed that the hydrogel demonstrates rapid self-healing properties and can repeatedly undergo self-assembly in response to brief cooling stimuli (Zhu et al., 2020).

2.2.2.3. Self-Assembled Hydrogels

Self-assembled hydrogels are a unique type of physical hydrogel that form through the spontaneous arrangement of small molecules into ordered structures, without the need for external intervention (Du et al., 2015). Various types of self-assembling molecules, such as peptides, recombinant proteins, DNA, synthetic small molecules, and copolymers, have been extensively studied. These molecules serve as the building blocks that can autonomously assemble into supramolecular nanostructures when placed in an aqueous

solution. The resulting self-assembled hydrogels possess remarkable biocompatibility, targeting capabilities, and biomedical safety, making them ideal for applications in the development of intelligent materials, biomedical agents, and drug delivery systems. The assembly process is driven by intramolecular forces, including van der Waals forces, electrostatic interactions, hydrophobic interactions, hydrogen bonds, and π–π stacking. The strength and dynamics of these noncovalent interactions profoundly influence the mechanical properties, bioactivity, and morphology of self-assembled hydrogels (Long et al., 2020).

Peptides have the ability to adopt various secondary structural motifs, including α-helices, β-sheets, β-hairpins, and coiled coils. Additionally, they can self-assemble into different nanostructures such as fibers, micelles, ribbons, and vesicles (Chen et al., 2024). These self-assembled peptide hydrogels offer modularity, enabling the attachment of different bioactive modules to a peptide sequence. Peptide amphiphiles (PAs), which are derivatives of peptides, consist of a hydrophilic peptide connected to a hydrophobic group, typically an alkyl chain. The hydrophobic tails of PAs facilitate the aggregation of the hydrophilic ends, resulting in the formation of three-dimensional networks. These amphiphilic hydrogels with entangled network structures have been extensively studied as drug carriers due to their noncytotoxic nature and high drug-loading capacities. Moreover, the three-dimensional nanofibrous networks formed by the self-assembly of PAs can serve as biomimetic hydrogel scaffolds for tissue engineering, leveraging their biocompatibility and high porosity (Sis et al., 2022).

2.2.3. Characterization

Based on the components of their network and how the network strands and junctions are linked, hydrogels can be classified into monolithic, fibrous, granular and polymer-particle hydrogels (Chen et al., 2024).

2.2.3.1. Monolithic Hydrogels

Monolithic hydrogels are the most common hydrogels, characterized by cross-linked polymer networks formed by a single species of monomers. These hydrogels exhibit a homogeneous three-dimensional structure and gel-like behavior, typically achieved at high polymer concentrations and cross-linking densities. The properties of monolithic hydrogels are highly dependent on factors such as monomer type, network mesh size, and cross-linking density (Bertsch et al., 2023; Chen et al., 2024)

2.2.3.2. Fibrous Hydrogels

Fibrous hydrogels are formed through supramolecular assembly, resulting in a fibrous architecture that mimics the mechanical properties of the ECM, such as its strain-stiffening behavior (Chen et al., 2023).

Fibrous hydrogels thus have some key characteristics: i) cytocompatibility, enabling cell encapsulation without the requirement for potentially toxic crosslinkers, ii) high porosity and high mechanical responsiveness, and iii) modularity, facilitating the integration of biochemical signals, such as cell-adhesive motifs (Włodarczyk-Biegun, 2016; Shoenmakers et al., 2018).

2.2.3.3. Granular Hydrogels

Granular hydrogels, also called microgels, are made up of jammed microparticles based on hydrogels. In contrast to colloidal hydrogels, these granular hydrogels exhibit viscoelastic properties on a macroscale due to their densely packed arrangement and the presence of micron-sized voids. These voids create a porous and interconnected network that facilitates cell migration and proliferation (Riley et al., 2018).

2.2.3.4. Polymer-particle Hydrogels

A polymer–particle hydrogel is alternatively formed by cross-linking or embedding particles within polymeric networks. The particle-crosslinked hydrogels are composed of networks developed from the multivalent and dynamic interactions (which include electrostatic, hydrophobic and coordination) among polymers and (nano)particles. The affinity of particle-crosslinked hydrogels towards mechanical properties relies explicitly on three key parameters: i) the interaction between (nano)particles alongside polymers, ii) size of the (nano)particles concerning the persistence length amount of polymers and iii) density of cross-linking (Meis et al., 2021).

Polymer-particle hydrogels can be prepared to have tunable mechanical properties and tissue adherence features, apart from biocompatibility, biodegradability, and non-immunogenicity. Also modified is their ability to integrate sites for conjugating bioactive signaling molecules, such as physical barriers that prevent postoperative adhesion. Moreover, cargo release from polymer-particle hydrogels may be retarded because of the smaller mesh size as compared to other varieties of hydrogels like monolithic hydrogels, fibrous gels, and granular gels. They can also be used for cell delivery vehicles with

enhanced cell viability since the high mechanical strength of polymer-particle hydrogels protects the cells from the pressures during injection (Chen et al., 2023).

2.2.3.5. Interpenetrating Network Hydrogels

Interpenetrating polymer networks (IPN) are defined as polymers consisting of at least two polymer networks that are interwoven at a molecular level without any covalent attachment. Semi-IPNs occur when a linear polymer penetrates a cross-linked network. On the other hand, full IPNs occur when both components are cross-linked. The major benefit of IPNs is the development of dense hydrogel matrices with improved mechanical features, efficient drug loading, and controlled physical properties. Common polymers for IPN hydrogels are natural polymers and their derivatives (polysaccharides and proteins) and synthetic polymers having hydrophilic functional groups. IPNs can comprise synthetic hydrophilic polymers or a combination of natural proteins and synthetic polymers (Vedadghavami et al., 2018; Liao et al., 2024).

2.2.4. Properties

This section summarizes the main properties of hydrogels to be used in the tissue regeneration. These properties encompass the biocompatibility, biodegradability, swelling, and mechanical performance.

2.2.4.1. Biocompatibility

Biocompatibility, a vital factor in the clinical application of hydrogel scaffolds for tissue engineering and regenerative medicine, encompasses two key elements. Firstly, the material must be able to withstand interaction with the organism without causing any rejection or damage. Additionally, the reactions during this process should be devoid of inflammation and carcinogenicity (Chyzy & Plonska-Brzezinska, 2020; Crawford et al., 2021). To address the issue of poor biocompatibility, researchers employ various techniques such as the use of synthetic conjugated polymers and the incorporation of ions to enhance tissue repair and reconstruction (Mantha et al., 2019; Zhang et al., 2022).

2.2.4.2. Biodegradability

The advantageous feature of hydrogel materials lies in their ability to biodegrade. This process involves the gradual breakdown of the material within the body through various mechanisms such as dissolution, enzymatic digestion, and cell engulfment. When used for tissue repair, the regenerated tissue completely replaces the implanted hydrogel,

leaving no residual material behind (Xia et al., 2021). It is important for hydrogel materials to exhibit controlled biodegradability that matches the rate of cell growth and tissue repair. If degradation occurs too quickly, the mechanical integrity may be lost before complete tissue regeneration, whereas slow degradation can delay the healing process. Therefore, determining the appropriate degradation rate is a critical factor in hydrogel design. Fortunately, researchers have made significant advancements in this area, employing techniques such as surface modification, polymer blending, and incorporation of alkaline particles to enhance biodegradability (Gupta et al., 2024).

2.2.4.3. Swelling

Hydrogels are materials with an interconnected structure that can absorb significant amounts of water and swell in biological settings without breaking down. The process of hydrogel expansion begins with the diffusion of water into the hydrogel network, followed by the unraveling of polymer chains, and culminates in the growth of the hydrogel framework. To measure the swelling characteristics of hydrogels, the swelling ratio is defined as the weight-swelling ratio between the swollen gel and the dry gel. Various factors, including the cross-linking ratio, ionic environment, synthesis method, and polymer's chemical structure, can affect equilibrium and swelling kinetics (Chyzy & Plonska-Brzezinska, 2020; Chen et al., 2023).

2.2.4.4. Mechanical performance

The hydrogel possesses mechanical characteristics on both macroscopic and microscopic levels. At the macroscopic scale, the hydrogel scaffold provides stability and maintains volume (Mantha et al., 2019; Belgodere et al., 2023). On a microscopic level, cells attached to the hydrogel matrix have the ability to perceive mechanical stimuli, converting them into biochemical signals that regulate vital physiological processes (Li et al., 2022). Hydrogels have vast potential for use in bone tissue engineering. Enhancing the mechanical performance of hydrogels is crucial for improving the efficacy of bone defect repair (Zhao et al., 2022). Insufficient mechanical properties of the hydrogel material can lead to easy deformation of the repaired defect and a lack of early-stage support. To enhance the mechanical performance of hydrogels, researchers have employed various methods, including the construction of double network structures (Guo et al., 2021), the utilization of composite nanotechnology (Cui et al., 2019), the introduction of conductive materials (Arambula-Maldonado et al., 2023), and the reinforcement of fiber networks (Brusentsev et al., 2023).

2.2.5. General Applications of Hydrogels

Hydrogels have been explored for several applications, such as drug delivery, antimicrobial activity, in the biosensors field, and tissue engineering. In this subsection, these applications are briefly analyzed.

2.2.5.1. Drug Delivery

Hydrogels have been extensively explored for drug delivery owing to their biocompatibility and porosity. The tunability of the polymeric network structures along with pore size, allows the delivery of the drug at a predecided concentration to a specific region for predefined periods. Also, in these solid networks, diffusion-, swelling-, or stimulus by breaking chemical bond-controlled fashion with drugs is trivial; otherwise, feasible mechanisms (Narayanaswamy & Torchilin, 2019).

2.2.5.2. Antimicrobial activity

The application of antimicrobials is of special importance in the treatment of bacterial-associated diseases. The advantages of hydrogels include but are not limited to local delivery, sustained and controlled release, targeted delivery, and excellent biocompatibility. Antimicrobial hydrogels may act as carriers for antimicrobial agents: inorganic nanoparticles, antibiotics, polymers, and peptides among others, or be used as antimicrobial materials by themselves (Li et al., 2018; Chen et al., 2023).

Although the loading of antimicrobial agents into hydrogels is the most common and easy way to do so, intrinsic antimicrobial hydrogels have several advantages related to their enhanced functionality. Polymers and peptides are the two primary classes of intrinsically antimicrobial hydrogels. Antimicrobial polymer-based hydrogels are synthesized by covalently binding active agents to a polymer matrix. Peptide-based ones are formed through the self-assembly of AMPs into hydrogel networks. The antimicrobial activity of these materials is strongly linked to the cationic charge, as well as cross-linking density and hydrophobicity, which enables interactions, between polymers or peptides (Yang et al., 2018).

2.2.5.3. Biosensors

Hydrogels are growingly considered in the field of biosensing for their three-dimensional structure that permits simultaneous accommodation of multiple recognition elements, their ability to amplify molecular sensitivity into macroscopic effects such as variations

in swelling, and the possibility to control diffusion of molecules through their polymeric matrices. In biosensors, hydrogels are typically used in one of two ways: as a surface layer coating the sensor (e.g., an electrode) or as an immobilization matrix that retains bioelements. Hydrogels allow the enzymes and other bioelements to be functional in highly aqueous and biocompatible environments (Bae et al., 2020; Chen et al., 2024).

2.2.5.4. Tissue Engineering

Tissue engineering, an ever-growing discipline, merges materials, living cells and growth factors to augment repair or regeneration of damaged or lost tissues. Hydrogels have achieved vast popularity as scaffold materials in tissue engineering because their properties are mechanical and structural and are similar to those of ECM. Thus, these scaffolds act as artificial ECMs, organizing cells into a three-dimensional structure and delivering stimuli to promote the growth and formation of desired tissues (Mantha et al., 2019; Chyzy & Plonska-Brzezinska, 2020). Natural and synthetic materials can be used to form hydrogel scaffolds for tissue engineering. Hydrogel scaffolds find application in tissue engineering as: i) fillers, ii) three-dimensional structures that act as a base for cell growth, and iii) carriers for the delivery of bioactive molecules that can bring about the formation of tissues (Chen et al., 2024).

2.3. Dental Tissue regeneration

Dental tissue regeneration is a multidisciplinary field that exhibits a complex and exciting symbiosis between medicine, materials science, engineering, and biology. It is widely recognized that oral and craniofacial tissues have a limited ability to spontaneously regenerate and return to their original state after severe damage (Ye, Wei & Zeng, 2022). In this context, by combining the tissue engineer's main pillars, complying sscaffolds, stem cells, and bioactive molecules, dental and craniofacial tissue engineering has been researched based on the collaborative action of these components, regarding to the enhancement of the reparative potential of the resident cells of the tissue while promoting the migration of more stem cells towards the site of injury and propagating the overall regenerative process.

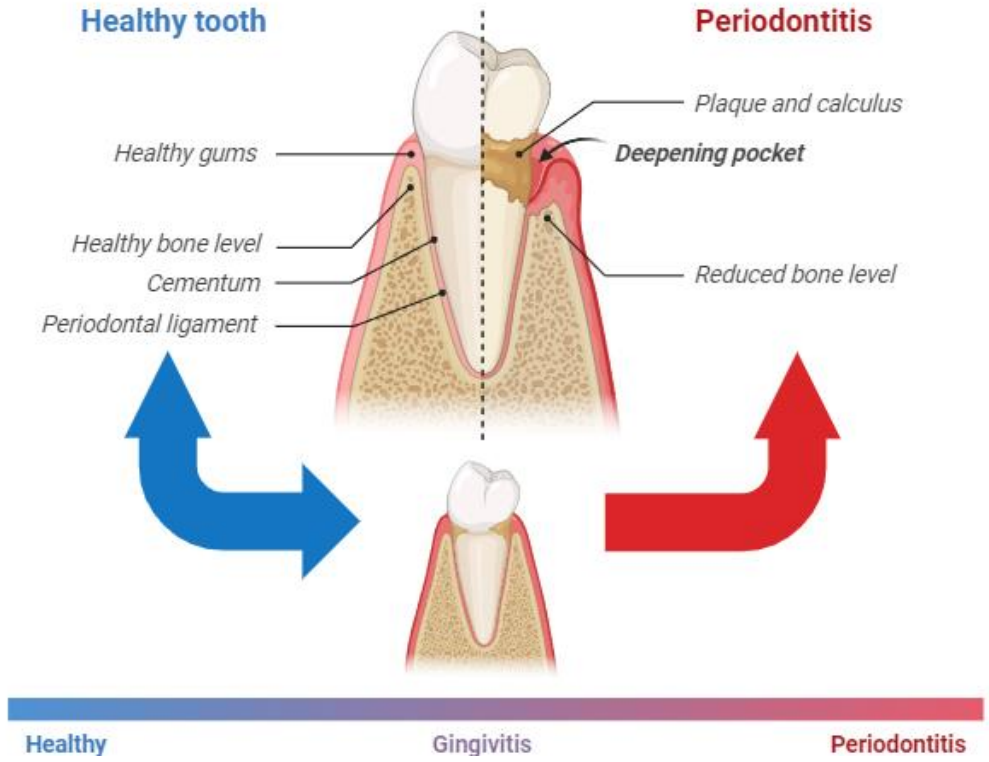
In this section, the potential of hydrogel-based systems in dentistry, a research field that has known an interesting growth, in order to promote periodontal, dental pulp regeneration, and enamel remineralization is overviewed.

2.3.1. Periodontal Regeneration

Periodontal disease is a complex condition influenced by multiple factors, where harmful bacteria trigger the host's immune response, resulting in damage to the tissues that support and surround the teeth, including the gingiva, periodontal ligament, alveolar bone, and cementum. These four types of tissue comprise the periodontium, a crucial support system necessary for restoring and properly functioning periodontal structures (Ausenda et al., 2019; Li et al., 2022). In addition to securing the teeth within their alveolar sockets, the periodontium plays a vital role in stabilizing them by absorbing and distributing forces from chewing, while also acting as a defense against various pathogens. The initial indication of periodontal disease is gingivitis, an inflammation of the gums triggered by the accumulation of dental plaque, with the host's response influencing the progression of the disease. While better oral hygiene practices can reverse gingivitis, neglecting treatment may lead to periodontitis, which involves the breakdown of collagen fibers, loss of alveolar bone, and the development of soft tissue pockets between the tooth root and gingiva. If left untreated, this condition can advance to severe periodontitis, resulting in loose teeth, chewing difficulties, and tooth loss (Figure 2) (Xu et al., 2019; Santos et al., 2023).

Figure 2

The different stages of periodontal disease.



The development of hydrogel-based materials applied to the dentistry field has known a thrilling growth over the last decade. They are expected to provide a new perspective for promoting periodontal tissue regeneration and repairing periodontal bone defects (Guo et al., 2024).

In tissue engineering, scaffolds serve a crucial function by attempting to mimic the ECM composition, offering three-dimensional structural support and direction for both endogenous and exogenous cells (Yamada et al., 2022). Particularly esteemed in this domain, hydrogels possess a distinctive three-dimensional network structure and significant water content, closely resembling the natural ECM. A variety of novel hydrogel scaffolds have been created to enhance tissue regeneration by improving the interaction between the scaffold materials and the target tissue cells (Sun et al., 2024).

In developing hydrogels intended as scaffolds for periodontal regeneration, it is crucial to consider the material, structural design, and functionalization methods to ensure the adherence (Naahidi et al., 2017). The key considerations in the hydrogels development include biocompatibility, biodegradability and porosity, as follows:

- Biocompatibility: It is critical for hydrogel scaffolds to be compatible with the target tissue. The unreacted monomers, reaction initiators, or other components left after synthesis in the material could potentially damage the tissue. Thus, carefully selecting materials and synthesis methods, followed by comprehensive purification, is essential to reduce the possible negative impacts.
- Biodegradability: Hydrogel scaffolds must be able to degrade and be resorbed in a controlled way within the body.
- Porosity: One of the primary characteristics of hydrogel scaffolds is their porosity, which permits the delivery of nutrients and oxygen to cells, as well as the removal of metabolic waste. Well-connected pores will enhance cell infiltration into the scaffold, and a uniform distribution of cells will contribute to tissue regeneration. Factors such as pore size, shape, and volume distribution are critical in determining a scaffold's porosity and affect how far cells can penetrate (Chen et al., 2018). Additionally, mechanical strength is necessary for the scaffold to effectively integrate into the damaged area and bear weight. Furthermore, the surface characteristics of the hydrogel scaffold play a crucial role in affecting cell adhesion and proliferation through their interaction with adjacent tissue. By thoroughly evaluating and refining these properties, hydrogel scaffolds can create an optimal three-dimensional microenvironment that significantly enhances the periodontal tissue regeneration (Santos et al., 2023; Sun et al., 2024).

Stem cells are recognized for their ability to self-replicate and their flexibility in transforming into various functional cell types, offering considerable promise in areas such as cell repair, regenerative medicine, and pharmacology. Extensive research has focused on the capacity of stem cells to regenerate periodontal tissue, which can be categorized into two primary groups: odontogenic and non-odontogenic. The odontogenic category comprises dental pulp stem cells, stem cells derived from human exfoliated deciduous teeth, periodontal ligament stem cells (PDLSCs), apical papilla stem cells, and gingival mesenchymal stem cells (GMSCs). In contrast, non-odontogenic stem cells include bone marrow-derived stem cells (BMSCs), adipose-derived stromal cells, embryonic stem cells, and induced pluripotent stem cells (Ding et al., 2021; Sun et al., 2024).

The conventional hydrogels' absence of a microporous structure frequently obstruct cell spreading and proliferation. Although one potential modification involves increasing the

hydrogel's porosity by lowering the polymer concentration, this could jeopardize the stability of the hydrogel. Developing microporous hydrogels is a practical alternative, promoting motility for both the host and encapsulated cells. A prior investigation introduced fast-curing microporous hydrogels based on gelatin and GelMA (Edwards et al., 2022). These hydrogels, which cure in just 2.5 minutes following injection via photopolymerization and enzymatic cross-linking, facilitated uniform cell distribution and notable cell spreading and proliferation within a week. Furthermore, these materials were capable of transporting human marrow-derived stem cells (hMSCs) that had been primed with interferon-gamma, enhancing the release of anti-inflammatory agents like prostaglandin E2 and interleukin-6.

Throughout the past years, research has indicated that a range of medications and growth factors can facilitate the regeneration of periodontal tissue. Nonetheless, the application of these bioactive agents is frequently restricted due to their rapid degradation rates and short half-lives. A widely used approach for drug delivery employs hydrogels, which, because of their distinctive physical properties, are well-suited for this role, enabling sustained release of medications (Onaciu et al., 2019). Within hydrogels, drugs can be encapsulated, integrated into their structures, or adsorbed onto their surfaces. Several factors affect the release of drugs from hydrogels, such as the composition and shape of the hydrogel, the method of manufacturing, external conditions, and the interactions between the hydrogel and the bioactive compound (Zięba et al., 2020). By optimizing these variables, it is possible to control both the rate and duration of drug release with precision. An ideal hydrogel for drug delivery should possess several essential biophysical and chemical properties. It must be biocompatible to avoid causing adverse reactions or immune rejection and should be gradually degraded within the body. The by-products resultant from degradation must be non-toxic and non-inflammatory, with a degradation rate that aligns with the drug release to ensure the medication remains effective for the necessary period. Additionally, the hydrogel should securely adhere to periodontal tissue to prevent any movement during treatment or everyday activities (Sun et al., 2024).

The table 2 highlights the hydrogel-based systems reported for periodontal regeneration.

Table 2

Systematization of the hydrogel-based materials used in periodontal regeneration.

Hydrogel	Main Results	Reference
Alginate/polyvinyl alcohol scaffolds with hydroxyapatite nanoparticles	Superior cell attachment and promoted growth and differentiation of periodontal ligament stem cells into osteoblasts	Bahrami et al., 2019
Collagen hydrogel scaffolds combined with bone morphogenetic protein-2	Induce effective periodontal repair	Kato et al., 2015
Polyethylene glycol and Chitosan composite gel with acetylsalicylic acid	Promoted the proliferation and osteogenic differentiation of periodontal ligament stem cells and enhanced bone regeneration	Zhang et al., 2019
Gelatin methacryloyl and poly(ethylene glycol) dimethacrylate encapsulating periodontal ligament stem cells	Cell proliferation, spreading, and osteogenic differentiation increased as the Gelatin methacryloyl volume ratio increased	Ma et al., 2019
Chitosan, *β*-glycerol phosphate, gelatin, aspirin, and erythropoietin	The hydrogels exhibited no toxicity *in vitro*. Aspirin and erythropoietin could be continuously released from the hydrogels for at least 21 days	Xu et al., 2019
Chitosan biguanidine, carboxymethylcellulose, bone morphogenetic protein-2, vascular endothelial growth factor with Human dental pulp stem cells	Hydrogels were non-toxic and significantly increased dental pulp stem cells proliferation. Hydrogels with bone morphogenetic protein-2 and vascular endothelial growth factor showed significantly higher gene and protein expression of alkaline phosphatase, Collagen I, and osteocalcin; increased alkaline phosphatase activity and calcium deposition	Divband et al., 2021
Chitosan, gelatin, glycerol phosphate, bone morphogenetic protein-6, induced pluripotent stem cells	Synergistic effects of induced pluripotent stem cells and bone morphogenetic protein-6 increased bone and cementum formation	Chien et al., 2018

Chitosan, β-glycerol phosphate	These hydrogels promoted periodontal tissue regeneration, which was observed through significantly increased new bone and cementum formation	Zang et al., 2014
Chitosan, β-glycerol phosphate, bone morphogenetic protein-7, ornidazole	Hydrogels loaded with ornidazole exhibited antimicrobial activity against *P. gingivalis*	Zang et al., 2019
Alginate, chitosan, β-TCP, melatonin	Melatonin-loaded hydrogels accelerated the formation of new bone and enhanced the quality of newly formed bone, allowing complete periodontal regeneration	Abdelrasoul et al., 2022
Self-assembling peptide hydrogel RADA16 from PuraMatrix™	RADA16 resulted in significantly increased bone volume, trabecular thickness, and reduced trabecular separation	Takeuchi et al., 2014

Periodontal tissue regeneration is very complex. It is because of their capacity to incorporate cells into their structure that many hydrogels are researched as scaffolds and/or drug delivery systems. They are able to degrade on the same timeline as the formation of new tissue. Additionally, hydrogels can be incorporated with several bioactive molecules, which induce cellular processes: migration, proliferation, differentiation, vascularization, and mineralization (Santos et al., 2023; Zhang et al., 2023). Besides, hydrogels feature outstanding biocompatibility, water retention, controlled release, and support for cellular interaction in the process of periodontal regeneration.

By the analysis of table 2, it can be concluded that the majority of the hydrogel-based systems studied to periodontal regeneration are derived from natural sources (alginate, chitosan or collagen) with chitosan as the major source used (Zang et al., 2014; Chien et al., 2018; Xu et al., 2019; Zang et al., 2019; Divband et al., 2021). These hydrogels promoted periodontal regeneration with increased new bone formation and cementum formation.

Chitosan is a highly versatile biomaterial and has been reported to be biocompatible, osteoconductive, and evokes a minimal foreign-body response and fibrous encapsulation due to structural similarity with glycosaminoglycan, a natural component of ECM (Divband et al., 2021).

Most of the hydrogel materials were developed to release substances like aspirin or melatonin (Xu et al., 2019; Zhang et al., 2019; Abdelrasoul et al., 2022). These hydrogels exhibited antimicrobial activity (Zang et al., 2019).

Xu et al. (2019) developed an interesting injectable thermosensitive hydrogel that continuously released aspirin and erythropoietin, facilitating the healing of inflammation and alveolar bone repair. Aspirin and erythropoietin released 86.6% and 69.4% of their doses, respectively, within the first three days of the drug release trial, with aspirin's faster release rate aiding in the early stages of periodontitis treatment, where controlling inflammation quickly is vital.

With respect to the periodontal tissues, neither *in vitro* nor *ex vivo* tests can fully mimic the standard conditions of resistance of occlusal mechanical forces by periodontal ligament, cementum, and alveolar bone. Therefore, hydrogels should simultaneously act on both hard and soft tissues due to their composite nature (Elango et al., 2020). Many options have been studied *in vitro* with good results, which can be followed by preclinical studies, but this closed relationship blocks further investigations of hydrogels in clinical trials. Therefore, *in vivo* models are required to mimic the occlusal state for the support of functional regeneration design (Bermúdez et al., 2021). Also, one of the most challenging aspects would be the reconstitution of the insertion apparatus (Sharpey's fiber between cementum and alveolar bone), as its absence then turns into a negative impact on the capacity of the tooth to support occlusal forces (Liang et al., 2020).

The high standards applied to claiming hydrogels for bone, periodontal ligament, and cementum healing and regeneration make most of the purposes explained pending in vitro or preclinical animal studies. Of the hydrogels available for alveolar regeneration, PEG with BMP-2 and PLGA with BMP-2 have been proven to be effective in animal models for regenerating bone defects around implants by producing more bone formation and osseointegration (Ayala-Ham et al., 2021).

2.3.2. Dental pulp Regeneration

The primary treatment options for the pulp tissue that has been damaged encompass the direct and indirect pulp capping to preserve the pulpal tissue's vitality. In contrast, endodontic treatment, which involves the three-dimensional shaping, cleaning, and filling of the soft tissue space within the tooth using an inert, biocompatible material, results in the loss of pulp vitality, adversely affecting the integrity of the tooth structure. This loss

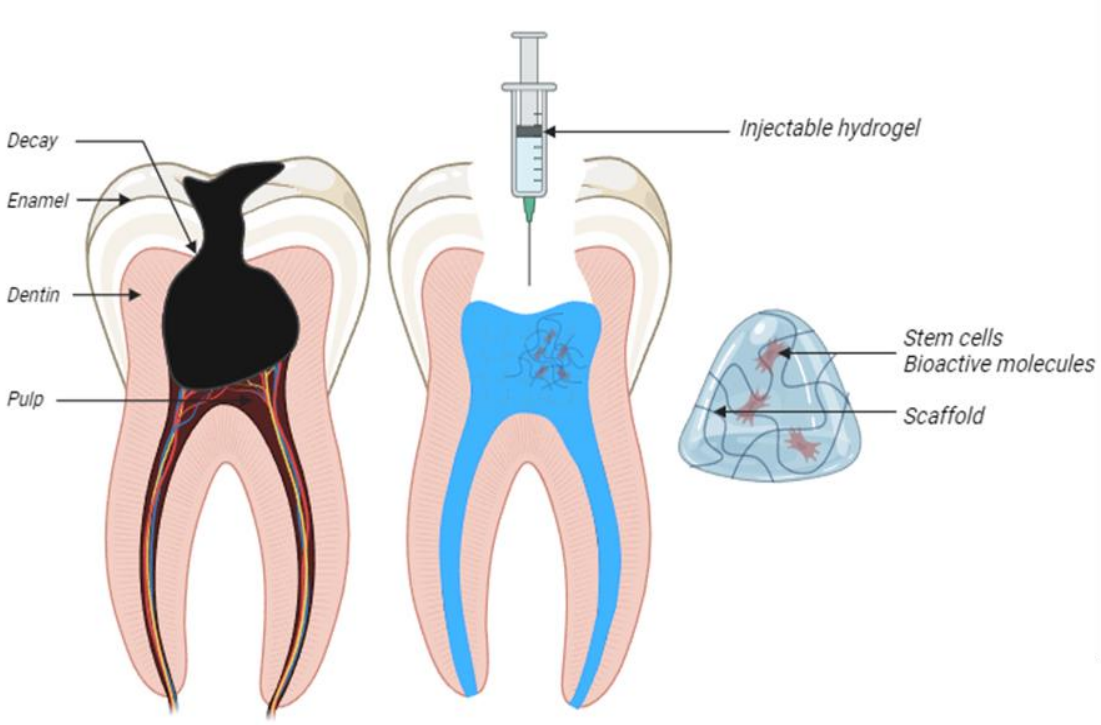
of vitality can particularly impact the maturation of young permanent teeth and their apexogenesis - a vital pulp therapy procedure performed to encourage physiological development and formation of the root end, raising significant concerns among researchers regarding the regeneration of the dentin-pulp complex through alternative tissue engineering methods (Ye, Wei & Zeng, 2022). The regeneration of the dentin-pulp complex through regenerative endodontic procedures (REP) or revascularization depends on the stimulation of resident stem/progenitor cells' differentiation. REP entail inducing intracanal bleeding and forming a blood clot, which serves as a scaffold for the migration and differentiation of stem/progenitor cells from the apical dental papilla (SCAP) for regeneration (Abbass et al., 2020; Chen et al., 2024).

The successful pulp regeneration requires two essential prerequisites: effective disinfection of the root canal and an appropriately sized apical foramen.

Regeneration of the dentin-pulp complex may also incorporate strategies for repairing or replacing damaged pulp tissues through tissue engineering methods. These various tissue engineering strategies fundamentally rely on a combination of three essential elements: cells, bioactive molecules, and scaffolds (Figure 3) (Bhoj et al., 2015; Ha et al., 2019; Jang et al., 2020). Within the realm of dentin–pulp complex regeneration, the stem/progenitor cells that have been explored include stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem/progenitor cells (PDLSCs), dental pulp stem cells (DPSCs), stem cells from apical papilla (SCAP), and dental follicle stem/progenitor cells (Bakhtiar et al., 2018; Mantha et al, 2019). The primary morphogens commonly utilized alongside dental stem/progenitor cells to promote a range of cellular activities and facilitate the formation of different tissue structures include BMP, vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and transforming growth factor (TGF), even at minimal concentrations. Research has demonstrated that VEGF and FGF can significantly promote angiogenesis - growth of blood vessels from the existing vasculature - and neovascularization in severed human dental pulps, while BMPs are believed to stimulate the formation of new dentin (Moussa & Aparicio, 2019; Haugen et al., 2020; Li, Fan & Fan, 2024).

Figure 3

The hydrogel action for pulp regeneration.



The table 3 summarizes the hydrogels applied for pulp regeneration therapy.

Table 3

Systematization of the hydrogel-based materials used in pulp regeneration.

Hydrogel	Main Results	Reference
Chitosan with Stemcell from the apical papilla	Chitosan hydrogel can improve the effect of pulp regeneration through cell homing method	Moreira et al., 2021
RGD-alginate hydrogels	Enhance cell adhesion and proliferation	Bhoj et al., 2015
RAD/Dentonin hydrogel	Promoted the proliferation, migration, odontogenic differentiation, and mineralization deposition of dental pulp stem cells	Liu et al., 2021
Hyaluronic acid with human bone marrow mesenchymal stem cells	Hyaluronic acid can maintain cell viability and proliferation and promote osteogenic/dentin differentiation of human bone marrow mesenchymal stem cells	AlHowaish et al., 2022

Polyethylene glycol with dental pulp stem cells	Promote the aggregation of dental pulp stem cells and their odontogenic differentiation	Lu et al., 2015
Cinnamaldehyde with dental pulp stem cells	Cinnamaldehyde cross-linked collagen scaffolds are beneficial to human dental pulp stem cells adhesion, proliferation, and differentiation	Kwon et al., 2016

Multiple hydrogel biomaterials have potential in the research of pulp regeneration. The injectable hydrogels combined with stem/progenitor cells for dentin–pulp complex regeneration are considered a promising approach in the field of tissue engineering, primarily, because they can be injected inside the tooth and easily and successfully adapt to the contour of the pulp chamber (Lu et al., 2015; Kwon et al., 2016; Moreira et al., 2021; AlHowaish et al., 2022). Gelation - association of polymer units to form a gel network with a firm three-dimensional structure - *in situ* through cross-linking of hydrogel precursors, makes these materials able to appropriately fill all irregularities and defects (Abbass et al., 2020; Bertsch et al., 2023).

All of the hydrogels shown in table 3 promote the proliferation and differentiation of stem cells, with the hydrogel-based systems developed by Liu et al. (2021) and Kwon et al. (2016) exhibiting interesting features.

Liu et al. (2021) reported an attractive material, a self-assembled peptide RAD/Dentonin with an A-based nanofiber network structure to attach to hydrogels (L-gel or D-gel) by heat-cooling technology. This hydrogel-based material showed good biocompatibility and can promote the adhesion, proliferation, migration, odontogenic differentiation and mineralization of human dental pulp stem cells (hDPSCs).

Kwon et al (2016) showed that the cross-linking of collagen scaffolds by cinnamaldehyde had beneficial effects with respect to attachment, proliferation and differentiation of hDPCs. Consequently, the application of cross-linking agents such this may represent a new strategy for dentine-pulp complex regeneration.

Hydrogels should maintain the desired volume and structural integrity for the required time to perform this function. For dentin–pulp complex regeneration, hydrogels act as carriers of stem/progenitor cells with odontogenic potential (Song et al., 2024). The following cells are evidence of this opinion: DPSCs (Jang et al., 2020), odontoblasts-like cells, SCAP (Ha et al., 2020), endothelial cells and primary dental pulp cells. They can also act as carriers for the local delivery of antibiotics (clindamycin) and bioactive

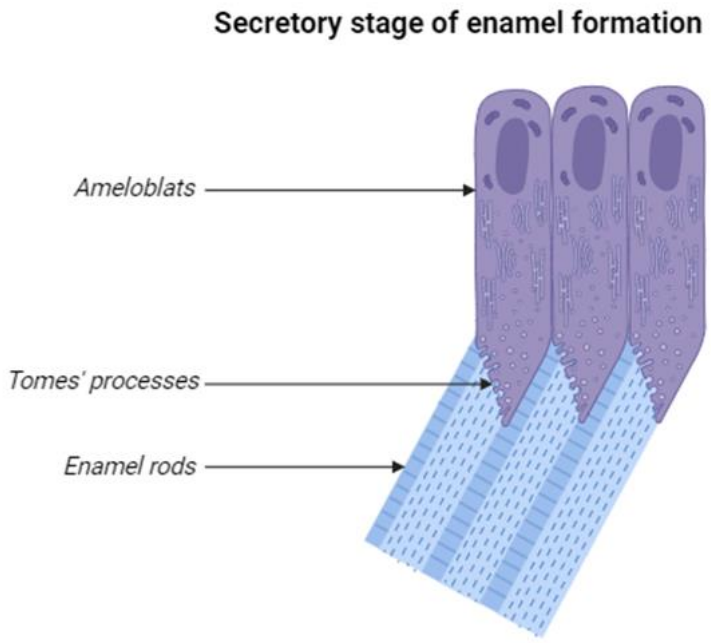
molecules aiming to promote tissue regeneration. Examples are VEGF (Bhoj et al., 2015), FGF (Bhoj et al., 2015), BMP, TGF-β1, stem cell factor (Mu et al., 2020), dentonin sequence, and RGD cell-binding motifs. Once implanted, being biodegradable, hydrogels allow the release of bioactive molecules that influence the surrounding environment (Li, Fan & Fan, 2024).

2.3.3. Enamel Remineralization

Natural enamel initially forms in a gel-like ECM. Under this condition, the ameloblasts secrete proteins, essentially amelogenin, which will bind inorganic ions like calcium ions and phosphate, which are abundant in their surroundings (Figure 4). This process results in the formation of highly-oriented and parallel enamel crystallites. Consequently, regenerating enamel lies in materials–capable to mimetize the conditions for crystal mineralization of hydroxyapatite (HAP) in an environment at normal body temperature (Liao et al., 2024).

Figure 4

The enamel formation process.



In the typical approach to treating dental caries, the cavity is filled with a polymer material to replace the decayed portion. Unfortunately, this filling material lacks bioactivity, which can potentially result in the development of secondary caries. In the past few decades, there has been a growing interest in non-invasive therapeutic techniques that promote the remineralization of superficial dental structures, and their medicinal benefits have been

widely acknowledged. Given the strong adhesive properties of the hydrogel, it is logical to combine it with a remineralization agent to enhance its effectiveness (Song et al., 2023; Song et al., 2024).

Comprised of meticulously organized hydroxyapatite nanocrystals, enamel is a highly mineralized substance. Various methods have been developed to facilitate enamel remineralization, such as the introduction of mineral ions (such as calcium, phosphate, and fluoride) through dental varnishes, filling composites, glass ionomers, toothpaste, dentifrice, or mouth rinse solutions. Additionally, the deposition of fluorapatite-like phases within the enamel has been explored. However, the effectiveness of these approaches is limited due to the short duration of contact with the tooth. To address this issue, hydrogels have been utilized to deliver remineralization-promoting agents and extend the contact time (Song et al., 2023; Zhang et al., 2023).

The table 4 summarizes the various hydrogels used in the remineralization.

In the enamel regeneration, the majority of the hydrogel-based materials in development are natural hydrogels.

The natural hydrogels exhibit an excellent biocompatibility and controllable degradability. Their major limitation relies in the relatively low mechanical strength which limits their application in enamel remineralization. In contrast, synthetic polymer hydrogels have much better mechanical properties and can be designed to tune a wide variety of structures and functions. However, many synthetic polymers have poor degradability and may be toxic.

Table 4

Systematization of the hydrogel-based materials used in enamel regeneration.

Hydrogel	Main Results	Reference
Chitosan hydrogel loaded with amelogenin	Facilitated faster mineral induction and resulted in the organized growth of hydroxyapatite crystals	Au-Ruan & Au, 2014
Maleic chitosan, thiolated alginate, *β*-glycerophosphate, calcium phosphate, and calcium carbonate	Exceptional mineralization and possesses highly crystallized hydroxyapatite	Zhang et al., 2021
Chitosan hydrogel incorporating leucine-rich amelogenin peptide	Surface hardness recovering over 87% of the hardness value of sound enamel	Mukherjee et al., 2016
Poly(carboxybetaine acrylamide) and amorphous calcium phosphate	Inhibited the adhesion and biofilm formation of *Streptococcus mutans* and the remineralization of demineralized enamel was achieved	He et al., 2022
Enamel matrix derivative-calcium chloride agarose hydrogel	Enamel matrix derivative has the ability to control the nucleation and growth of the crystals. The Ca^{2+} present within hydrogels offers a good environment conducive to remineralization. The rod-like, well-organized, fluoridated hydroxyapatite crystals, which are more acid-resistant than hydroxyapatite, were formed on etched enamel	Cao et al., 2014
Chitosan-agarose hydrogel	Regulate crystal growth orientation and obtain a parallel hydroxyapatite crystal structure and high hardness of the regenerated enamel	Musat et al., 2021
Alginate-carboxymethyl chitosan hydrogels	Oxidized alginate-carboxymethyl chitosan hydrogel can regenerate enamel tissue	Mohabatpur et al., 2022
QP5 peptide and bioactive glass	QP5 promotes enamel remineralization by guiding calcium and phosphate ions provided by Bioactive glass. Bioactive glass can regulate the mineralized microenvironment to be alkaline, simulating the pH regulation of ameloblasts during enamel maturation	Liu et al., 2022 Ding et al., 2020

In the enamel regeneration, the majority of the hydrogel-based materials in development are natural hydrogels.

The natural hydrogels exhibit an excellent biocompatibility and controllable degradability. Their major limitation relies in the relatively low mechanical strength which limits their application in enamel remineralization. In contrast, synthetic polymer hydrogels have much better mechanical properties and can be designed to tune a wide variety of structures and functions. However, many synthetic polymers have poor degradability and may be toxic.

Among the studies summarized in table 4, it is important to emphasize that Cao and coworkers reported the first study of an enamel matrix derivative in enamel remineralization (Cao et al., 2014). On the other hand, Zhang and coworkers synthesized a peculiar polysaccharide-based hydrogel through a Michael addition reaction, which was tested in the mineralization using the "enzyme-promoted" approach, being observed interesting advantages in the enamel sealing (Zhang et al., 2021).

Amelogenin is the most predominant enamel matrix protein and is involved in remineralization, which seemed to motivate the development of chitosan-based hydrogels incorporating amelogenin, that showed promising results in the enamel remineralization (Au-Ruan & AU, 2014; Mukherjee et al., 2016).

Agarose hydrogels provide mineral precursors as reservoirs and transport them dynamically to the enamel surface, contributing to hydroxyapatite formation on the enamel surface. Besides, the agarose hydrogel matrix can coordinate enameled parallel HAP crystal growth on the enamel surface (Musat et al., 2021). Indeed, research on chitosan hydrogel for enamel remineralization has grown these last few years (Liao et al., 2024). In order to improve the performance of chitosan hydrogels in controlling mineralization orientation and achieve *in situ* formation of enamel surface parallel layers of HA crystal during remineralization, amelogenin may be included into chitosan hydrogels. Another approach is that chitosan and agarose can be combined together by hydrogen bonds formation to prepare chitosan-agarose hydrogels in which a property of agarose contributes to the regulation of crystal growth orientation so as to obtain a parallel HA crystal structure and high hardness of the regenerated enamel.

Alginate is a natural polysaccharide that has been widely used in tissue engineering. However, alginate has a low degradation rate (Soltan et al., 2019). Mohabatpur et al

(2022) developed a self-cross-linkable hydrogel composed of oxidized alginate and carboxymethyl chitosan. This hydrogel showed antibacterial properties against *Streptococcus mutans* and *Streptococcus sobrinus*. Besides that, the *in vitro* enamel regeneration studies showed alkaline phosphatase production and mineral deposition.

In table 4, it is also summarized a study involving an interesting material, a peptide QP5-loaded biomimetic hydrogel composite with bioactive glass – QP5 is an amelogenin-based peptide – which can mimetize the enamel process because QP5 guides ionic calcium and phosphate from bioactive glass to initiate the enamel remineralization enamel. Further, bioactive glass can control the mineralized microenvironment to be alkaline since the pH value during ameloblast maturation is regulated by NaP. The composites based on bioactive glass show better biosafety, better binding to the enamel surface, ion release and buffering capacity for pH value. The enamel lesions treated with bioactive glass also showed lower lesion depth and loss of minerals in both in vitro and *in vivo* studies (Ding et al., 2020; Liu et al., 2022).

Therefore, the niche of hydrogel-based materials for enamel remineralization still faces ambitious challenges, such as: the synthesis of HAP crystals with natural enamel structure and properties; the material should have good mechanical properties to resist the mechanical force in the oral environment; and a degradation rate that is controllable and corresponds to the regeneration of enamel, besides to exhibit a good biocompatibility. This is still a challenging task, but IPN hydrogels were synthesized as a highly promising approach for enamel remineralization and are expected to receive much attention in the future.

3. CONCLUSION

This narrative review summarizes the studies in the field of hydrogel-based materials applied to dentistry regarding the dental tissues regeneration – periodontal, dental pulp regeneration, and hard tissue regeneration (enamel remineralization). The general features of the hydrogel-based systems are also overviewed – classification, preparation strategies, characterization system, peculiar properties and general applications.

The field of hydrogel-based systems applied to dentistry is still in the early stage. The trend has increased over the past five years, with research outputs showing very promising results.

By exploiting the diverse properties of hydrogels, researchers have recently developed various application-oriented strategies based on these materials.

In the area of periodontal tissue regeneration, hydrogels have an ideal ability to be compatible with surrounding biological tissues, exhibiting appropriate water retention, and controlled release, which can also induce cell differentiation, and cellular interactions as anti-inflammatory responses and immune reaction regulators during periodontal regeneration. The hydrogel-based systems can achieve a flowable fit into the defect area within the original periodontal structure in a short time before solidifying under body temperature (thereby promoting histocompatibility) and retaining implant materials in the lesion for homogeneous cellular, medicinal, and bioactive factor delivery. With hydrogels being printable, natural-like periodontal tissue scaffolds can be reproduced, thus greatly facilitating hydrogel application in periodontal tissue regeneration.

As far as hydrogels are concerned in dental pulp regeneration, they are better than many other biological materials because of their excellent physical and chemical properties, which can match the highly personalized structures of the dental pulp cavity, personalized medicine. The cell compatibility of hydrogels is good for most cells, and these materials can be used as a medium to combine more than two types of cells for regenerating nerve- and blood vessel-rich pulp tissue. Hydrogel microspheres can be used as a good sustained-release carrier for releasing drugs over different time periods.

Regarding hard tissue regeneration, the current knowledge is not so vast compared to periodontal or pulp regeneration. Based on the principles of biomineralization using hydrogel and biomimetic polypeptides, this combination has opened new possibilities for regenerating tooth-hard tissues. While hydrogels have proven effective for preventing

caries, treating dentin hypersensitivity, and pulp capping, their application in translational research and clinical practice is still a challenge.

In tissue engineering, hydrogels act not only as carriers that deliver stem cells, drugs, and therapeutic agents but also play a role in regulating cell behavior during the process of regeneration to some degree. The synthesis difficulty, allied to the lack in some extent of safety studies constitute the major challenges in the translational research. An easier regulatory approval and the implementation of appealing preparation processes in the companies that meet the criteria from an economic point of view will also promote the commercialization.

Even though the use of synthetic hydrogels in cellular tissue regenerative engineering has been on the rise because of mechanical properties along with controllable components, synthetic hydrogels do not have good biocompatibility and biosafety like natural hydrogels. The perfect hydrogel scaffold material should possess excellent biological properties, simulate the extracellular matrix, get involved in releasing signal molecules, and regulate stem cell behavior. Also, it should have great physical and chemical properties, which can be tuned by chemical functionalization, including suitable strength, flexibility, appropriate porosity and degradation rate.

Indeed, the application of hydrogel-based systems in dentistry exhibits a tremendous potential due to their tunable characteristics, which is aligned with personalized therapies. We firmly believe that this exciting research field will quickly grow in the coming years, with the preparation of tunable materials that can be produced on high-scale, accomplishing the safety and commercialization requirements.

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