Hydrogel-Based Scaffolds in Oral Tissue Engineering

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Regenerative therapy in dentistry has gained interest given the complexity to restore dental and periodontal tissues with inert materials. The best approach for regeneration requires three elements for restoring functions of affected or diseased organ tissues: cells, bioactive molecules, and scaffolds. This triad is capable of modulating the processes to replace lost or damaged tissues and restore function, as it has an impact on diverse cellular processes, influencing cell behavior positively to induce the complete restoration of function and morphology of such complex tissues. Hydrogels (HG) have shown advantages as scaffolds as they are soft and elastic three-dimensional (3D) networks formed from hydrophilic homopolymers, copolymers, or macromers. Besides simple or hybrid, HG show chemical, mechanical and biological activities such as the incorporation of cells in their structures, the retention of high-water content which enhances the transportation of cell nutrients and waste, and elastic and flexible characteristics that emulate the native extracellular matrix (ECM). HG can induce changes in cellular processes such as chemotaxis, proliferation, angiogenesis, biomineralization, and expression of specific tissue biomarkers, enhancing the regeneration process. Besides some of them have anti-inflammatory and anti-bacterial effects. This review aims to show an extensive overview of the most used hydrogels in tissue engineering, emphasizing those that are studied for the regeneration of oral tissues, their biological effects, and their clinical implications. Even though most of the HG are still under investigation, some of them have been studied *in vitro* and *in vivo* with outstanding results that may lead to preclinical studies. Besides there are HG that have shown their efficacy in patients such as hyaluronan HG that enhances the healing of gingival tissue.

Keywords: hydrogel, oral tissue, dental regeneration, periodontal regeneration, tissue engineering

INTRODUCTION

In recent years, advances have been made in oral tissue engineering and regenerative dentistry thanks to the growing amount of research in fields such as, stem cell biology, genetic, molecular engineering, and pathologies that affect the dental organ and its supporting tissues (Miran et al., 2016). Nevertheless, regeneration is still ruled by the need for three elements that have the goal of restoring functions of affected or diseased organ tissues (Cavalcanti et al., 2013; Lee et al., 2014; Fukushima et al., 2019): ECM, which serves as a scaffold; active biomolecules, that regulate cell

growth and differentiation; and mesenchymal cells, needed for the new tissue formation (Sakata et al., 2015). Thus, in tissue engineering, regeneration will be achieved if a structural scaffold is provided, conferring mechanical properties (Goker et al., 2019), but also, inducing migration of cell populations. Besides, biomaterials used in tissue engineering must be degraded in a time range comparable to the growth of the new tissue (Colombo et al., 2014).

Consequently, dental biomaterials have passed from passive bioinert structures to bioactive materials that have the final goal to return form and function to oral tissues (Schmalz and Smith, 2014). Despite progress, oral tissue regeneration is challenging as the dental organ is made up of different tissues as well as the periodontium. In this regard, hydrogels (HG), have been gaining interest since they possess three-dimensional (3D) polymeric networks with characteristics similar to tissues (Athirasala et al., 2018).

According to Slaughter et al., HG are defined as 3D insoluble polymer matrices created from crosslinked hydrophilic homopolymers, copolymers, or macromers (Slaughter et al., 2009). Due to their thermodynamic compatibility, these polymers are soft and elastic with water and have been used in many biomedical applications (Peppas et al., 2000; Slaughter et al., 2009). In the case of biological HG, they can be formed mainly from agarose, alginate, chitosan, hyaluronan, fibrin, and collagen (Lee and Mooney, 2001; Malafaya et al., 2007).

Such HG, either simple or hybrid, have shown excellent chemical, mechanical and biological activities (Moussa and Aparicio, 2019). They work incorporating cells in their structures, and at the same time, degrading themselves to let place to new healthy tissue. HG, been porous structures, can retain high-water content enhancing the transportation of cell nutrients and waste. Besides, HG are elastic and flexible emulating the native ECM (Mantha et al., 2019).

HG are considered the biomaterial of choice for tissue engineering in dentistry and biomedicine. According to Eelkema et al., at present, HG are used in personal care products, biomaterials, coatings, and plant fertilizers. They also are considered for future applications such as sensing, drug delivery, soft robotics, and biohybrid or biointerfacing materials (Chai, 2009). When HG are compared with other types of biomaterials, they are superior showing proper mechanical strength, porous structure, enhanced biocompatibility, and adjustable biodegradability (Chai et al., 2017). HG have emerged as a promising biomaterial for therapeutic delivery of cells and bioactive molecules for tissue regeneration in dentistry, showing the capacity to conform to the three-dimensional defect and the adaptability for minimally invasive surgical procedures. Given that HG have been considered the biomaterial of choice for tissue engineering applications (Fan and Wang, 2017), review aims to show an extensive overview of the most used hydrogels in tissue engineering, emphasizing those that are studied for the regeneration of oral tissues , their biological effects, and their clinical implications. This is the first review that compiles the use of hydrogels in regenerative dentistry, taking into consideration all dental and periodontal tissues.

HYDROGEL-BASED SCAFFOLDS USED IN TISSUE ENGINEERING

The success of tissue regeneration relies on the design of biofunctional scaffolds. Lately, HG are considered the best candidates given their characteristics that emulate the natural ECM, enabling proliferation, vascularization, and survival of cells. Here we summarize the most common HG used in tissue engineering, their characteristics and its current uses.

Collagen-Based Hydrogels

Collagen-based HG mimic interactions between cells and ECM *in vivo* and can be biofunctionalized. Collagen is the most studied fibrous protein, it confers unique properties to the ECM allowing cell adhesion, migration, and proliferation (Lodish et al., 2000). One of the most common methods to obtain it for therapeutic purposes is by decellularization of organs or tissues (Magno et al., 2020). Currently, collagen-based HG can be found commercially in different concentrations, alone or mixed with various molecules, such as cinnamaldehyde (Kwon et al., 2017), riboflavin (that confers resistance, compression, shortens the setting time, and also confers photocurable properties), and chondroitin sulfate-polyethylene glycol (CS PEG) adhesive (Chae et al., 2014). Collagen can be biofunctionalized using growth factors (Momose et al., 2016a, 2016b), bone morphogenetic proteins (BMPs) (Yamamoto et al., 2003), cytokines, or exomes potentiating their use (Barthes et al., 2021).

Collagen-based HG can mimic cell-cell and cell-matrix interactions *in vivo* and regulate more orderly cell growth, for this reason, it have been applied to the engineering of cardiac tissues (Kaiser et al., 2018), corneal and corneoscleral regions (Chae et al., 2014), alveolar bone, and periodontal tissues (Kato et al., 2015). Also in the aesthetic area, cosmetic formulations are widely used due to their moisturizing, regenerating, and film-forming properties, for filling wrinkles and facial expression lines (Li et al., 2005).

Hyaluronic Acid Hydrogels

Hyaluronic acid or hyaluronan (HyA) HG can be combined to improve their characteristics and have multiple clinical uses in human. HyA is a biopolymer that can be modified and processed to form HG for biomedical applications (Burdick and Prestwich, 2011). It has great biological importance since it has a high affinity to CD44 and is degradable through oxidative species and various enzymes such as hyaluronidase, beta-D glucuronidase and beta D-N-acetyl-hexosaminidase (Stern, 2004). HyA HG can combine with DNA/polyethyleneimine molecules and MMP-sensitive peptides to modulate the delivery of particles to cells (Gojgini et al., 2011). HyA can be directly combined with 2-Aminoethyl methacrylate (AEMA) (Niloy et al., 2020a), platelet lysate (HAPL) (Almeida et al., 2018a), polyethylene glycol diacrylate (PEGDA) + Gelatin (HySistem-C) (Jones et al., 2016a, 2016b), and catechol (enhancing adhesion *in vivo*) (Shin et al., 2015). HyA HG systems have been successful for clinical use in human patients in areas such as ophthalmology (Chang et al., 2021), orthopedics (Pitarresi et al., 2013), oncology (Suo et al., 2019), gynecology (Chen et al., 2017), dentistry (Ni

et al., 2019), and plastic surgery (Borzacchiello et al., 2015) for the repair of skin (as dermal fillers) and neural tissues, (Yeom et al., 2010), in gingival tissue (Jentsch et al., 2003), corneal tissue (Williams et al., 2017), for inter-articular viscosupplementation (Narkbunnam et al., 2012) and prevention of postoperative adhesions (Li et al., 2014a).

Gelatin Hydrogels

Gelatin based HG offer a variety of benefits for tissue engineering that enable their use as scaffold and drug delivery system. Gelatin is an insipid protein derived from collagen used in food and pharmaceutical industries (Mariod et al., 2011). It is obtained from the skin, scales, bones, ligaments, and tendons of bovine and porcine livestock (Okonkwo et al., 2007). Its abundance, low price, biodegradability, and non-toxicity (Ulfa et al., 2015) make it ideal for therapeutical use. Gelatin-based HG retain their structure as basic polymer chains, which can be combined, allowing biocompatibility, low immunogenicity, rapid biodegradation, allowing their use in biomedical applications such as in the manufacture of contact lenses, matrices for tissue engineering, and systems drug administration (Hoffman, 2012).

Nowadays, methacrylate gelatin HG (GelMA), is also used and polymerizes under ultraviolet light, in the presence of a photoinitiator, in a thermosetting cross-linked HG (Yue et al., 2015). It is biocompatible and preserves the RGD binding motifs to cells and the MMP binding domain, allowing its biodegradation (Sun et al., 2018). Besides, can be used as an injectable 3D bioprinted scaffold through electrospun fibrous membrane by light-induced crosslinking, extrusion, and microfluidics (Radhakrishnan et al., 2017). GelMA has been used also for the construction of cell-loaded microspheres for bone regeneration *in vivo* (Zhao et al., 2016), as multicomposite with polyethylene glycol (PEG), gelatin, and heparin (PGH) scaffold to induce chondrogenesis (Chen et al., 2020), in combination with sodium alginate, and poly (ethylene glycol)-tethacrylate (PEGTA) as a bio-link to print highly organized and perfusible 3D biologically relevant vessels with significant potential in tissue construct engineering vascularized (Jia et al., 2016).

Another HG used is the microbial transglutaminase (mTG) enzymatically crosslinked gelatin. mTG catalyzes the formation of covalent N e-(g-glutamyl) lysineamide bonds between individual gelatin strands to form a permanent network of polypeptides, thus, creating physiologically biocompatible scaffolds adequate for live surgery procedures, as well as a slow-release HG to deliver antibiotics and prevent bacterial colonization on the surface of implants for orthopedic surgery, as is feasible for intraoperative manipulation and can resist the pressure force in the insertion of intramedullary implants (Yung et al., 2007).

Chitosan Hydrogels

Chitosan (CS)-based scaffolds provide mucoadhesive characteristics through interactions between opposite charges and can be combined with other synthetic polymers such as methyl acroloyl glycin (CS-MAG), acquiring photosensitive properties (Qi et al., 2013), with PEG (Li et al., 2009), with fibrinogen and different types of proteins such as BMPs or amelogenin, among others, demonstrating a tissue binding capacity, able to control the release of many drugs or organo-specific signaling molecules. These chitosan-based HG have been gaining ground in biomedicine, cosmetics, immunotherapy, cell therapy, and tissue engineering. At present, it is used in the repair of arteriovenous (Kim et al., 2020), bone (Liu et al., 2017), skin (Huang et al., 2019), dental (Park et al., 2013), and even ocular tissues (Ozcelik et al., 2013).

Alginate Hydrogels

These type of HG lack of biological activity but can be biofunctionalized for medical applications. The brown algae produces a series of anionic biopolymers known as alginates (unbranched polysaccharides of mannuronic β-d acid (M) and α-L guluronic acid (G) covalently linked in different sequences and blocks along the polymer chain) that retain water, which offers advantages in terms of temperature and pH, enabling encapsulation and cell recruitment (Augst et al., 2006).

The crosslinked alginate HG have low mechanical rigidity and are progressively absorbed *in vivo* due to the release of divalent cations that crosslink the HG in the surrounding environment in exchange with monovalent cations (Lee and Mooney, 2012). Although alginate HG lack biological activity, they can suffer chemical and biochemical modifications (Augst et al., 2006; Bidarra et al., 2014) that enable biomedicals applications such as drug delivery (Loebsack et al., 2001) (Supramaniam et al., 2018), vehicle for Schwan cell transplantation (Mosahebi et al., 2001), and bulking agent (Loebsack et al., 2001).

Agarose-Based Hydrogel

Agarose is a linear polysaccharide found in marine algae, composed of -β- d galactopyran, -3,6-anhydrous β-l-galactopyranose units with thermo-reversible capacity when solubilized in water that can be combined with chitosan, gelatin, fibrin, ECM, minerals, and synthetic particles for biomedical applications (López-Marcial et al., 2018). Agarose HG are thermosensitive to a temperature between 32 and 40°C, which enable their use as bioprinting materials *in vitro* and *in vivo* for skin, peripheral nerves, and skeletal tissue (Gopinathan and Noh, 2018).

Extracellular Matrix-Based Hydrogel

ECM scaffolds are widely used in tissue engineering given their well-preserved native characteristics. Decellularized ECM-based scaffolds are generally of porcine or bovine origin and that preserve the biochemical structure, nanostructure, and bioinductive properties of the native matrix (Badylak et al., 2009), structural and functional proteins, such as glycosaminoglycans, proteoglycans, and growth factors (GF) (Voytik-Harbin et al., 1998). ECM-derived materials are FDA approved and have been used in millions of patients (Zantop et al., 2006) since they can be solubilized in injectable HG by enzymatic digestion mainly by pepsin-mediated solubilization. Then the matrix obtained is transported to physiological pH and salt conditions to adapt to *in vivo* conditions, as well as to

inactivate pepsin. The digested ECM is cross-linked on a nano-fibrous hydrogel after incubation at 37°C (Saldin et al., 2017; Spang and Christman, 2018).

The biggest disadvantage of the ECM HG are the manufacturing time and the addition of aggressive proteins for digestion. Nevertheless, they are widely used for various tissue engineering applications (Saldin et al., 2017) such as photothrombotic cortical and other soft tissue ischemic lesion in rats (Koči et al., 2017) and percutaneous transendocardial injections for cardiac repair (Traverse et al., 2019).

Keratin Hydrogels

Keratin is a structural fibrous protein associated with epithelial cells, is also found in hair, wool, claws, and nails (Coulombe et al., 2000). It is considered one of the most abundant natural polymers (Donato and Mija, 2020) and its products are keratein and keratose (obtained when extracted at low pH in the presence of reducing agents) (Hill et al., 2010; Rouse and Van Dyke, 2010). The extracted keratin proteins can self-assemble preserving the RGD Leu Asp-Val (LDV) and Leu-Asp-Ser (LDS) motifs, which favor cell union and growth (Verma et al., 2008; Ajay Sharma et al., 2017a). Keratin HG influence cell behavior, allowing innate response modulation, as well as epithelial cell polarization (Salas et al., 2016). Furthermore, these HG promotes adhesion, proliferation, and differentiation of ad-MSCs in adipocytes, osteoblasts, vascular endothelial cells, and myocytes *in vitro* and improves skin wound healing *in vivo* (Lin et al., 2019).

Peptide-Based Hydrogel

Peptide-based HG are biomedical materials that have great stability due to their self-assembly capacity, their high-water content allows an application based on infiltrates. These can be obtained by various methods, such as enzyme controlled hydrogelation, Spontaneous self-assembly, hydrogelation, Chemical, and physical crosslinking enhanced hydrogelation, presenting microporous structure, mechanical stability, biocompatibility, injectability, and tissue elasticity (Li et al., 2019). Furthermore, they can be combined with other types of molecules that make them suitable for biomedical applications such as drug delivery (Paladini et al., 2013), antitumor therapy (Altunbas et al., 2011), 3D bioprinting, 3D culture neural tissue, tissue engineering, and wound healing (Altunbas et al., 2011).

Synthetic Hydrogels

Synthetic hydrogels are a group of materials with diverse biomedical applications that possess thermostability and durability in comparison with natural hydrogels (Varaprasad et al., 2017). Furthermore, they are reasonably inexpensive (Munim and Raza, 2019). They can be divided into degradable and non-degradable HG.

Polylactic acid (PLA) is a synthetic, hydrophobic, bio-based polymer obtained from the bacterial fermentation of renewable carbohydrates of vegetable origin, it is biocompatible and biodegradable (Jain et al., 2016). It has adequate mechanical properties, is safe and non-toxic

(Reichert et al., 2020). PLA can be conjugated to PEG and polysaccharides to form HG(92).

Polyvinyl alcohol (vinyl alcohol) (PVA) is a polymer consisting of repeating units of vinyl alcohol, with a constant relative weight allowing a high polydispersity index (Goodship and Jacobs, 2009). Biomaterials based on PVA have biocompatibility and are sensitive to pH changes, allowing commercial availability and facilitating the synthesis of HG (Peixoto et al., 2006). These PVA HG have particular physical properties that preserve microstructure, being ideal for tissue substitution and other biomedical applications (Jiang et al., 2011) such as administration through nanoparticles that encapsulate the drug that respond to changes in temperature, changes in pH, and the presence of an oscillating magnetic field (Koetting et al., 2015).

PEG is widely used to produce constructs for biological applications due to hydrophilicity, non-toxicity, low protein adhesion, and non-immunogenicity (Yang et al., 2005; Buxton et al., 2007). The PEG HG must be cross-linked to achieve a high-water content construction. Furthermore, the terminal hydroxyl groups of PEG molecules can be easily functionalized by thiol, carboxyl and acrylate groups. It also can bind to other molecules or bioactive agents (Zhu, 2010). PEG-based HG can be synthesized by radiation crosslinking and covalent crosslinking allowing reactive chain ends (Zhu and Marchant, 2011).

HYDROGELS FOR DENTAL TISSUE REGENERATION

HG have emerged as an alternative for the regeneration of dental organ tissues (**Figure 1**) and their characteristics make them a promising alternative for clinical application (**Table 1**). Tooth loss is a global health problem that affects socially and economically (Righolt et al., 2018). It causes affections in vital functions such as biting, chewing, smiling, speaking, and also have psychosocial implications. Dental caries and periodontal disease are major causes of tooth loss (Angelova Volponi et al., 2018). Caries is the most common disease reported worldwide (Petersen, 2003; Kassebaum et al., 2015) and is given by an imbalance in the demineralization-remineralization equilibrium caused by the presence of dental biofilm leading to a clear loss of tooth minerals, progressing to a cavity through the dissolution of the subsurface mineral tooth structure, followed by progression to irreversible cavitation of the enamel and/or dentine layers (Han et al., 2017; Jablonski-Momeni et al., 2019; Pandya and Diekwisch, 2019).

When a tooth is cavitated in the dentin limit with the pulp chamber, the odontoblast layer secretes bioactive molecules to induce dentinogenesis. Nevertheless, the processes of reparative dentine formation, as well as enamelogenesis, are still unclear, and their regeneration is a clinical challenge (Smith et al., 1995; Bleicher, 2014; Wang et al., 2014). Additionally, in the case of pulp involvement, one of the main intentions of clinical treatment is the preservation of pulp vitality by using direct capping with biocompatible materials to form reparative dentin (Huang, 2011;
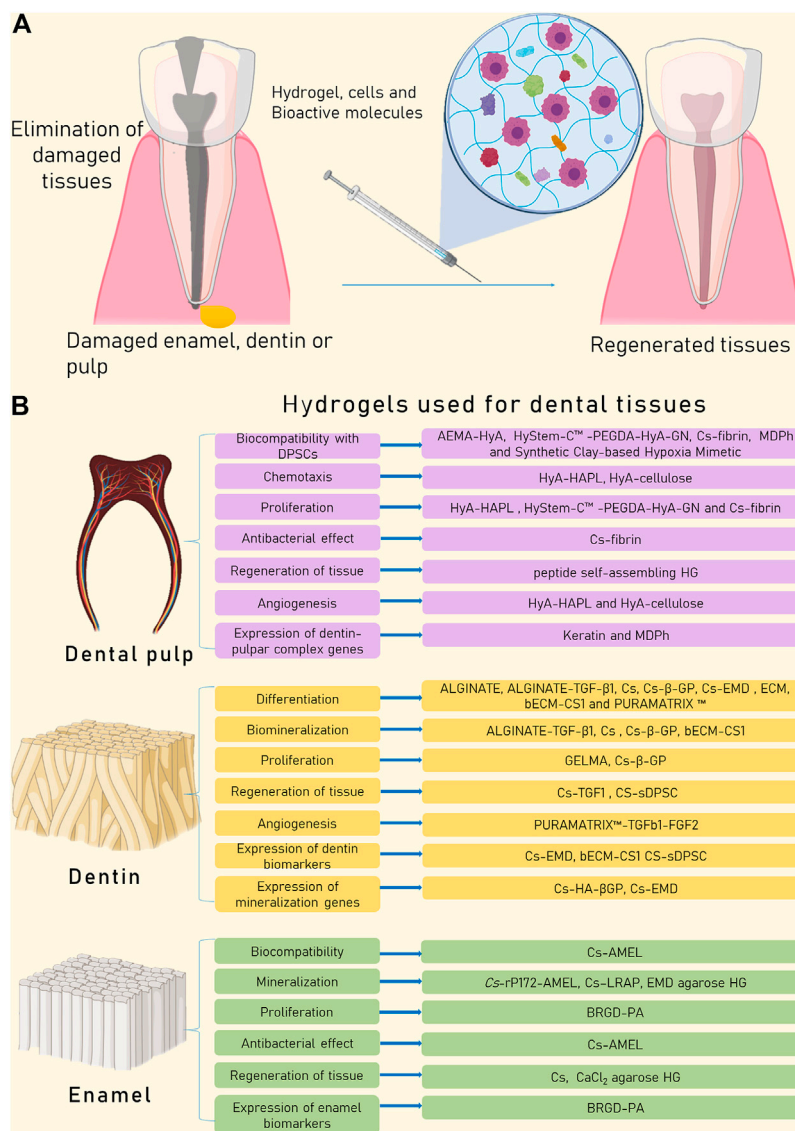
FIGURE 1 | The combined use of cells, bioactive molecules, and scaffolds such as HG is considered the best approach to achieve tissue regeneration. **(A)** Dental organs can be damaged principally by caries and trauma, injuring enamel, dentin, or pulp. Advances in the field of HG could be beneficial to find the ideal scaffold to regenerate every lost tissue. **(B)** HG can induce changes in cellular processes such as chemotaxis, proliferation, angiogenesis, biomineralization, and expression of specific tissue biomarkers, enhancing the regeneration process.

Hilton et al., 2013). Although this treatment is in some cases effective, so far there is not a successful treatment to induce the full regeneration of the enamel, dentin, and pulp.

Hydrogels-Bassed Scaffolds for Dental Pulp Regeneration

The dental pulp is one of the most difficult tissue to regenerate, given its complex nature. The dental pulp is a dynamic connective tissue that has an innate capacity to respond to infections or injuries (Morotomi et al., 2019). This property has provided the basis for the design of regenerative treatments. Research on pulp regeneration increases steadily in the

literature, applying biological principles to protect the pulp (Nakashima et al., 2017). According to Ahmadian *et al.*, successful pulp therapy depends on the form and site of injury, the age of the tooth, and the therapeutic approach and capping material (Ahmadian et al., 2019). The investigations have focused on the use of HG, in studies with pulp tissue cells, *in vitro* and *in vivo*. This knowledge is applied in clinical treatments, which include; indirect or direct pulp capping, pulpotomies, and pulp revascularization, with emphasis on dentin formation, sensory nerves, and blood vessel development (Moussa and Aparicio, 2019). Here we present an overview of all HG studied for dental pulp regeneration.

TABLE 1 | Hydrogel-based scaffolds for dental tissue regeneration.

	PULP		
Hydrogel type	Type of study	Effect	Reference
Hyaluronic acid (HA) hydrogels	Review	These hydrogels collaborate as a scaffold support, as they are biocompatible and safe in human dental cell therapy.	Ahmadian et al. (2019)
2-Aminoethyl methacrylate (AEMA)–modified Hyaluronic acid (HA) hydrogels	*In vitro.* 2-aminoethyl methacrylate (AEMA) modified hyaluronic acid (HA) hydrogels were synthesized by UV polymerization. Tests were performed by MTT assay, Hoechst 33342 live cell staining, and propidium iodide staining of dead cells.	Improves expression of NANOG and SOX2 in dental pulp stem cells (DPSC) and these cells presented spherical morphology. presents potential for regeneration.	Niloy et al. (2020a)
Hyaluronic acid (HA) HG incorporating platelet lysate (HAPL) hydrogel	*In vitro.* HA hydrogels incorporating PL (HAPL) were prepared. hDPSC were placed on the hydrogel. Cell metabolism and DNA quantification were evaluated as well as calcium and alkaline phosphatase activity (ALPL) and RUNX2 gene expression.	The incorporation of PL increased cellular metabolism and stimulated the deposition of mineralized matrix by hDPSCs.	Almeida et al. (2018a)
hyaluronic acid (HA)with cellulose nanocrystals (CNC) and enriched with platelet lysate (PL) hydrogel	*In vitro and ex vivo.* chemotactic and proangiogenic activity was evaluated by Recruitment and cell in human umbilical vein endothelial cell co-cultures.	Incorporating CNC improved the stability of materials against hydrolytic and enzymatic degradation. stimulated chemotactic and proangiogenic activity.	Silva et al. (2018)
HyStem-C ™ polyethylene glycol diacrylate (PEGDA), hyaluronan (HA), and gelatin (Gn) hydrogel	*In vitro.* hDPSC viability was evaluated embedded in PEGSSDA-HA-Gn hydrogels at 2% (p /v). Fibronectin (Fn) was added to determine the effect of protein concentration of the extracellular matrix.	Gelation time decreases with increased concentration. The hydrogel is biocompatible with hDPSCs, and they facilitate cell propagation.	Jones et al. (2016a, 2016b)
fibrin and chitosan(Cs) Hydrogel	*In vitro.* Various fibrin-chitosan formulations were examined by rheological analysis, as well as antimicrobial effect, cell viability, extracellular matrix deposition, and collagen production.	The fibrin-chitosan hydrogel is cytocompatible at pH 7.2. the chitosan presented antibacterial effect, the hydrogel was biocompatible and stimulated the production of type I/III collagen.	Ducret et al. (2019)
keratin hydrogels	*In vitro e in vivo.* Biocompatibility was evaluated as a pulp response, the gel was implanted in rat molars performing a partial pulpotomy. After 28 days, it was analyzed by histological and immunohistochemical methods to identify the formation of DMP-1.	The gel is dimensionally stable, hydrophilic and biocompatible allowing pulp healing by expression of DMP-1.	Ajay Sharma et al. (2017a)
Multidomain Peptide Hydrogels	*Ex vivo.* MDP hydrogel was injected into the interface of the odontoblasts and dentin or into the core of the pulp of the jaw cuts and subsequently cultured for up to 10 days. Histology and alizarin red staining were evaluated as well as immunohistochemistry.	Scaffolds placed in pulp tissue are biocompatible and preserve the local tissue architecture.	Moore et al. (2015)
Synthetic Clay-based Hypoxia Mimetic Hydrogel	*In vitro. Resazurin*-based toxicity assays, and viability of human DPCs on a synthetic hydrogel were performed clay-based 5–0.15% as well as charged hydrogels. Production of VEGF from DPC of the supernatant was measured	Biocompatible in DPC. Cell monolayer and cell cluster formations were observed. stimulated VEGF production in DPC.	Müller et al. (2018)
Enzyme-cleavable hydrogel	*In vitro e in vivo.* FGF, TGFβ1, and VEGF were evaluated. Cell morphology and propagation in three-dimensional cultures were visualized. Subcutaneous transplantation of the hydrogel into dentin casts was performed in immunosuppressed mice.	Formation of vascularized soft connective tissue similar to dental pulp.	Galler et al. (2012)

TABLE 1 | (*Continued*) Hydrogel-based scaffolds for dental tissue regeneration.

DENTINE			
Hydrogel type	Type of study	Effect	Reference
Alginate	*In vitro*. Measurement of endogenous transforming growth factor TGF-β1 to the dentin-pulp complex as a signal of restorative processes.	Release of TGF-β1 Odontoblast-like cell differentiation. TGF-β1 may indicate both the induction of cell differentiation and the up-regulation of its matrix secretion in the human dentin-pulp complex.	Dobie et al. (2002)
bEMCCollagen 1	*In vitro*. evaluation of the level of odontogenic differentiation of DPSCs on hydrogel scaffolds derived from bone extracellular matrix (bECM) vs those seeded on Col-I.	Demonstrated that the levels of mRNA expression of the DSPP, DMP-1 and MEPE. In addition, increased mineral deposition was observed in bECM hydrogel scaffolds and was demonstrated by Von Kossa staining.	Paduano et al. (2016)
ChitosanHydroxyapatite	*In vitro*. Proliferation and osteogenic differentiation of DPSCs in injectable thermo-sensitive chitosan/b-glycerophosphate/hydroxyapatite hydrogel.	DPSCs exhibit high expression of alkaline phosphatase and high regulation in expression of Runx-2, Collagen 1, and osteocalcin.	Chen et al. (2016)
Chitosan + (VEGF/CS/βGP)	*In vitro*. Effects on DPSCs, cultured on the CS/β-GP hydrogel, assessing adhesion proliferation, and vitality	Sustained release of VEGF could promote odontogenic differentiation as the high expression of ALP, RUNX-2, OCN and OSX was observed after 7 and 14 days, which allowed the high formation of mineralized nodules demonstrated with the red alizarin staining (ARS).	Wu et al. (2019)
Glycol-Chitosan Supplemented whith Enamel Matrix	*In vitro*. Effect of GC-TRS and collagen on cell viability was examined by diphenyltetrazolium bromide assay. Expression of markers for odontogenic/osteogenic differentiation was analyzed byqPCR.	It promotes odontogenic differentiation of DPSC, which expressed dentin sialophosphoprotein and dentin matrix protein 1, and osteopontin messenger RNA was significantly regulated.	Park et al. (2013)
Gelatin Methacryloyl (GelMA)	*In vitro*. cell viability, and proliferationwith simple engineering strategies for reconstruction of tissue similar to dental pulp.	Viscosity was shown to allow greater printability of the bio-links, in formulations containing higher concentrations of alginate. At concentrations of 100 μg ml-1, these soluble dentin molecules significantly enhance the odontogenic differentiation of apical papilla stem cells encapsulated in bioprinted hydrogels.	Athirasala et al. (2018)
Gelatin Methacryloyl (GelMa)	*In vitro*. Hydrogels were micropattern by photolithography, to generate microgrooves and ridges, where SCAP was seeded and analyzed for self-alignment by fluorescence microscopy using F-actin/DAPI. Furthermore, odontogenic differentiation was analyzed by the expression of the alkaline phosphatase protein.	Micropatterned hydrogels enhance the odontogenic potential of SCAPs *in vitro*, demonstrating an increase in ALP expression on stamped substrates within 7 days in culture.	Ha et al. (2020)
Self-assembling Peptide HydrogelK(SL)3RG(SL)3KGRGDS (K, lysine; S, serine; L, leucine; G, glycine; R, arginine; D, aspartic acid)	*In vivo*. Mice 8- to 10 week-old females, strain NIH-III were used as subcutaneous transplan recipients of dentin cylinders loaded hidrogel on the dorsal surface of each animal.	Material for tissue regeneration, releasing VEGF, TGF-β1 and FGF-1, which promotes the differentiation of DPSCs in Odontoblast-like cells.	Galler et al. (2011a)
Recombinant Human Collagen (rh Collagen) Peptide (Puramatrix)	*In vivo*. Roots of human premolars injected with Puramatrix™ or rhCollagen containing SHED were implanted subcutaneously into immunodeficient mice (CB-17 SCID).	SHEDs, were differentiated to odontoblasts expressed (DSPP, DMP-1, MEPE), after 21 days of interaction allowing the ability to form new tubular dentin.	Rosa et al. (2013)
Chitosan	*In vivo*. Microsphere loaded porous chitosan membrane with TGF-1 (MS-TGF) for a sustained release, in the pulp coating and the formation of restorative dentin in a Beagle breed dog model, At 60 days after stimulation.	The MS-TGF group generated reparative dentin with a thickness of (142 ± 29) m, 3 times thicker than with Dycal and 6 times thicker TGF-free chitosan membrane.	Li et al., (2014b)
GelatinL-lactic acid oligomers	*In vivo*. Hydrogelimplanted subcutaneously in the backs of twelve 10 week-old BALB/c immunocompromised nude mice.	The controlled release of simvastatin from hydrogels promotes the odontoblastic differentiation of DPSCs, improving the activity of alkaline phosphatase, calcium deposition and morphogenic secretion of bone protein-2 and dentin sialoprotein, which promoted calcification both *in vitro* and *in vivo*.	Miyazawa et al. (2015)

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TABLE 1 | (*Continued*) Hydrogel-based scaffolds for dental tissue regeneration.

	ENAMEL		
Hydrogel type	Type of study	Effect	Reference
CaCl$_2$ agarose hydrogel	*In vitro.* hydrogel biomimetic mineralization model on an etched enamel surface in the presence of 500 ppm fluoride, was analyzed with scanning electron microscopy, X-ray diffraction.	The generated tissue had enamel prismlike layers containing well-defined hexagonal hydroxyapatite crystals. The modulus of elasticity and the nanohardness of the regenerated enamel prismlike tissue were similar to those of natural enamel.	Cao et al. (2014a, 2014b)
rP172 hydrogel	*In vitro.* HG was applied to artificial caries lesions on extracted human third molars and tested for static gel remineralization in the presence of artificial saliva, pH cyclic treatment at pH 5.4 acetic buffer and pH 7.3 gel remineralization.	Remineralization activity on early artificial caries in a cyclic treatment model and multispecies oral biofilm model. Repetitive application of the hydrogel significantly improved enamel hardness continuously over time.	Fan et al. (2012)
CS-AMEL hydrogel	*In vitro.* Two models for enamel defects, erosion and early caries, were asessed in this study. After pH cycling and treatment with CS-AMEL hydrogel enamel defects were analized.	Repair of artificial incipient caries by re-growing oriented crystals and reduction of the depth of the lesions by up to 70%.	Ruan et al. (2016)
Ch-LRAP hydrogel	*In vitro*. Effect of hydrogel was analized by scanning electron microscopy , X-ray diffraction ,focus-ion beam, transmission electron microscopy, and Vickers microhardness test.	Stimulates the growth of organized *de novo* aprismatic crystals on demineralized enamel surfaces that showed significant hardness of the demineralized enamel surface, after 3 days of treatment.	Mukherjee et al. (2016)
EMD-agarose Hydrogel	*In vitro.* Human enamel slices were demineralized with 37% phosphoric acid for 1 min. They were covered with hydrogel. Results were obtained by Scanning electron microscopy, energy-dispersive X-ray spectroscopy, and X-ray diffraction.	EMD promotes *in vitro* biomimetic mineralization and facilitated enamel prism like tissue formation on demineralized human enamel.	Cao et al. (2014a, 2014b)
BRGD-PA hydrogel	*In vitro.* LS8 and primary EOE cells were cultured within PA hydrogels injected into the enamel organ epithelia of mouse embryonic incisors. The expression of AMEL, AMBN, integrin α5, and integrin α6 was detected by QPCR and immunodetection	EOE cell proliferation with differentiation into ameloblasts as evidenced by their expression of enamel specific proteins. Nanofibers within the forming extracellular matrix, in contact with the EOE cells engaged in enamel formation and regeneration.	Huang et al. (2008)
Chitosan hydrogel	*In vitro*. Human premolars extracted were sectioned into 1mm thick slices. After remineralization, the enamel surfaces were ultrasonically cleaned for 2 min and analyzed with SEM. Energy Dispersive Analysis X-Ray spectrometer.	The regeneration of enamel erosions in an enamel-like layer in this study supports the use of chitosan in cases of dental erosions.	Ibrahim et al. (2018)

Hyaluronic Acid Hydrogels

HyA favors stem cell therapy in dentin/pulp lesions, although *in vivo* studies showed a high degradation rate and poor mechanical resistance (Ahmadian et al., 2019). HyA can be modified with 2-Aminoethyl methacrylate (AEMA) to form a non-cytotoxic HG for dental pulp stem cells (DPSCs). This HG mimics the *in vivo* 3D environment to cell growth and maintains their native morphology and stemness, increasing the expression of stemness factors such as NANOG and SOX2, in comparison with cells grown on conventional culture plates (Niloy et al., 2020b). On the other hand, the HyA HG biofunctionalized withHAPL enhances the recruitment of DPSCs isolated from permanent teeth and stimulates the mineral matrix deposition, evidencing its potential for repairing damaged pulp/dentin tissue (Almeida et al., 2018b). Also, when HyA is combined with cellulose nanocrystals improves the stability of the material against hydrolytic and enzymatic degradation, producing an HG that can be injected *in situ*.

Besides, the addition of HAPL to this HG results in the release of the chemotactic and pro-angiogenic growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) which function as chemotactic and pro-angiogenic agents promoting DPSCs recruitment and proliferation, in co-cultures of DPSCs and human umbilical vein endothelial cells. Besides, in a chorioallantoic membrane assay (CAM) the arrangement of DPSCs nearby the new vessels promoted by the CAM infiltration into the HG was seen (Silva et al., 2018).

In the case of the commercially available HyStem-C™ injectable HG PEGDA, hyaluronan, and gelatin (Gn)], the modification of PEGDA by adding a disulfide bond results in a biocompatible HG that can hold DPSCs, meanwhile, increased ratios of HyA: Gn improves cell viability for 14 days. Besides, when fibronectin is added, cell proliferation increases over time (only when

concentrations of 1.0 and 10.0 µg/ml in PEGDA-HyA-Gn HG are used) (Jones et al., 2016a, 2016b).

Other Natural Hydrogels

Chitosan (Cs) is a biodegradable natural biopolymer of amino polysaccharides used in pharmaceuticals, biomedicine, tissue repair, and tissue engineering, given its biocompatibility (Li et al., 2009). Its residual products are not toxic nor immunogenic (Tamura et al., 2011). Cs-based HG (CsHG) can be easily modified or coupled with various molecules and proteins that potentiate the repair effect or act synergistically (Giri et al., 2012). In oral research, CsHG has been used both *in vitro* and *in vivo*, adding hydroxyapatite (HA), β-glycerophosphate (β-GP), VEGF, or extracellular enamel matrix.

CsHG combined with fibrin shows an excellent capacity to maintain DPSCs viability, fibroblast-like morphology, proliferation rate, and type I/III collagen production. Besides, it has an antibacterial effect against *Enterococcus faecalis* (Ducret et al., 2019); meanwhile, Keratin HG has shown biocompatibility with the dental pulp of upper molars in partial pulpotomy treatments, and after 28 days the expression of dentin matrix protein 1 (DMP-1) was detected, suggesting that keratin HG may be a source of biomaterial for biological treatment of pulp tissue (Ajay Sharma et al., 2017b).

Synthetic Hydrogels

The multidomain peptide HG (MDPh), in *ex vivo* organ culture, shows compatibility with the dental pulp and stimulates odontoblasts to be synthetically active. Also, MDPh can stimulate the expression of dentin-matrix proteins as dentin sialophosphoprotein (DSPP). An important finding is that when MDPh is used in dental pulp did not induce mineralization, but preserved pulp tissue architecture (Moore et al., 2015). On the other hand, the synthetic clay-based HG can maintain the viability of dental pulp cells (DPCs), without increasing VEGF levels in the supernatant when DPCs are cultured on the clay-HG loaded with hypoxia mimetics agents such as dimethyloxalylglycine (DMOG), desferrioxamine, L-mimosine, and $CoCl_2$. In contrast, the supernatant of DMOG-loaded HG alone stimulated VEGF production (Müller et al., 2018). In the case of the enzyme-cleavable HG (made from self-assembling peptide nanofibers), it can be combined with basic fibroblast growth factor (FGF2), transforming growth factor (TGF-β1), and VEGF, maintaining DPSCs morphology and enhancing their spreading in 3D cultures. Besides, the subcutaneous transplantation of this HG within dentin cylinders into immunocompromised mice enhances the formation of a vascularized soft connective tissue similar to the dental pulp (Galler et al., 2012).

Hydrogels-Bassed Scaffolds for Dentin Regeneration

In the regenerative dentistry area, it has been implemented the use of HG for dentin regeneration, testing different biomaterials such as alginate alone or in combination with GelMa, Cs, glycol-chitin, ECM, self-assembled peptides, and collagen. Dentin is

considered the second hardest mineralized tissue in the body after enamel and corresponds to the largest part of the tooth crown. During odontogenesis, cells from the neural crest migrate into the mesenchyme and reach the branchial arch where they contribute to the formation of maxillary dental germs. The odontoblast precursors migrate from the front-nasal bud where interactions between the epithelial cells of the dental lamina and mesenchymal cells contribute to the formation of pulp. Initially, these cells do not appear to be functional, but later, they can constitute a deposit for the renewal of apoptotic odontoblasts, becoming prepolarized osteoblasts (Mao and Prockop, 2012). Dentin is formed by two simultaneous processes, the formation of a collagen matrix, which is associated with soluble and insoluble signaling molecules that stimulate odontogenesis (Chun et al., 2011), and the formation of mineral crystals in this matrix. These processes are orchestrated mainly by Ca^{2+} where they interact with ATPase, Na^+, calcium channels, and intercellular binding proteins. This specialized tissue has specific functions such as root formation and the formation of new dentin throughout life, this is a physiological process known as secondary dentin formation; meanwhile, the repair induced by environmental stimulus generating an interface between pulp and damaged dentin (Couve et al., 2014) or restoration (Sloan and Waddington, 2009) is known as tertiary dentin. The HG used for dentin are summarized below.

Alginate Hydrogels

Alginate HG have been applied for wound healing, as they can structurally emulate ECM and can be combined with various drugs or molecules for sustained release (Lee and Mooney, 2012). Currently, printable alginate HG combined with dentin matrix have been designed, showing that this biomaterial increases cell viability, due to their ability to retain soluble dentin molecules within itself, improving their regeneration capacity (Athirasala et al., 2018). Besides, when is combined with GelMa (known as micropatterned HG) induces a synergistic effect, increasing the odontogenic potential of stem cells from apical papilla (SCAPs) *in vitro*, increasing alkaline phosphatase (ALP) expression in stamped substrates within 7 days of culture (Ha et al., 2020). Alternatively, the effect of the alginate HG loaded with TGF-β1 on root discs shows a sustained release of this growth factor, increases dentin matrix deposition, and induces odontoblast cell differentiation with subsequent secretion of tubular dentin matrix on the cut surface (Dobie et al., 2002).

Chitosan-Based Hydrogel

CsHG coupled with HA and β-GP induces a high ALP expression and a positive regulation in the expression of Runx-2, type I collagen (COL1), and osteocalcin (OCN) in DPSCs (Chen et al., 2016). Also, the effects of the CsHG/β-GP as a sustained release system for VEGF in DPSCs has been evaluated, demonstrating that this biomaterial enables adhesion, cell proliferation, and odontogenic differentiation with high expression of ALP, RUNX-2, OCN, and osterix (OSX) after 7 and 14 days, allowing the formation of mineralized nodules *in vitro* (Wu et al., 2019).

In the case of ChHG supplemented with enamel matrix derivatives (EMD), it promotes the odontogenic differentiation

of DPSCs, showing a porous and well-interconnected structure, allowing proliferation, odontogenic differentiation of DPSCs and the expression of DSPP, DMP-1, and osteopontin (OPN) (Park et al., 2013). *In vivo* studies using a porous material loaded with microspheres of ChHG with TGF-β1, a sustained release over 7 days of more than 40% was shown. After days of stimulation, reparative dentin was formed, resulting in 3 times thicker dentin than the Dycal group and 6 times thicker than ChHG without TGF-β1(137).

Extracellular Matrix-Based Hydrogel

The ECM provides physical scaffolding to cells and structural support to tissues, offering specific signals that are needed for regeneration and homeostasis, through its proteins, proteoglycans, and anchor sites (Hussey et al., 2018). ECM allows the release of cytokines and signaling molecules that induce cell activation and differentiation (Johnson et al., 2019). EMC-based HG are 3D networks capable of absorbing large amounts of water or physiological fluids from the implantation site (Rosales and Anseth, 2016). *In vitro* studies have shown odontogenic differentiation of DPSCs in HG scaffolds derived from decellularized bone ECM (bECM) or COL1, demonstrating that the levels of mRNA expression of DSPP, DMP-1, and matrix extracellular phosphoglycoprotein (MEPE) increases significantly in cells grown in bEMC *vs* those grown in COL1 scaffolds. In addition, increased mineral deposition has been observed in bECM HG scaffolds (Paduano et al., 2016).

Peptide Hydrogel

Peptides are short amino acid chains that can be used in biomaterials without the need to replicate the entire natural sequence. In this regard, the supramolecular self-assembled and patented HG, Puramatrix™, has been used for *in vivo* research, injected into the human root canals in the back of immunodeficient mice (CB-17 SCID), containing human SHED (stem human exfoliated deciduous teeth), resulting in differentiation to odontoblasts that express DSPP, DMP-1, and MEPE, after 21 days of interaction, allowing the formation of new tubular dentin (Rosa et al., 2013). Additionally, conjugates with TGF-β1, FGF2, VEGF in DPSCs have also been used, showing not only dentin reparation after 6 weeks, but also vascularized and soft connective tissue like dental pulp within the dentin cylinder. Dental Sialoprotein (DSP) was used to detect odontoblastic-like cells at the dentin interface, confirming cell differentiation (Galler et al., 2012).

Collagen-Based Hydrogel

Collagen is the most abundant protein within the ECM (Ricard-Blum, 2011). Collagen-based HG have been used as immunomodulators, drug carriers, healing agents, chelators, and in tissue regeneration (Wei et al., 2019). This type of HG can promote adhesion, proliferation, and differentiation in cardiac, corneal epithelial, endothelial, liver, bone, and mesenchymal stem cells (MSCs) (Agmon and Christman, 2016) and have been studied in a Yucatecan mini-porcine model, where the porcine DPSCs were transferred through the

collagen HG directly to the root canal, previously prepared through pulpectomy, then, after 4 months, the analysis showed that vascularized soft tissue was recovered, creating a structure similar to a dentin bridge covering it. In addition, immunohistochemical analysis detected the expression of nestin, DSPP, DMP-1 and bone sialoprotein in cells similar to odontoblasts (Zhu et al., 2018).

Hydrogels-Bassed Scaffolds for Enamel Regeneration

HG-based scaffolds, provide the essential support needed during tissue regeneration (Abbass et al., 2020) and can be used for repairing enamel. This tissue forms the outermost covering of teeth, being a nanostructured mineralized tissue. The ameloblasts, epithelial cells derived from the enamel organ of the developing tooth, are the cells involved in the production of enamel (Chatzistavrou et al., 2012) in a highly regulated process known as amelogenesis, in which proteins are secreted into the extracellular space to then initiate biomineralization (Roveri and Iafisco, 2010). Amelogenin (AMEL) is the most abundant protein, acting as master control of the orientation and elongation growth of enamel rods during the mineralization process. Then, is ameloblastin (AMBN) as the second most abundant non-amelogenin enamel-specific glycoprotein, acting as a cell adhesion molecule for ameloblasts (Moradian-Oldak, 2009). Lastly, enamelin and tuftelin proteins (found in lower proportions) that control apatite nucleation and growth in conjunction with amelogenin. AMEL and other enamel proteins are eventually degraded by proteinases such as the matrix metalloproteinase 20 (MMP-20) and kallikrein 4 (KLK4) at different stages of amelogenesis (Moradian-Oldak, 2009; Moradian-Oldak, 2012). At the end of the process, the enamel is 96% crystalline calcium phosphate in form of HA and 4% organic components (products of degradation of enamel proteins) and water (Baldassarri et al., 2008).

Since mature enamel does not contain cells, it cannot be remodeled by itself. Therefore, biomaterials mimicking enamel are now under revision to regenerate decayed or traumatized dental organs (Mann, 1997). Nevertheless, HA crystals isolated from mature enamel are longer and different in shape from those synthetic. In this regard, the synthesis of enamel-like hydroxyapatite nanorods has been only possible by applying extreme laboratory conditions such as high temperature, high pressure, and extremely acidic pH (Chen et al., 2005; Yamagishi et al., 2005). Moreover, replacing enamel is difficult since none of the materials can mimic all its physical, mechanical, and aesthetic properties (Moradian-Oldak, 2012). Thus, the development of improved biomaterials that could be used in physiological conditions is needed. Here we present the emerging biomaterials in HG presentation that are used for enamel regeneration.

Chitosan and Amelogenin-Based Hydrogel

In an exploratory study, a biocompatible recombinant amelogenin (rP172) HG was prepared to release phosphate, Ca^{2+} and F^-. This HG applied to artificial enamel caries lesions, displayed remineralization

activity on early artificial caries in a cyclic treatment model and multispecies oral biofilm model. Repetitive application of the HG significantly improved enamel hardness continuously over time (Fan et al., 2012).

Ibrahim, et al., have used CsHG in enamel slices etched with 37% phosphoric acid. After application, morphological changes on the enamel surface were observed by scanning electron microscopy showing the regeneration of an enamel-like layer in the treated samples with a difference between the Ca/P ratios. They showed that Cs provided a substrate to immobilize the nano-building units acting as a template for the structured assembly of the regenerated enamel layer (Ibrahim et al., 2018).

The amelogenin-CsHG (CS-AMEL) is biocompatible, biodegradable, and has antimicrobial and adhesion properties (Ruan et al., 2013; Ruan and Moradian-Oldak, 2014). It has been used for biomimetic repair of human enamel with erosive or carious lesions in two pH-cycling systems. CS-AMEL HG showed effectiveness at pH 4.6 (similar to the pH of the mouth after food consumption) and pH 6.5 (average pH during the nighttime). This HG forms a new organized layer of enamel-like crystals on the surface of erosive lesions and could repair artificial incipient caries by regrowing oriented crystals and reducing the depth of the lesions by up to 50–70% under pH-cycling conditions (Ruan et al., 2016).

Cs with leucine-rich amelogenin peptide (Ch-LRAP) HG system acts as an organic template on acid-treated enamel surfaces and allows faster mineral induction and results in organized crystal growth of hydroxyapatite. The demineralized tooth slices treated with a single application of Ch-LRAP HG for 3 days showed a dense mineralized layer comprising very well-organized enamel-like apatite crystals with a continuous growth at the interface between the repaired layer and natural enamel. There was an improvement in the surface hardness after treatment of the demineralized sample with almost 87% recovery of the hardness value (Mukherjee et al., 2016).

Agarose-Based Hydrogel

An HG biomimetic mineralization model consisting of an enamel slice, $CaCl_2$ agarose HG, ion-free agarose HG, and a phosphate solution was designed to regenerate enamel prism-like crystals. The $CaCl_2$ agarose HG regulated the pattern, size, and mineral phase of the growing crystals through cooperative interactions with calcium, phosphate, and fluoride ions. The regenerated apatite crystals were found to be highly oriented along the c-axis with good crystallinity (Cao et al., 2014a, 2014b). Agarose HG has been also combined with the enamel matrix derivative (EMD) and applied to demineralized enamel, showing enamel prism-like crystals with hexagonal structures denser, thicker, and more orderly packed than the crystals formed without EMD (being calcium, phosphorus, and fluorine the main elements found in their composition), confirming that they were fluorinated hydroxyapatite. Moreover, the degree of crystallinity of the hexagonal crystals formed is improved in the presence of EMD. Nevertheless, the degree of structural perfection in the regenerated enamel prism-like tissue without EMD was higher than that with EMD (Cao et al., 2014a, 2014b).

Peptide-Based Hydrogel

The self-assembled peptide amphiphile (PA), containing the peptide motif Arg-Gly-Asp, or "RGD" (BRGD-PA), were used in ameloblast-like cells (line LS8) and primary enamel organ epithelial (EOE). Cells were cultured within PA HG, and the PA was injected into the enamel organ epithelia of mouse embryonic incisors. *In vitro*, LS8 cells and primary EOE cells responded to the BRGD-PA nanostructures with enhanced proliferation and higher amelogenin, ameloblastin, and integrin expression levels. In the organ culture model, the site of BRGD-PA injection showed EOE cell proliferation with differentiation into ameloblasts, expressing enamel-specific proteins such as AMEL and new discovered proteins involved such as thrombospondin 2 (TSP2). Ultrastructural analysis showed the nanofibers within the forming ECM, in contact with the EOE cells engaged in enamel formation and regeneration (Galler et al., 2011b).

HYDROGELS FOR PERIODONTAL REGENERATION

HG can be easily administered into irregular shapes of tissue defects as those caused by periodontal disease (Li and Guan, 2011; Shi et al., 2017; Xu et al., 2019). Periodontal disease is a prevalent infectious disease worldwide, that causes the damage of periodontal support tissues, which can eventually lead to tooth loss. Periodontal treatments aim to first control the infections and then reconstruct the architecture and function of periodontal tissues including cementum, periodontal ligament (PDL), and alveolar bone (Liu et al., 2019). Nevertheless, clinical periodontal therapy fails in regenerating the lost periodontal tissues, thus alternative treatments are needed (Sowmya et al., 2017a).

The engineering of periodontal tissues is a challenging approach given the complex hierarchical architecture comprising of a soft tissue interspersed between two distinct hard-tissue structures (Carranza et al., 2006). The attempts for simultaneous regeneration of the lost tooth-supporting structures are ultimately dependent on the interplay among scaffold, cells, and bioactive signals (Sowmya et al., 2017b) (**Figure 2**). Another limiting factor is the stiffness of scaffolds since they may lead to poor adaptation of the construct on the root surface (Ivanovski et al., 2014). Hence, HG can be used as an alternative, as they are a widely employed carrier in drug-controlled release, and possesses distinctive qualities, such as easy preparation, low cost, low toxicity and eases of use (Ahadian et al., 2015; Merino et al., 2015) (**Table 2**).

Hydrogels-Bassed Scaffolds for Periodontal Ligament Regeneration

Currently, various materials have been used to regenerate the periodontal ligament (Tomokiyo et al., 2019), among them, are fibrin, transglutaminase gelatin, alginate, Cs, chitin-HyA, chitin-PGLA, peptides, and synthetic ECM. The PDL is conformed of fibers arranged in different spatial directions. PDL allows anchoring, provides firmness and cushioning capacity, and is located in the space between the dental organ and alveolar bone (Mehrotra and Singh, 2019). It is a specialized connective tissue, mainly composed of extrinsic collagen fibers known as Sharpey´s fibers, mesenchymal cells, undifferentiated fibroblasts, as well as by the vasculature of the dentinal arteries, which allows rapid
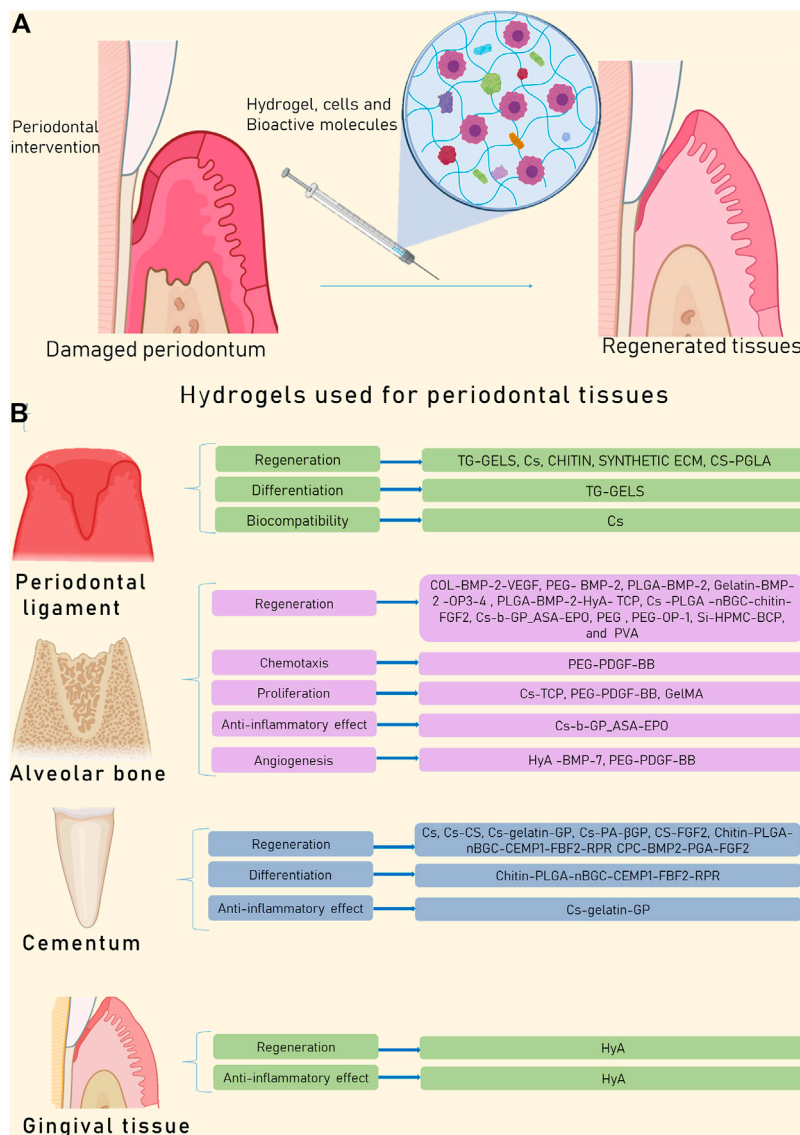
FIGURE 2 | The complete regeneration of the periodontium is not possible yet given the complex characteristics of the tissues. **(A)** HG are a promising alternative to restore the structure and function of the periodontal complex. **(B)** HG in periodontal cells can induce differentiation, proliferation, chemotaxis, and angiogenesis, enabling the formation of new tissue. Besides some HG in periodontium show anti-inflammatory effect.

healing and remodeling (Yamamoto et al., 2016). Chronic periodontitis and aggressive periodontitis cause loss of function of PDL and later loss of support of the dental organ, ending in their premature loss. The HG used for periodontal ligament regeneration are presented below.

Transglutaminase Gelatin-Based Hydrogel (TG-Gels)

High rigidity matrix-based materials such as TG-gels can allow osteogenic differentiation *in vitro*. New findings demonstrate macrophage modulation and stem cell recruitment in highly rigid HG for regeneration of the periodontal complex, where macrophages tend to undergo M1 polarization. Furthermore, when they were prepared in 3D and combined with IL-4 or stromal cell-derived factor 1

(SDF1), they repaired periodontal defects 4 weeks after surgery, allowing regeneration of hybrid tissue composed of bone, periodontal ligament, and cementum (He et al., 2019).

Chitosan Hydrogels

CsHG have been studied for regeneration of the periodontal complex. *In vivo*, CsHG with PDL cells transplanted in rat intraosseous periodontal defects is highly biocompatible and biodegradable. Histological analysis showed that the CsHG is degraded after 4 weeks of implantation and instead a cell complex, cement, PDL, and new bone formation were formed at the margin of the defect without any adverse reaction in the surrounding tissue (Park et al., 2003).

TABLE 2 | Hydrogel-based scaffolds for periodontal tissue regeneration.

PERIODONTAL LIGAMENT			
Hydrogel type	Type of study	Effect	Reference
Chitin-hyaluronic (The CS-HA hybrid)	*In vitro*. Modified hyaluronic acid (HA) and chitosan (CS) were employed to create a hybrid CS-HA hydrogel scaffold for periodontal regeneration. for cell culture was tested using NIH3T3 and MG63 cell lines.	The Alamar Blue test showed increased cellular viability and high CD44 expression, suggesting that the cells had higher migration when seeded onto the CS-AH scaffolds.	Miranda et al. (2016)
Fibrin-based	*In vitro/In vivo. In vitro* studies, a PEGylated fibrin hydrogel was combined with DPSCs. evaluated their cell proliferation, cell morphology, matrix degradation, collagen production and mineral deposition. *In vivo* study transplanted PEGylated fibrin constructs into dentin discs in immunosuppressed mice and examined for new tissue formation.	*In vitro* the cells were able to proliferate within PEGylated fibrin. In addition, the histological analysis revealed fibrin degradation and production of a collagen matrix. *In vivo* transplantation produced vascularized soft connective tissue.	Galler et al. (2011b)
Transglutaminase gelatin (TG gels)	*In vitro/In vivo*. TG gels containing cytokines and /or cells, for polarization of murine Mφs. in a critical periodontal defect in rats.	The presence of IL-4 could induce Mφs towards an anti-inflammatory M2 phenotype, which could promote the osteogenesis of BMSC *In vitro*. *In vivo* TG gel periodontal regeneration was significantly improved, allowing regeneration of hybrid tissue composed of bone, periodontal ligament, and cement.	He et al. (2019)
Alginate/Hyaluronic Acid	*In vitro/In vivo*. Hyaluronic acid (based stem cell delivery system loaded with TGF-β1 ligand, which encapsulates PDLSCs; and investigated the chondrogenic differentiation of cells encapsulated in alginate /HA hydrogel microspheres.	The results showed that PDLSC in contact with the hydrogel high levels of expression of Col II, Aggrecan and Sox-9 genes related to chondrogenesis. In addition, significant high expression of Col II was verified in PDLSC implanted subcutaneously in nude mice after 8 weeks.	Ansari et al. (2017)
Chitosan	*In vivo*. Periodontal tissue regeneration using enzymatically solidified chitosan hydrogels with or without cell loading.	In terms of periodontal regeneration, alveolar bone height, alveolar bone area, and epithelial growth, both CHIT and CHIT+Cell hydrogels, showed a significant increase in functional ligament length.	Park et al. (2003)
Chitosan nanoparticles and PLGA (CS-PLGA) with recombinant human FGF-2	*In vivo*. The evaluation of this scaffold was carried out in a periodontal defect model in rabbit.	Demonstrated complete regeneration of hybrid periodontics tissues.	Sowmya et al. (2017b)
Synthetic extracellular matrix	*In vivo*. Periodontal defects were induced at six sites in eight miniature pigs in the premolar/molar area (–4 weeks).	Demonstrating formation of Sharpey´s fibers and hybrid tissue in the area of the periodontal.	Fawzy El-Sayed et al. (2012)
	In vivo. Periodontal defects were induced at four sites in eight miniature pigs in the premolar/molar area (–4 weeks).	G-MSCs in conjunction with IL-1ra-loaded HA-sECM show a significant periodontal regenerative potential.	Fawzy El Sayed et al. (2015)

TABLE 2 | (*Continued*) Hydrogel-based scaffolds for periodontal tissue regeneration.

ALVEOLAR BONE			
Hydrogel type	**Type of study**	**Effect**	**Reference**
Collagen with bone morphogenetic protein 2 (BMP-2) and vascular endothelial growth factor (VEGF)	*In Vivo* The synergistic effect of VEGF and BMP-2 on maxillary alveolar bone regeneration was evaluated in a canine model by microcomputed tomography and histological staining.	Stimulates early bone formation in alveolar defects. However, the effective concentration of BMP-2 and VEGF to add to the scaffold is unknown.	Kim et al. (2016)
The polyethylene glycol (PEG) with BMP-2	*In Vivo*. 48 implants were partially placed in adult mini-pigs. At 9 weeks, soft tissue healing was assessed and the extent of the new vertical bone was assessed with microCT and histomorphometry.	Induces an increase in the height and volume of the alveolar bone on a dental implant.	Wen et al. (2017)
HyA with bone morphogenetic protein 7 (BMP-7)	*In Vitro, In Vivo*. HA hydrogels were prepared with human OB ± BMP-7. Cell viability, osteogenic gene expression, mineralized tissue formation and BMP-7 release *in vitro* were evaluated. . In an *in vivo* model, subcutaneous ectopic mineralized tissue formation was assessed by μ-CT and histology	Demonstrated formation of mineralized collagen matrix activates formation of vascularized bone-like tissue, providing a favorable environment for bone formation.	Hamlet et al. (2017)
Poly (lactic-co-glycolic acid) (PLGA) with BMP-2	*In Vivo*. 4 beagle dogs (13–15 kg) were usedSequential fluorescent labeling and histological analysis were performed to assess new bone formation and osseointegration in the defect area.	Improves bone formation around osseointegration implants.	Jo et al. (2015)
Gelatin with BMP-2 and OP3-4	*In Vivo*. OP3-4 was injected subperiosistically with BMP-2 in the right maxillary diastema. The mice were sacrificed 28 days later. Bone formation was analyzed for radiopaque area and bone mineral content and histological analysis based on fluorescence.	Induces bone formation, reducing inflammation caused by BMP-2.	Uehara et al. (2016)
PLGA with BMP-2, hydroxyapatite and tricalcium phosphate	*In Vivo*. Gel /HA /β-TCP compounds alone, infused with BMP-2 solution (BMPi) or microspheres (BMPm), were placed in rat jaws using a mini titanium implant for 4 weeks, efficacy was assessed by micro-computed tomography, bone fluorochrome and histology	Showed osteogenic potential and increased supraalveolar crest bone.	Chang et al. (2017)
Chitosan with biphasic calcium phosphate.	*In Vitro*. Osteoblast proliferation was evaluated, evaluated by scanning electron microscopy analysis. cell studies were performed with human osteoblasts observing expression of alkaline phosphatase activity	Promotes high proliferation and adhesion of osteoblastic cells and increases alkaline phosphatase activity	Iviglia et al. (2016)
Chitosan with Quitinpoli PLGA /nano bioactive glass-ceramic (nBGC) /cementum protein 1, chitin-PLGA /fibroblast growth factor 2	*In Vivo*. Cytocompatibility and cementum, fibrogenic and osteogenic differentiation of stem cells from human dental follicle were evaluated. *In vivo*, histological and immunohistochemical microcomputed tomography analysis was performed.	Stimulates alveolar and periodontal regeneration.	Sowmya et al. (2017a)
Chitosan with sodium glycerophosphate b (b-GP), aspirin, and erythropoietin.	*In Vivo*. Aspirin and erythropoietin (EPO) release was evaluated continuously, as well as immunohistochemical staining and micro-CT analysis and histological.	Effective to control inflammation, improves alveolar regeneration.	Xu et al. (2019)
PEG with osteogenic protein (OP-1)	*In Vivo*. Bone defects were created in jaws of minipigs. Samples were processed for histology and histomorphometry 3 weeks after implantation.	High concentrations, this protein induces faster bone maturation.	Catros et al. (2015)
PEG with growth factors platelet-derived growth factor-BB (PDGF-BB)	*In Vivo*. Four mandibular alveolar ridge defects were created in dogs. After 8 weeks, titanium dental implants were placed. After 8 weeks of, the animals were sacrificed and histomorphometric analysis of the alveolar ridge was performed.	Promotes cell proliferation, angiogenesis and chemotaxis in osteogenic processes.	Vierra et al. (2016)
Gelatin with transforming growth factor (TGF-b1) and PDGF-BB,	*In Vivo*. Regeneration of periodontal tissue was evaluated after the controlled release of growth factors in PRP. DNA, mitochondrial activity and ALP activity were evaluated. and by ELISA release of TGF-β1. and MTT assay	Promotes cellular activity by increasing osteogenesis.	Nakajima et al. (2015)

TABLE 2 | (Continued) Hydrogel-based scaffolds for periodontal tissue regeneration.

GelMA with encapsulated human periodontal ligament stem cells hPDLSCs	In Vitro, In Vivo. The microstructure of the GelMA hydrogels was examined. Critical alveolar defects were performed in vivo in rats, and the evaluation of new bone formation was observed by Micro-CT.	Stimulates proliferation, migration and osteogenic differentiation. efficient for alveolar regeneration.	Pan et al. (2019b)
Octapeptide of phenylalanine, glutamic acid, and lysine	In Vitro The ability to host human mesenchymal stem cells in three dimensions and induce their osteogenic differentiation was evaluated. Bone protein synthesis, including Col-1, osteocalcin and alkaline phosphatase, and mineralization were evaluated	Showed viability, proliferation and differentiation of human mesenchymal stem cells to osteoblasts, synthesizing bone proteins such as collagen-1 (Col-1), osteocalcin and ALP.	Castillo Diaz et al. (2016)
Biphasic Calcium Phosphate Hydroxypropyl Methyl Cellulose HG (Si-HPMC /BCP)	In Vivo. Efficacy for the treatment of periodontal defects (canine fenestration and premolar furcation) in canine models. bone formation was evaluated in the furcation mode	At 3 months, bone formation was observed. Si-HPMC hydrogel improves intergranular cohesion and acts as a barrier.	Struillou et al. (2011)
The Polyvinyl alcohol (PVA)	In Vitro Tensile tests, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray diffraction (XRD) were performed.	These data suggest bioactivity of the hydrogel is associated with the surface distribution of hydrophilic/hydrophobic components. Hydrogels can have potential applications in regeneration.	Tang et al. (2015)

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Hydrogel type	Type of study	Effect	Reference
Chitosan (Cs)Hydrogels	In vivo Chitosan hydrogels were prepared and transplanted into rat intraosseous periodontal defects. After 4 weeks, the jaws were removed, for histological, histomorphometric, and immunohistochemical evaluations.	It was observed to degrade after 4 weeks of implantation, with no adverse reaction. Stimulate the formation of cellular cement in the defect.	Park et al. (2003)
Chitosan (Cs) /Collagen (CS)Hydrogels	In vivo Intraosseous defects were created in the maxillary and mandibular first and third premolars. and a histological examination was performed.	Stimulates a significant amount of new cement along the notch and root surface to the level of the junctional epithelium.	Park et al. (2003)
Chitosan (Cs)/gelatin/glycerolphosphate(GP) Hydrogels	In vivo A chitosan/gelatin/glycerol phosphate hydrogel was evaluated for transplanted stem cells. In animal models, bone defects were performed in maxillary molars. I evaluate the new regeneration of PDL (shown by Masson's trichrome staining and a qualification assay).	Increases cementum formation, due to increased expression of CAP in vitro. Furthermore, in vivo it showed cementum formation and decreased inflammation progress.	Chien et al. (2018)
Chitosan (Cs)-PA-βglycerolphosphate βGP	In vitro/in vivo. Physicochemical properties and in vitro biocompatibility were evaluated. and its morphology with scanning electron microscopy and in vivo study in dog class III furcation defects	Short gel time, higher viscosity, higher water absorption, proper degradation time, porous structure and no cytotoxicity. In addition, it promoted newly formed cement in class III furcation defects in dogs.	Zang et al. (2014)
Collagen (CS)	In vivo. Periodontal defects were created. the hydrogel was implanted in each defect. The percentage of length of new bone and cement was evaluated. In vivo. Class II furcation defects were surgically created. the defect was filled with the hydrogel. Histometric parameters were evaluated 2 and 4 weeks after surgery	The hydrogel does not stimulate ankylosis and the newly formed periodontal ligament showed normal morphology, demonstrating regeneration of the periodontium.	Miyaji et al. (2007), Kosen et al. (2012)
Collagen (CS)/fibroblast growth factor2(FGF2)	In vivo. The collagen was injected into a sponge. Subsequently, the hydrogel with FGF2 was implanted in class II furcation defects in dogs. Histometric parameters were evaluated at 10 days and 4 weeks after surgery	Periodontal junction was repaired, with cement-like tissue, similar to the periodontal ligament, indicating that FGF2 guided the self-assembly and restored the function of the periodontal organs.	Momose et al. (2016a, 2016b)
Chitin-PLGA-nBGC-CEMP1- fibroblast growth factor2(FBF2) RPR	In Vivo. Cytocompatibility and cementum, fibrogenic and osteogenic differentiation of stem cells from human dental follicle were evaluated. In vivo, histological and immunohistochemical microcomputed tomography analysis was performed.	Stimulates cementum differentiation of hDFC. In vivo, I encourage the formation of new cement.	Sowmya et al. (2017a)
CPC-BMP2-PGA-FGF2	In vivo. Three Macaca fascicularis were used. Periodontal defects in the mandible were created After 3 months, tissue regeneration was assessed by histomorphometry and radiographic measurements.	Showed descending epithelial growth, while cementum and ligament regenerated significantly.	Wang et al. (2019)

TABLE 2 | (*Continued*) Hydrogel-based scaffolds for periodontal tissue regeneration.

GINGIVAL TISSUE			
Hydrogel type	Type of study	Effect	Reference
Hyaluronic acid	*In vivo*. Conducted a randomized controlled clinical pilot study. carried out the Evaluation of the efficacy of a biogel based on hyaluronic acid on periodontal clinical parameters.	HA esterified gel showed an effect in reducing gingival inflammation when used as an adjunct to mechanical plaque control at home and could be used successfully to improve periodontal clinical indices.	Pilloni et al. (2011)
	In vivo. In a randomized study, 50 male subjects with plaque-induced gingivitis. The clinical indices (API, Turesky index, PBI) and the crevicular fluid variables (peroxidase, lysozyme) were determined at the beginning of the study and after 4, 7, 14 and 21 days.	The result was a significant decrease in the activities of peroxidase and lysozyme after 7, 14 and 21 days.	Jentsch et al. (2003)
	In vivo. Hyaluronic acid was used to repair the interdental papilla in the aesthetic zone.	HA gel as gingival filling material can be considered as a treatment option for augmenting interdental papilla in aesthetic área.	(288)
	In vivo. Effect of hyaluronan in 19 patients affected with mild chronic periodontal disease, with shallow pockets in at least two different quadrants, comparing the material with normal oral hygiene.	Subgingival placement of 0.2% HA gel along with SRP provided a significant improvement in gingival parameters.	(266)

Chitin-Based Hydrogels

Chitin-derived biomaterials can mimic the structure of the ECM, providing excellent scaffolding for cells, and creating a favorable immune environment for accurate periodontal healing and regeneration (Xie et al., 2020). In this regard, the chitin-HyA hybrid HG generates a perfect scaffold material that can be used to support osteoblasts, gingival fibroblasts, cementoblasts, and periodontal fibroblast cells (Miranda et al., 2016; Orellana et al., 2018). Also, HG composed of chitin nanoparticles and PLGA can benefit tissue regeneration when is functionalized with recombinant human FGF-2, since it can achieve total closure of the periodontal defect in a rabbit model, where histologically periodontal ligament-like characteristics can be seen (Sowmya et al., 2017b).

Synthetic Extracellular Matrix Hydrogels

These new generation HG have been designed from synthetic HyA scaffolds (Espandar et al., 2012) to improve the results of periodontal treatment. These HG can be modified by adding IL1 receptor antagonist (IL-1ra) that binds with high affinity to cell surface IL-1 receptors (Gorth et al., 2012). As it is known that in periodontitis there is an increase in IL-1 (Suwatanapongched et al., 2000), a favorable effect in periodontal wound healing is seen when the HG containing IL-1ra is administered (Zhang et al., 2004). *In vivo* studies confirm the periodontal regenerative potential of this HG with gingival margin stem cells (GMSCs) demonstrating the formation of Sharpey´s fibers and hybrid tissue in the area of the periodontal defect (Fawzy El Sayed et al., 2015).

Hydrogels-Bassed Scaffolds for Alveolar Bone Regeneration

Tissue engineering in alveolar bone regeneration has developed new alternatives for the treatment of bone defects. The alveolar bone is essential to guarantee the success of dental organ preservation, and also if the use of dental implants is required (Warnke et al., 2004). The close relationship between alveolar bone, periodontal ligament, and cement, hinders successful regeneration. To solve this problem, It is necessary to develop HG in combination with biomolecules (Lee and Shin, 2007). Alveolar scaffolds must be osteoinductive or osteoconductive, have mechanical resistance, adequate degradation, avoid the collapse of the area to be regenerated, good flexibility, adapt to different morphologies, and be easily managed. However, until now, biomaterials with all of these characteristics are not available (Kaigler et al., 2011). Here we summarize the HG used for alveolar bone regeneration.

Hydrogels Biofunctionalized With BMPs

HG with different BMPs have been used, due to their commercial availability and their easy incorporation into different HG. For instance, the collagen HG with BMP-2 and VEGF, *in vivo* has shown a favorable early bone formation in dogs with alveolar defects. However, the effective concentration of BMP-2 and VEGF to add to the scaffold is unknown, and long-term studies are required. (Kim et al., 2016). The polyethylene glycol (PEG) HG with BMP-2 *in vivo* induces an increase of the height and volume of the alveolar and cortical bone over an implant placed in the jaw of pigs, indicating that it could enhance the retention of the implants when the alveolar crest is small. (Wen et al., 2017). In the case of HyA HG with bone BMP-7, it has the capability of forming a mineralized collagen matrix *in vitro*. Interestingly, *in vivo*, this HG can form vascularized bone-like tissue after 4 weeks in a rat model, since it provides a favorable environment for bone formation. (Hamlet et al., 2017). The PLGA HG with BMP-2 has been used in beagle dogs creating irregular bone defects around implants resulting in the improvement of bone formation and osseointegration when compared with control without BMP-2 (Jo et al., 2015).

The Gelatin HG with BMP-2 and the osteoprotegerin mimic peptide OP3-4 (YCEIEFCYLIR) was created to avoid the side effects of BMPs, such as inflammation and carcinogenesis. This combination in a murine model applied by subperiosteal injection shows the induction of bone formation, reducing inflammation caused by BMP-2 (Uehara et al., 2016). The PLGA HG with BMP-2, hydroxyapatite and tricalcium phosphate has been used in rats for supra-alveolar crest augmentation treatment, showing osteogenic potential and increasing supra-alveolar crest bone (Pilloni et al., 2011).

Chitosan Hydrogels

CsHG can be combined with biphasic calcium phosphate. The incorporation of ceramic particles in this material makes it stable at different pH and increases the elastic modulus and maximum tensile strength. Besides, it promotes high proliferation, osteoblastic cell adhesion and increases ALP activity (Iviglia et al., 2016). Other additions that have been made to the CsHG such as Quitinpoli PLGA/nano bioactive glass-ceramic (nBGC)/CEMP-1, chitin-PLGA/FGF2 as PDL layer, and platelet-rich plasma-derived growth factors and chitin-PLGA/nBGC. This three-layer nanocomposite HG is cytocompatible and favors osteogenic differentiation of stem cells from the human dental follicle, and its content of growth factors helps the formation of bone trabeculae, stimulating alveolar and periodontal regeneration (Sowmya et al., 2017a). CsHG can be used also with β-GP, aspirin, and erythropoietin. *In vivo* experiments revealed that this HG is effective in controlling inflammation, enhances alveolar regeneration, and could be used in the future for clinical application (Xu et al., 2019).

Other Hydrogels

The PEG HG has been used in defects in the mandibular alveolar ridge of dogs showing unpredictable bone formation and, in most cases, a negative impact on alveolar regeneration (Vierra et al., 2016). The PEG with osteogenic protein (OP-1) in a mini-pig alveolar defects model shows that in high concentrations, this protein induces faster bone maturation (Catros et al., 2015). In the case of HG with growth factors such as platelet-derived growth factor-BB (PDGF-BB), it promotes cell proliferation, angiogenesis, and chemoattraction in osteogenic processes (Pan et al., 2019a). Meanwhile, gelatin HG with TGF-β1 and PDGF-BB promotes cellular activity by increasing osteogenesis (Nakajima et al., 2015)

The GelMA HG with encapsulated human periodontal ligament stem cells (PDLSCs) presents a highly porous and interconnected network providing an optimal niche for proliferation, migration, and osteogenic differentiation. *In vivo*, performing alveolar bone defects in rats, the PDLSCs encapsulated in GelMA HG show to be more efficient for alveolar regeneration and it may represent a new strategy for therapy of alveolar bone defects (Pan et al., 2019b).

The use of biodegradable HG with octapeptide of phenylalanine, glutamic acid, and lysine has shown the formation of an ideal niche, apt for cell physiological functions. *In vitro* tests showed viability, proliferation, and differentiation of human mesenchymal stem cells to osteoblasts, synthesizing bone proteins such COL1,

OCN, and ALP. Furthermore, mineralization within the HG was evident, showing potential to regenerate hard tissues such as alveolar bone (Castillo Diaz et al., 2016).

Regarding Biphasic Calcium Phosphate Hydroxypropyl Methyl Cellulose (Si-HPMC/BCP) HG, due to its BCP particle components helps to osteoconduction process, while Si-HPMC improves intergranular cohesion. This biomaterial is easy to use and simplifies the filling process for bone lesions. However, more research is needed to be used on alveolar defects. (Struillou et al., 2011).

The PVA HG has chemical stability, biocompatibility and is porous facilitating cell adhesion. *In vitro* tests showed excellent biocompatibility, and in X-ray Diffraction tests, it was observed that it has affinity to form HA. This HG may have potential applications in alveolar bone regeneration, as it was also associated with the surface distribution of hydrophilic/hydrophobic components (Tang et al., 2015).

Hydrogels-Bassed Scaffolds for Dental Cementum Regeneration

Cementum neoformation is esencial for periodontal regeneration. Precementum formation starts following the fragmentation of Hertwig's root sheath, the matrix of proteins formed subsequently provides an adequate attachment substrate to support differentiation of cementoblasts from ectomesenchymal progenitors of the dental follicle (Hammarström et al., 1996). Cementum deposition continues with the apical development of the root allowing the integration of Sharpey's fibers collagen bundles, into the newly generated cementum (Menicanin et al., 2015). The formed tissue is a hard and avascular connective tissue located on the dental root that emerges from the alveolar bone to the dental organ. It has been described two types of cementum depending on the content of inner cells: cellular and acellular (Veis, 2002). Dental cementum participates in key processes such as maintaining stability and chewing charge distribution, thus, when cementum is infected or suffers trauma, treatments require to provide a disinfected root surface to promote the reinsertion of collagen fibers and avoid the formation of pathological spaces that would promote reinfection. Consequently, the success of periodontal regenerative therapy relies on the health of the dental cementum, its health, and its capacity to regenerate (Grzesik and Narayanan, 2002).

Dental cementum is composed of 50% of organic matrix, of which, 90% is COL1. Another cement-related collagen protein is type III collagen, involved in the development, repair, and regeneration of periodontal tissues (Alvarez-Pérez et al., 2006). Besides, within its organic composition, there are two specific proteins of this tissue 3-Hydroxyacyl coenzyme A dehydratase 1 (HACD1)/Cementum Attachment Protein (CAP) and CEMP-1. HACD/CAP has been implicated in the nucleation, regulation, and direction of hydroxyapatite crystal growth and regeneration of critical bone defects in rat calvaria, meanwhile, CEMP-1 regulates the activity of cementoblasts by inducing differentiation and mediates in the mineralization process, migration, and proliferation of gingival fibroblasts (Arzate et al., 1992; Bermúdez et al., 2015; Villegas-Mercado et al., 2018); Furthermore, CEMP1 also

promotes the nucleation of octacalcium phosphate crystals, which is a mineral phase and a precursor of HA crystals (Alvarez-Pérez et al., 2006). The other 50% are minerals, mostly HA [Ca_{10} $(PO_4)_6$ $(OH)_2$] (Nanci and Bosshardt, 2000). Here we show the HG able to sustain at the same time biomineralization for cementum regeneration and proliferation of cells for periodontal ligament formation.

Chitosan-Based Hydrogels

CsHG with or without fluorescently labeled PDL cells were prepared and transplanted into rat intrabony periodontal defects; untreated defects were used as empty controls. After 4 weeks the results showed that PDL cells remained viable upon encapsulation within CsHG before transplantation but histological analysis demonstrated that the CsHG were degraded after 4 weeks without affection of the adjacent tissue. Interestingly, CsHG without cell loading showed a newly formed cellular cementum limited to the apical part of the defect (Yan et al., 2015). In another study, one-wall intrabony defects of beagle dogs were surgically created in the bilateral maxillary first and third, and the mandibular second and fourth premolars and treated with a Cs/collagen sponge, at 8 weeks after the operation, a significant amount of new cementum formed along the notch and root surface to the level of the junctional epithelium was found (Park et al., 2003).

In 2018 Chien et al., applied an injectable and thermosensitive Cs/gelatin/glycerol phosphate HG to enhance stem cell delivery and engraftment. Synergistic effects of iPSCs and BMP-6 increased cementum formation which was confirmed by CAP expression since it was significantly up-regulated in vitro. Besides, iPSCs-BMP-6-HG in vivo showed the formation of cementum and minimized the progress of inflammation in rats with maxillary-molar defects. These findings suggest that this combination can promote stem cell-derived graft engraftment with an anti-inflammatory effect, which resulted in highly possible periodontal regeneration (Chien et al., 2018). Interestingly, a thermosensitive CsHG using autoclaved Cs powder (121°C, 10 min) and β-GP (Cs-PA/GP) were compared to the physicochemical properties and biocompatibility in vitro. According to their results, the Cs-PA/GP HG had a shortened gelation time, higher viscosity, increased water absorption, appropriate degradation time, porous structure, and no obvious cytotoxicity on human periodontal ligament cells. Besides, the Cs-PA/GP HG promoted cell-like newly formed cementum in dog class III furcation defects (Zang et al., 2014).

Collagen-Based Hydrogels

In 2017, Miyaji et al., studied collagen HG on periodontal wound healing in beagles. Sixty-four periodontal dehiscence type defects were created on the buccal roots of four beagles for collagen HG implantation. After 8 weeks new cementum was found in a higher proportion when compared with the control group indicating that periodontal regeneration was stimulated by collagen HG implantation (Miyaji et al., 2007). Another study in surgical-created defects was developed by Kosen et al., where collagen HG/sponge scaffold was prepared by injecting collagen HG, cross-linked to the ascorbate-copper ion system, and placed in class II

furcation defects in beagle dogs. At 2 weeks, the collagen HG/sponge scaffold displayed high biocompatibility and biodegradability with numerous infiltrating cells. In the experimental group, reconstruction of alveolar bone and cementum was frequently observed 4 weeks after surgery. The study showed that cell-rich connective tissue had reformed along the root surfaces after the application of HG. Also, the 4-week specimens in the HG group showed no ankylosis, and the newly formed periodontal ligament showed functional morphology, demonstrated by the connection of newly formed cementum to the alveolar bone with Sharpey´s fibers (Kosen et al., 2012)

Momose et al., evaluated the application of collagen HG scaffold in combination with FGF2 in furcation defects in beagle dogs. Collagen HG was associated with FGF2 and injected into sponge-form collagen and then implanted into class II furcation defects in dogs. This combination improved cell and tissue growth where cells and blood vessel-like structures were found on day 10. At 4 weeks, periodontal attachment, consisting of cementum-like tissue, periodontal ligament-like tissue, and Sharpey's fibers, was repaired, indicating that FGF2-loaded scaffold led to self-assembly and then re-established the function of periodontal organs (Momose et al., 2016a, 2016b).

Other Hydrogels

Sowmya et al., in 2017 used the strategy of a tri-layered nanocomposite HGl scaffold that was developed by assembling chitin PLGA/nano bioactive glass-ceramic (nBGC)/CEMP-1 as the cementum layer, chitin–PLGA/FGF2 as the PDL layer, and chitin–PLGA/nBGC/platelet-rich plasma-derived growth factors as the alveolar bone layer layers in the order of periodontal tissue location, i.e., cementum, PDL, and alveolar bone. The cementogenic differentiation of human dental follicle stem cells on the tri-layered nanocomposite HG scaffold with growth factors was comparable to the cellular differentiation on the tri-layered nanocomposite HG scaffold cultured in induction medium. In vivo, complete healing with the formation of new cementum was more pronounced in tri-layered nanocomposite HG scaffold with growth factors in comparison to the other groups (Sowmya et al., 2017a).

The calcium phosphate cement (CPC)/propylene glycol alginate (PGA) with BMP-2 and fibroblast growth factor (FGF)-2 gel (CPC/BMP+PGA/FGF) was studied in a periodontal defect model in Macaca fascicularis where three-wall periodontal defects were surgically created in the mandible. Results showed that epithelial downgrowth, meanwhile cementum, and ligament were regenerated significantly when compared to the control group (Wang et al., 2019).

Hydrogels-Bassed Scaffolds for Gingival Tissue Regeneration

The gingival tissue can be completely regenerated (Freedman, 2012). The gingival tissue is a fibromucous membrane that covers the mandibular and maxillary bone, designed to withstand constant trauma from chewing. Clinically it is divided into

free, inserted, and papillary. According to its structure, it is made up of stratified squamous epithelium, rich in keratinocytes. The gingival epithelium is divided into oral epithelium, sulcus epithelium, and junction epithelium (Andrian et al., 2006). HG used for gingival tissue regeneration are presented below.

Hyaluronic Acid-Based Hydrogels

Various injuries can cause periodontal damage, in the clinical area, several studies have been able to demonstrate the capacity of new materials based on polysaccharides such as HyA since it is known to be a powerful antioxidant, biocompatible, anti-inflammatory, with viscoelastic and bacteriostatic properties. The efficacy of topical application of HyA HG in the treatment of gingivitis has been evaluated and it turned out to be a potentially useful supplement in therapy against this disease (Pistorius et al., 2005). The gel containing 0.2% hyaluronate has a beneficial effect in the treatment of gingivitis, the result was a significant decrease in the activities of peroxidase and lysozyme after 7, 14, and 21 days (Jentsch et al., 2003), complemented with scaling and root planing (SRP) in patients with chronic periodontitis (Gontiya and Galgali, 2012).

In terms of better healing after nonsurgical therapy, supplemental use of 0.8% hyaluronan HG after complete mechanical debridement potentially has significant clinical benefits (Bolt and Bhupinder, 2007). In the area of dental esthetics, it has been used to repair the interdental papilla, where it is infiltrated until extravasation, after 3 months of follow-up, a completely healthy gum was observed and the papilla was filled (Zatta da Silva et al., 2019).

CLINICAL IMPLICATIONS

Given that human dental tissues cannot be biologically repaired once formed, strategies such as the use of cells and biomolecules in 3D scaffolds for regeneration are required. Nonetheless, pulp tissue vulnerability needs a different perspective, since it lacks collateral circulation and requires the revascularization and reinnervation of the new tissue (Bermúdez et al., 2021). Therefore, the development of strategies with the potential for clinical implementations is still a far task to achieve.

From a clinical viewpoint, HG combined or alone can enhance remineralization or increase thickness and mechanical properties of the regenerated enamel and dentin. Furthermore, HG present outstanding chemical, mechanical and biological activities (Moussa and Aparicio, 2019) such as the capacity of incorporating cells in their structures and at the same time degrading themselves to let place to new healthy tissue. Besides, they can retain high-water content which enhances the transportation of cell nutrients and waste, and also are elastic and flexible emulating the native ECM (Mantha et al., 2019). Nevertheless, most of them remain under experimental investigation on *in vitro* cell models and preclinical models and need to validate their dose and efficiency to be used. Nowadays, only ChHG with TGF-β1and Puramatrix™ have shown promising results in dental tissues *in vivo*. ChHG with TGF-β1showed a sustained release over 7 days of more than 40% of the TGF-β1 *in vivo*, resulting in reparative dentin formation (Li et al., 2014b). In the case of Puramatrix™, when

is injected into the human root canals in the back of immunodeficient mice (CB-17 SCID), containing human SHED, induces the differentiation to odontoblasts that express DSPP, DMP-1, and MEPE, allowing the formation of new tubular dentin and when conjugates with growth factors TGF-β1, FGF2, VEGF in DPSC, vascularized and soft connective tissue similar to a dental pulp was observed within the dentin cylinder after 6 weeks (Almeida et al., 2018a). These results could, in the future, change the clinical approach, avoiding the use of inert materials for treating dental tissues.

Concerning periodontal tissues, during *in vitro* or *ex vivo* tests, normal conditions such as resistance of occlusal mechanical forces of PDL, cementum, and alveolar bone cannot be completely achieved. Due to their complex composition, HG should be capable to interact with hard and soft tissues at the same time (Elango et al., 2020). Many options have been studied *in vitro* with promising results that may lead to preclinical studies (Nagy et al., 2018), but this closed relationship hinders the use of HG in clinical studies. Thus, *in vivo* models are mandatory for representing the occlusal state to support the functional regeneration design (Bermúdez et al., 2021). Besides, one of the biggest challenges is the reconstitution of the insertion apparatus (Sharpey's fiber connecting cementum and alveolar bone) since its absence impacts negatively the capacity of the tooth to support the occlusal forces (Liang et al., 2020).

Regarding HG used to promote bone, PDL, and cementum healing and regeneration, most of them have only been evaluated *in vitro* or in preclinical animal studies. Among the HG reported for alveolar regeneration, the PEG HG with BMP-2 (Wen et al., 2017) and PLGA HG with BMP-2 (Jo et al., 2015) have proved their value to regenerate bone defects around implants in animal models resulting in the improvement of bone formation and osseointegration. Another promising HG is the collagen HG/sponge achieving the formation of bone, cementum, and periodontal ligament with functional morphology (Kosen et al., 2012). Also, its combination with FGF2 results in periodontal attachment, consisting of cementum-like tissue, periodontal ligament-like tissue (Momose et al., 2016a, 2016b).

In the case of gingival tissue wound healing stimulated by the application of HG, it should benefit the clinical outcome of other oral surgical procedures by guaranteeing that the tissue is sealed off from the contaminated oral environment, conducting to a more rapid connective tissue reorganization and causing a faster gain in wound strength, reducing the potential for scarring and scar tissue contraction, thus gingival restoration and stability is crucial to obtain predictive results (Villa et al., 1943). Fortunately, HG have proved their value in regenerating soft tissues. For instance, the 0.2% hyaluronate HG decreases the peroxidase and lysozyme activity (Jentsch et al., 2003), enhancing wound healing after SRP in patients with chronic periodontitis (Gontiya and Galgali, 2012). Besides, 0.8% hyaluronan HG (Bolt and Bhupinder, 2007) has been used to repair the interdental papilla with completely healthy gum and the papilla after 3 months (Zatta da Silva et al., 2019).

Finally, although the results obtained with HG are promising, we must not lose sight of the fact that oral tissues function unified, and during their development, signal pathways can crosstalk and participate in more than one tissue. Thus, the stimuli given by one

HG can be useful for another closely related one (Bermúdez et al., 2021). For instance, some promising HG alone or combined might induce biomineralization in enamel, dentin, cementum, and alveolar bone, and others might function for neovascularization and neoformation of the dental pulp and soft periodontal tissue. Despite this, more research is needed to determine which combinations of biofunctionalizing molecules, cells, and HG can improve clinical results.

CONCLUDING REMARKS

HG-based scaffolds used for oral tissue regeneration offer several advantages including the capacity of incorporating cells in their structures and at the same time degrading themselves to let place to new healthy tissue. Besides, they can be injectable and retain high water content which enhances the transportation of cell nutrients and waste. They are elastic and flexible emulating the native ECM. Additionally, HG can be combined with biofunctionalizing molecules to induce cellular processes such as chemotaxis, proliferation, differentiation, neovascularization, and biomineralization.

Even though most of the HG are still under investigation, some of them have been studied *in vitro* and *in vivo* with promising results that may lead to preclinical studies. Besides, there are HG that have shown their efficacy in healing gingival tissue in patients, such as hyaluronan HG.

Finally, the use of HG could become a gold standard for the treatment of damaged oral tissues by replacing the use of inert materials that are currently highly used in dental practice, if limitations for *in vivo* studies are exceeded and well-designed clinical studies support their value to be broadly used.

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