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Present and future of tissue engineering scaffolds for dentin-pulp complex regeneration

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Abstract

More than two thirds of the global population suffers from tooth decay, which results in cavities with various levels of lesion severity. Clinical interventions to treat tooth decay range from simple coronal fillings to invasive root canal treatment. Pulp capping is the only available clinical option to maintain the pulp vitality in deep lesions, but irreversible pulp inflammation and reinfection are frequent outcomes for this treatment. When affected pulp involvement is beyond repair, the dentist has to perform endodontic therapy leaving the tooth non-vital and brittle. On-going research strategies have failed to overcome the limitations of existing pulp capping materials so that healthy and progressive regeneration of the injured tissues is attained. Preserving pulp vitality is crucial for tooth homeostasis and durability, and thus, there is a critical need for clinical interventions that enable regeneration of the dentin-pulp complex to rescue millions of teeth annually. The identification and development of appropriate biomaterials for dentin-pulp scaffolds are necessary to optimize clinical approaches to regenerate these hybrid dental tissues. Likewise, a deep understanding of the interactions between the micro-environment, growth factors, and progenitor cells will provide design basis for the most fitting scaffolds for this purpose. In this review, we first introduce the long-lasting clinical dental problem of rescuing diseased tooth vitality, the limitations of current clinical therapies and interventions to restore the damaged tissues, and the need for new strategies to fully revitalize the tooth. Then, we comprehensively report on the characteristics of the main materials of naturally-derived and synthetically-engineered polymers, ceramics, and composite scaffolds as well as their use in dentin-pulp complex regeneration strategies. Finally, we present a series of innovative smart polymeric biomaterials with potential to overcome dentin-pulp complex regeneration challenges.

Keywords

dentin-pulp complex; endodontics; multifunctional scaffolds; natural scaffolds; synthetic scaffolds; tissue engineering

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

1 | THE NEED AND CHALLENGE OF DENTIN-PULP COMPLEX REGENERATION

Worldwide, approximately 2.43 billion people (36% of the population) have dental caries/decay in their permanent teeth and about 620 million children (9% of the population) in their baby teeth (Vos et al., 2012). In the United States, dental caries is the most common chronic childhood disease. An astounding 92% of Americans ages 20 to 64 have had a cavity as an adult, and almost a quarter of that age group have untreated tooth decay (NICDR, 2014). Although surface decay can usually be treated and arrested with a filling, a tooth with deep decay or one that has been severely injured in an accident or the trauma of recurrent dental work may become unhealthy, inflamed, or infected. Left untreated, an infection may spread to the surrounding tissues (W. Zhang & Yelick, 2010).

The currently available treatment scenarios in such serious conditions are either vital pulp therapy, if the pulp is still vital or root canal treatment if the pulp is irreversibly inflamed. The vital pulp therapy aims to induce a dentinal bridge formation with scarred tissue to maintain pulp vitality and function after experiencing reversible pulpal injury. This mechanism of tissue repair is based on replacing damaged odontoblasts by newly regenerated populations of odontoblast-like cells derived from pulp stem cells in the healthy portion of the pulp (G. T. Huang, 2009; Scheller, Krebsbach, & Kohn, 2009). However, pulp capping in decayed teeth has been considered questionable and thus must be restricted to a few well-chosen cases (Bashutski & Wang, 2008).

Successful outcomes for vital pulp therapy are very dependent on the type and location of the injury, the age of the tooth, and the treatment modality, that is, the capping material and the integrity of the cavity restoration (Tziafas, 2004). In addition, the clinical diagnosis of pulp vitality is also inaccurate, and there are currently no established evidence-based guidelines to help clinicians determine which cases warrant being treated with this conservative approach (G. T. Huang, 2008). In reality, vital pulp therapy often results in eventual endodontic therapy as 45% of pulp capping interventions fail after 5 years with increasing failure rates up to 80% after 10 years (Goldberg et al., 2009).

The American association of endodontists has estimated that 22.3 million endodontic procedures were performed annually (American Association of Endodontists, 2017, October 23). To date, having a root canal is the only treatment protocol available to retain a diseased tooth. It requires total pulp amputation followed by pulp-space disinfection and filling with an artificial rubber-like material (G. T. Huang, 2008, 2009). Despite reported clinical success, endodontically treated teeth become devitalized, brittle, and susceptible to postoperative fracture or other complications, including reinfections (J. Y. Kim et al., 2010). At 1–5 years, 10–50% of endodontically treated teeth have been reported to be associated with post-treatment diseases due to incomplete removal of contaminated debris (Landys Boren, Jonasson, & Kvist, 2015). Ten to 20 years after endodontic therapy, many other clinical complications can occur, including the development of periapical lesions due to recontamination of the root canal (Fristad, Molven, & Halse, 2004). Twenty-year follow up studies have also shown that 15% of the subjects developed periapical pathosis and the

extraction percentage reached to 21% based on cumulative teeth survival curves (Prati, Pirani, Zamparini, Gatto, & Gandolfi, 2018). Further, the loss of pulp vitality in young permanent teeth terminates dentin formation, root development, apical closure, and subsequent tooth maturation. Endodontically treated teeth may also be undermined if they are not properly restored, making them more vulnerable to masticatory forces. Besides, endodontic therapy also often results in discoloration of the tooth crown, mainly due to staining from endodontic filling materials (Goto, Ceyhan, & Chu, 2009). Of particular importance is that the long-term coronal seal also plays a crucial role affecting the outcome of root canal treatment as shown in long-term and meta-analysis studies (Eckerbom, Flygare, & Magnusson, 2007; Gillen et al., 2011; Ng, Mann, & Gulabivala, 2010; Ng, Mann, Rahbaran, Lewsey, & Gulabivala, 2007).

Thus, there are notable limitations in the current biomaterials-based strategies used for pulp capping and dentin regeneration. Some of the most significant limitations are the severe inflammatory reactions induced by the synthetic capping materials that can cause failure, the permeability of the newly formed “dentin-like”/scar bridge that might enable bacterial recontamination of the tissues with the end result of secondary pulp inflammation, and the compromised blood supply in the exposed axial wall that results in tissue necrosis. Besides, formation of the dentinal bridge can occur within the teeth with irreversible inflammation, which requires comprehensive retreatment (Bergenholtz & Spangberg, 2004; Farges et al., 2015). These limitations mainly derive from the fact that current biomaterials used in the clinics for healing dentin-pulp tissues lack specific temporal and spatial control over biologic signalling required for progenitor cells’ homing and differentiation to eventually fully restore structural and functional characteristics of the tissue. Moreover, the mechanistic understanding of the regenerative outcomes attained using current pulp capping biomaterials is for the most part missing. Finally, current biomaterials are notably limited to control infection and inflammation for promoting reparative tissue formation (Schmalz & Smith, 2014).

To remedy these limitations of the current treatment, research in dentistry has extended its focus to conduct and establish more effective, reliable, and safer methods than traditional pulp capping therapies by designing new regenerative methods for dental treatment and specifically for regenerating the dentin-pulp complex (Murray, Garcia-Godoy, & Hargreaves, 2007). Regenerative dentistry represents an attractive multidisciplinary therapeutic approach that counteracts traditional restorative/surgery techniques and benefits from recent advances in materials science, stem cell biology, molecular biology, and proteomics. A significant contribution of materials sciences to the field is fundamental to move regenerative dentistry from the laboratory to the clinic (Mitsiadis, Woloszyk, & Jimenez-Rojo, 2012).

One of the difficulties of developing new treatments is that bio-materials to be used as scaffolds for tissue regeneration need to have biocompatibility, interconnected porosity, conductivity for attachment, capability to promote proliferation and differentiation of committed cells, and the ability to incorporate inductive factors, appropriate mechanical properties, and biodegradability. A variety of biomaterials have been developed in labs to fulfil these requirements, the most important of which is enabling progenitor cell homing and differentiation by providing inductive cues for controlling tissue regeneration spatially

and/or temporally (Scheller et al., 2009). Several new materials have been investigated for this purpose including components of the extracellular matrix (ECM), other proteins, polysaccharides, peptides, natural or synthetic polymers, bioceramics, and recently, various innovative composites. Each material offers a unique chemistry, composition, structure, and degradation profile as well as the potential for modification. Thus, the role of the scaffold has changed from a passive carrier to a bioactive milieu with tailor-made properties for regenerating specific tissues.

For dentin-pulp complex regeneration, researchers should consider tailoring several aspects including revascularization, innervation, cell-matrix interactions, growth factor incorporation, controlled bio-degradation, remineralization, and contamination control (Galler, D'Souza, Hartgerink, & Schmalz, 2011). This paper reviews the main characteristics of materials that are naturally-derived and synthetically-engineered polymers, ceramics, and composite scaffolds, as well as their use in dentin-pulp complex regeneration strategies. In addition, Table 1 provides a comprehensive list of advantages and limitations of these materials. Finally, we highlight a series of innovative smart composite scaffolds with the potential to overcome dentin-pulp complex regeneration challenges.

2 | BIOMATERIALS-BASED SCAFFOLDS FOR REGENERATING THE DENTIN-PULP COMPLEX

2.1 | Natural and naturally-derived polymeric scaffolds

Naturally-derived polymers, such as polysaccharides and proteins, including those from the ECM of natural dentinal tissues, are obtained from natural sources and form hydrogels that might incorporate up to 99% water content. Their ability to swell in water allows them to exhibit an environment that resembles the highly hydrated state of natural tissues (Ratner & Bryant, 2004). Even though naturally-derived polymers are characterized by having batch-to-batch variations and poor mechanical properties (Yang, Leong, Du, & Chua, 2001), they are highly biocompatible, readily available, inexpensive, and easy to fabricate into hydrogels. So they have gained widespread attention and investigated as scaffolds for the dentin-pulp complex regeneration (Ko, Sfeir, & Kumta, 2010; Table 1).

2.1.1 | Polysaccharides—Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages that on hydrolysis give the constituent monosaccharides or oligosaccharides. Structurally, they range from linear to highly branched. Alginate, chitosan, and cellulose are the most commonly used for dentin-pulp complex regeneration.

Alginate: Alginate is a polysaccharide derived from brown algae, certain seaweeds, or bacteria. It is a linear polysaccharide copolymer of (1,4)-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) monomers (Drury & Mooney, 2003), and its structure consists of blocks of M and G monomers. The composition and arrangement of the M and G monomers determine the structure and the properties of alginate. Alginates that contain higher percentage of G blocks express higher elastic modulus and higher solute diffusivity. Alginate gels readily set in the presence of divalent ions via ionic cross-linking. Alginate beads are

usually made by calcium ion cross-linking (Lin & Yeh, 2004). Its mechanical strength can be improved by increasing calcium content and thus, cross-linking density. Stable covalent cross-linking compared with ionic cross-linking offers higher mechanical strengths to alginate hydrogels (Sakai & Kawakami, 2007). Arginine-glycine-aspartic acid (RGD) modified alginate hydrogel promotes cell adhesion, spreading, proliferation, and differentiation (Sharma, Srivastava, Grover, & Sharma, 2014). Alginates are biocompatible, degrade in vivo by enzymolysis, gel under mild conditions, have low immunogenicity and large diversity (L. Zhang, Morsi, Wang, Li, & Ramakrishna, 2013). However, they have low mechanical stiffness and uncontrolled degradation rates in vivo (Sharma et al., 2014). Alginate has been used in various biomedical applications, including systems for drug, antibody, and/or growth factor (GF) delivery (Li et al., 2006) as well as cell encapsulation and seeding (Gerecht-Nir, Cohen, Ziskind, & Itskovitz-Eldor, 2004).

Alginate scaffolds have been used to transplant subcultured human dental pulp cells (HDPCs) subcutaneously in the backs of nude mice. Six weeks after implantation, the subcutaneous formation of radio-opaque calcified bodies was observed in situ. Expression of type I, type III collagen, and dentin sialoprotein (DSP) in the mineralizing transplants was detected. Isolated odontoblast-like cells initiated dentin-like hard tissue formation. The study showed that HDPCs in alginate scaffolds actively differentiated into odontoblast-like cells and induced calcification that mimicked dentin (Fujiwara, Kumabe, & Iwai, 2006; Kumabe et al., 2006).

Chitosan: Chitosan is a linear polysaccharide composed of randomly distributed units of β -(1–4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is derived from chitin, which is found in the exoskeleton of marine crustaceans such as shrimps and crabs, as well as insects and the cell walls of fungi (Issa, Koping-Hoggard, & Artursson, 2005). Chitosan is soluble at pH lower than 5.5 and gels at pH 6 or by interacting with a variety of divalent and polyvalent anions (Suh & Matthew, 2000). Chitosan is known to be biocompatible, biodegradable, antimicrobial, and osteoinductive. It has the ability to bind to GFs, glycosaminoglycans, and DNA and can be easily processed into membranes, gels, nanofibres, beads, scaffolds, and sponges (Tanase et al., 2013). Chitosan can prevent the absorption of needed vitamins and minerals and could be dangerous to those who are allergic to shellfish. Drug delivery (Hejazi & Amiji, 2003), GF encapsulation (S. E. Kim et al., 2003), and gene delivery (Roy, Mao, Huang, & Leong, 1999) are examples of its uses in biomedical applications.

Reports on the use of chitosan as scaffold to support pulp cell growth and differentiation are controversial. On the one hand, N. R. Kim et al. investigated the growth and differentiation of HDPCs on a variety of natural scaffolds, including two types of collagen, gelatin, and chitosan. They concluded that chitosan did not properly support HDPCs growth compared with collagen and gelatin (N. R. Kim, Lee, Chung, & Yang, 2009). On the other hand, Feng et al. showed that a granular three-dimensional (3D) chitosan scaffolds obtained by freeze-drying, support neural differentiation of dental pulp stem cells (DPSCs) due to their good conductivity and suitability for nerve cell attachment, survival, and differentiation (Feng et al., 2014).

Cellulose: Cellulose and its derivatives are the most abundant naturally-occurring polysaccharide found in nature. It has glucose-based repeat units connected by β -1,4-glucosidic linkages. Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms (Esposito et al., 2005). Cellulose is biodegradable in nature by microbial or fungal enzymes that can break down the glucosidic linkages; however, biodegradation is limited or absent in animals and humans owing to the lack of those enzymes (Helenius et al., 2006). Cellulose has high water-holding capacity, high crystallinity, fine fibre network, ease of moldability, biocompatibility, and high tensile strength (Svensson et al., 2005). The main concern for using cellulose in biomedical applications revolves around its undesirable immunoresponse. Cellulose derivatives have been used in gel base, bioadhesives, controlling the release of drugs, tissue regeneration, and treatment of oedema (Svensson et al., 2005).

There have been no attempts to date to differentiate pulpal cells or generate pulp/dentin tissues using scaffolds made of cellulose solely; however, Chen et al. conducted a study using hybrid chitosan/carboxymethyl cellulose (CMC) scaffolds. The results of this study will be presented in the composite scaffolds section.

2.1.2 | Extracellular matrix—The ECM is a complex structural entity surrounding and supporting cells that are found within mammalian tissues. The ECM is composed of three major classes of biomolecules: (a) structural proteins (collagen and elastin); (b) specialized proteins (for example, fibrillin, fibronectin, and laminin); and (c) glycosaminoglycans (GAGs; for example, hyaluronic acid [HA];) (Kular, Basu, & Sharma, 2014). The ECM not only provides structural support to the tissue but also provides a dynamic environment that can be selectively degraded and remodelled via (bio)chemical signals to direct cellular responses. ECM-derived proteins and peptides are biological materials that can be easily manipulated for constructing various types of polymeric and composite scaffolds. Promoting the controlled release of the incorporated GFs into these scaffolds is one of their primary advantages; however, scaffolds made of ECM molecules are difficult to process and sterilize and have considerable batch variations (Galler et al., 2011).

Hyaluronic acid: Scaffolds made of HA, one of the glycosaminoglycans present in the ECM of connective tissues, have excellent potential for tissue regeneration applications. HA is an unbranched polysaccharide of repeating disaccharides consisting of D-glucuronic acid and N-acetyl-D-glucosamine (Ouasti et al., 2011). HA hydrogels have been widely investigated for applications in tissue regeneration, but their application in dental pulp regeneration is limited (Inuyama et al., 2010).

HA hydrogels are injectable, biocompatible, and have low immunogenic potential (Ouasti et al., 2011), but they have poor mechanical strength and rapid in vivo degradation rates (Jia et al., 2006). In any case, HA and its derivatives are known to have excellent potential for tissue engineering because they can be chemically and structurally modified for various applications.

Inuyama et al. (2010) studied the effects of sponges made of HA or collagen on odontoblasts from the KN-3 line in vitro, as well as on amputated dental pulps of rat molars in vivo. In

vitro, cells adhered to the stable structures of both HA and collagen sponges. In vivo, the dentin defect treated with HA showed a cell-rich reorganizing tissue compared with collagen (Inuyama et al., 2010).

Collagen: Collagen is the most abundant component in extracellular matrices. It has many applications in tissue engineering, drug delivery, and reconstructive surgery in orthopaedics and dentistry. Collagen sponges have low cytotoxicity, and they enhance osteoblast differentiation under suitable conditions (Chevallay, Abdul-Malak, & Herbage, 2000; Silver & Pins, 1992). Collagen type I is frequently extracted and used as scaffolds in tissue engineering. Allogeneic collagen, such as bovine collagen, has excellent biocompatibility and bioactivity (Prescott et al., 2008) and low immunogenicity in humans (Sumita et al., 2006), even though its biological origin could be a concern (DeLustro, Dasch, Keefe, & Ellingsworth, 1990). The intrinsic mechanical properties of collagen have been proved to be sufficient for dental pulp regeneration (Lee & Mooney, 2001), and they can be further enhanced by forming hybrid scaffolds in combination with calcium phosphate ceramics (Lluch, Fernandez, Ferrer, & Pradas, 2009).

Sumita et al. compared the performance of collagen and polyglycolic acid (PGA) 3D scaffolds for tooth-tissue engineering. Collagen sponge scaffolds enhanced tooth production compared with PGA fibre meshes in vivo (Sumita et al., 2006). Also, Prescott et al. investigated the role of stem cells, scaffold, and GFs in the organization and differentiation of DPSCs in a simulated furcal perforation site in a mouse model. Combining DPSCs, a collagen scaffold, and dentin matrix protein 1 (DMP1) showed an organized newly derived pulp tissue (Prescott et al., 2008).

Gelatin: Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals. Its chemical composition is closely similar to that of its parent collagen (Hoque, Nuge, & Yeow, 2015), and it is commonly used as a biological substrate for cell cultures and as a drug delivery vehicle (Hoque et al., 2015). Gelatin is biocompatible and bio-degradable, exhibits low antigenicity, does not produce harmful by-products upon enzymatic degradation, and available at low cost. With motifs' incorporation, such as RGD peptides, gelatin can modulate cell adhesion and provide multiple accessible functional groups for (bio) chemical modification (Hoque et al., 2015). The mechanical properties of gelatin gels are very sensitive to temperature variation and time (Sachlos & Czernuszka, 2003).

Ishimatsu et al. examined the effects of controlled release of different dosages of fibroblast GF-2 (FGF-2) from gelatin hydrogels on regeneration of the dentin-pulp complex. They concluded that the dosage of released FGF-2 had an influence on the structure of the calcified tissue regenerated in dentin defects as the use of the loaded gelatin hydrogels enabled the regeneration of calcified tissues that appeared to be osteodentin (Ishimatsu et al., 2009).

2.1.3 | Proteins and peptides—Proteins such as fibrin and silk, that is, other than the structural components of extracellular matrices, have potential applications in tissue engineering due to their compositional versatility, availability and excellent biocompatible

and biodegradable properties (Wanpeng Cao, 1996; Werkmeister & Ramshaw, 2012). However, their application for the regeneration of the dentin-pulp complex has been scarce.

Peptides are short amino acid sequences of proteins known to have specific biological activities. They can be synthetically manufactured by solid-peptide synthesis. This enables the easy customization of their biological, physical, and chemical properties as new modified peptides, inspired in natural sequences, can be designed and engineered (Collier & Segura, 2011; Williams, 2011). The potential application of peptide-modified molecules that self-assemble into fibrillary structures has been already explored for regenerating the dentin-pulp complex.

Fibrin: Fibrin is a natural biomaterial produced by polymerizing the plasma protein fibrinogen by the enzymatic activity of thrombin. It is advantageous compared with most of synthetic polymers and collagen gels when bio-compatibility, cost, cell adhesion, and immune response are concerns. Fibrin has no undesirable immunogenic reactions when obtained from autologous sources. Moreover, fibrin hydrogels are injectable, reproducible, and can be moulded to specific 3D shapes (Jockenhoevel et al., 2001). Mechanical properties of fibrin scaffolds have been notably improved when combined with polyurethane, polyethylene, hyaluronic acid, or calcium phosphate ceramics (Lee & Kurisawa, 2013). Physical characteristics, cell invasion, and survival characteristics of fibrin scaffolds can be adjusted by manipulating the fibrinogen/thrombin concentration. Increasing fibrinogen content increases stiffness but reduces porosity (Linnes, Ratner, & Giachelli, 2007).

Platelet-rich plasma (PRP) therapy is an emerging technology in sports medicine, and it has been used since the mid-1990s in dental and oral surgery. Recently, the patient-derived fibrin (PRF) scaffold (based on PRP technology) operates as an autologous and biodegradable delivery system. PRP/PRF used along with collagen membranes was extensively applied for regeneration of periodontal ligament and alveolar bone as a part of guided tissue regeneration procedures in periodontics and oral surgery. PRP therapy was shown to be a successful promoter in the healing process (Ficek, Kaminski, Wach, Cholewinski, & Cieszyk, 2011; Khodakaram-Tafti, Mehrabani, & Shaterzadeh-Yazdi, 2017). PRP and its associated formulations have yet to show promise in dental pulp regeneration, though. Fibrin has low mechanical stiffness and undergoes rapid shrinkage and degradation; nonetheless, shrinkage can be reduced by fixing agents, like poly-L-lysine and degradation rate can be controlled by fibril cross-linking or by use of enzyme inhibitors (Jockenhoevel et al., 2001). Yang et al. evaluated fibrin glue mixed with PRF as a scaffold seeded with dental bud cells for tooth regeneration. PRF is rich in cytokines and GFs, especially platelet-derived GF (PDGF) and tissue GF- β (TGF- β) that have relevant roles in angiogenesis and hard tissue regeneration, respectively. Dental bud cells cultured on the PRF/PRP scaffold regenerated a complete tooth with enamel, dentin, cementum, pulp, blood vessels, and periodontal ligament (Yang et al., 2012).

Silk protein: Silk is a natural protein that forms fibres, which can be woven into textiles. Silk from the silkworm has been used as biomedical suture material for centuries. Recently, its use in medicine has expanded for designing scaffolds to be used in hard tissue and ligament engineering (Vepari & Kaplan, 2007). The protein fibre of silk is composed of

fibroin and sericin and is produced by certain insect larvae to form cocoons (J. Zhang et al., 2012). Fibroin is the structural centre of silk, and sericin is the sticky material surrounding it. Silk proteins can be coupled via carbodiimide chemistry to peptides such as RGD (H. J. Kim et al., 2008). Silk proteins have unique mechanical properties as well as a diversity of side chain chemistries that enable conjugation of growth and adhesion factors (Altman et al., 2003). Silk has high strength, robust flexibility, mechanical toughness, and slow degradation rates (Wang et al., 2008). It is biodegradable, biocompatible, non-immunogenic, and supports cell attachment and proliferation (Sharma et al., 2014). Moreover, it can be prepared in a range of material configurations, including films, hydrogels, and microspheres (J. Zhang et al., 2012).

Xu et al. tested four silk protein scaffolds with or without RGD peptide having different pore diameters, ranging from 250 to 550 nm. These scaffolds were seeded with rat tooth bud cells and implanted into rat hosts. After 20 weeks, all harvested scaffolds showed regeneration of mineralized tissues. Thus, silk scaffolds facilitated the regeneration of mineralized osteodentin with specified sizes and shapes (Xu et al., 2008). W. Zhang et al. characterized 4-day post-natal rat tooth bud cells grown on hexafluoro-2-propanol (HFIP) silk scaffolds. These silk scaffolds not only supported osteodentin formation but also guided its size and shape and supported soft dental pulp formation (W. Zhang, Ahluwalia, Litterman, Kaplan, & Yelick, 2011).

Self-assembling peptides: Scaffolds made of peptide-based molecules specifically designed to self-assemble in nanofibres are novel synthetic matrices for tissue engineering. They are promising candidates because of their ease of synthesis, chemical diversity, and potential for tailoring a broad range of relevant properties for tissue engineering applications (Hartgerink, Beniash, & Stupp, 2002). Self-assembling peptides, as an example of smart customizable matrices, have been applied in biomedical nano-technology, cell culturing, and tissue engineering for both soft and mineralized dental tissues (Rubert Perez et al., 2015; Sargeant, Aparicio, Goldberger, Cui, & Stupp, 2012). They are formed by 15 to 25 amino acids, undergo self-assembly, and entrap water, forming nano-fibrous hydrogels (Palmer & Stupp, 2008). These hydrogel systems are biocompatible and trigger cellular responses. The peptides can be designed to guide cellular responses via interaction with attached cell adhesion motifs, incorporated enzyme-cleavable sites and/or suitable GFs. The design of the peptide sequence might allow control over material stiffness, mineral nucleation, antibacterial activity, and biodegradability (Rubert Perez et al., 2015). However, peptides, which are manufactured by solid-peptide synthesis, are expensive materials, and they have complex design parameters (Gelain, Bottai, Vescovi, & Zhang, 2006).

Galler et al. encapsulated two dental stem cell lines with peptide-amphiphile (PA) hydrogel scaffolds. Cell-matrix interactions were customized by incorporating RGD and an enzyme-cleavable site. Stem cells from human exfoliated deciduous teeth (SHED) and DPSC were cultured in PA hydrogels for 4 weeks. Although SHED showed high proliferation rates, collagen production and soft tissue formation, DPSC exhibited an osteoblast-like phenotype with expressed osteoblast marker genes and deposited minerals (Galler et al., 2008). Galler et al. also investigated the incorporation of a heparin binding motif in the self-assembled peptide nanofibre scaffold to mediate the activity of different GFs in encapsulated DPSC. In

a murine model, the subcutaneously transplanted hydrogel within dentin cylinders resulted in formation of a vascularized connective tissue similar to dental pulp (Galler, Hartgerink, Cavender, Schmalz, & D'Souza, 2012).

2.2 | Synthetically-engineered polymeric and ceramic scaffolds

2.2.1 | Synthetic polymeric scaffolds—Polylactic acid (PLA), polyglycolic acid (PGA), and polylactide-co-glycolide (PLGA) are synthetic polyester polymers. They have been used in various biomedical applications like engineering microvessels (Nör et al., 2001) fixation and repair processes for tendons and ligaments and biodegradable scaffolds for hard and soft tissue engineering (Bohl, Shon, Rutherford, & Mooney, 1998). They have many desirable properties such as biocompatibility, biodegradability, mild inflammatory response, cheap, and reproducible. Additionally, their mechanical properties, viscosity, porosity, degradation, and release rates of incorporated biomolecules can be tailored. Thereby, great design flexibility is offered by adjusting some parameters of the polymers like copolymer ratio, molecular weight, and crystallinity.

On degradation, both PLA and PGA produce lactic acid and glycolic acid, respectively, which are naturally removed through metabolic pathways and eventually excreted through urine (Athanasίου, Niederauer, & Agrawal, 1996; Taylor, Daniels, Andriano, & Heller, 1994). However, they are synthetic polymers and so they lack the biochemical information that is physiologically intrinsic to biopolymers that compose the ECM. Also, local accumulation of acidic degradation products in tissues is still a concern (Ulery, Nair, & Laurencin, 2011). PLGA is a copolymer with similar desirable physical and mechanical properties. The degradation rate of PLGA copolymers can be adjusted by tuning the proportions of lactic and glycolic acid monomers.

Scaffolds made of all these polymers have shown to be conducive for seeding of stem cells like SHED, DPSCs, and dental pulp fibroblasts. On these scaffolds, stem cells have shown differentiation into odontoblast-like cells and endothelial cells generating tissues similar to dental pulp and dentin. The configuration of the scaffold matrix pore size and distribution, powdered or fibre mesh, and so forth, also had a significant effect on the type of generated tissue (Tonomura et al., 2010). There are many studies that have been conducted using synthetic polyester polymers to engineer different dental tissues (Table 1). Mooney et al. have described a technique to engineer new pulp-like tissues utilizing cultured cells and synthetic extracellular matrices. Fibro-blasts were seeded onto synthetic matrices fabricated from PGA fibres, approximately 15 microns in diameter. The pulp-derived fibro-blasts adhered to the fibres, proliferated, and formed a new tissue over 60 days in culture with a cellularity similar to that of native pulp (Mooney, Powell, Piana, & Rutherford, 1996). Bohl et al. compared three different synthetic matrices, scaffolds fabricated from PGA fibres, a type I collagen hydrogel, and alginate to discern the most suitable matrix for dental pulp tissue engineering. Culturing cells on PGA resulted in a very high cell density with a significant collagen deposition. No cell proliferation was observed on alginate, and the growth of cells in collagen gels was moderate after 45 days (Bohl et al., 1998). Buurma et al. tried autologous tissue grafting for oral tissues restoration by attaching disaggregated autologous cells to natural or synthetic polymeric scaffolds. They reported that human dental

pulp and gingival fibroblasts (HPF and HGF) adhered to PGA scaffolds and survived when implanted subcutaneously in mice. The transplanted cells secreted type I collagen and cellular fibronectin (Buurma, Gu, & Rutherford, 1999). Cordeiro et al. investigated the formation of vascularized soft connective pulp-like tissue and new tubular dentin when SHED were seeded in PLA scaffolds, incorporated in tooth slices and then subcutaneously transplanted into nude mice. They observed that the resulting tissue showed architecture and cellularity closely resembling those of a physiologic dental pulp (Cordeiro et al., 2008). El-Backly et al. regenerated dentin/pulp-like tissue using a DPSC/PLGA scaffold. Cells were isolated from rabbit teeth and seeded onto scaffolds prepared from 50/50 PLGA polymers and transplanted subcutaneously in rabbits. After 12 days, DPSC showed high proliferative and clonogenic capacities. Formation of osteodentin-like structures as well as tubular bilayered structures of vertically aligned parallel tubules resembling tubular-like dentin were also observed (El-Backly, Massoud, El-Badry, Sherif, & Marei, 2008). Sakai et al. differentiated SHED cells into functional odontoblasts and endothelium. SHED cells were seeded in tooth slice/PLA scaffolds and implanted subcutaneously into mice. SHED differentiated into functional odontoblasts that generated tubular dentin (Sakai et al., 2010). Haung et al. tested the possibility of regenerating vascularized human dental pulp in emptied root canal spaces using a stem/progenitor cell-mediated approach with human root fragments. Stem/progenitor cells from apical papilla and human DPSC (hDPSC) were isolated, seeded onto PLGA scaffolds, inserted into the tooth fragments, and transplanted into mice. The results showed that the root canal space was filled entirely by a pulp-like tissue with well-established vascularity. In addition, a continuous layer of dentin-like tissue was deposited onto the root canal wall (G. T. Huang et al., 2010).

2.2.2 | Bioactive ceramic scaffolds—Calcium phosphate ceramics, bioactive glasses, and glass ceramics constitute a group of materials that have long been used in different compositions, structures, and configurations to heal and regenerate bone defects in orthopaedics, maxillofacial, and craniofacial applications. These ceramics are bioactive because, once implanted, they mediate on their surface the formation of a layer of biologically equivalent hydroxyapatite (HAP), that is, the calcium phosphate mineral that constitutes human hard tissues (Wanpeng Cao, 1996). Thus, cells that interact with the HAP-coated ceramic in vivo will differentiate and produce hard tissues (Neo et al., 1992). The mechanism by which the HAP layer is formed on the surface of bioactive materials was first described by Larry L. Hench for bioactive glasses (Hench, 1998). These glasses are based on silicon oxide and incorporate different ions as network modifiers (see section for more details). A rapid exchange of sodium and calcium ions from the glass surface with protons from the biological fluids starts the process and, as a result of it, the pH of the fluid increases. The more alkaline environment induces breaking of the Si–O–Si bonds in the glass and silanol groups form at the glass-fluid interface. Then, abundant silanol groups condense and polymerized in a silica gel layer. Calcium and phosphate groups penetrate this silica-rich layer forming a calcium and phosphate oxide-rich film on top of the silica-rich layer. The calcium phosphate film grows by incorporating more calcium and phosphate ions from the surrounding fluids initially forming an amorphous calcium phosphate layer on top of the bioactive glass. Finally, this layer crystallizes and forms a carbonated HAP layer (Hench, 1998). All bioceramics, such as HAP, other calcium phosphates, and calcium

silicates, are also bioactive following similar, although not identical, ionic interchanges and mechanism of apatite formation than the one originally described for bioactive glasses (Mas-Moruno et al., 2013). In any case, the release of dissolution products, such as calcium phosphate and silicate ions from the glass ceramic, is playing an important role in forming the surface apatite layer and enhancing the osteoblastic activity of the material (Jones, 2013). Scaffolds made of bioactive ceramics still require specific modifications to obtain structures with the desired permeability, controlled dissolution rate, and specific surface characteristics to enhance cellular activity and eventually, regeneration of human hard tissues (Yuan et al., 2011).

Calcium phosphates (CaP): CaP materials, that is, HAP, tricalcium phosphate (TCP), and biphasic calcium phosphate (HAP/TCP), have been widely tested for bone regeneration. 3D CaP porous granules have been successfully used in dental tissue engineering as they provide favourable 3D substrate conditions for hDPSC growth and odontogenic differentiation (Nam, Won, Kim, & Kim, 2011). Because of the biochemical similarity to mineralized tissues, CaP have good properties of resorption, biocompatibility, low immunogenicity, osteoconductivity, and bone bonding to surrounding tissues (L. Zhang et al., 2013). They are brittle, which constitutes their main drawback that limits their application in high load-bearing areas (Yuan et al., 2011). Addition of SiO₂ and ZnO dopants to pureTCP scaffolds increased the mechanical strength of the bioceramic as well as the cellular proliferation on it (Fielding, Bandyopadhyay, & Bose, 2012).

Tomomura et al. used porous HAP/beta-TCP (b-TCP), powdered HAP/b-TCP, and PGA fibre mesh as scaffolds to transplant cultured porcine dental pulp-derived cells into the backs of nude mice. After 6 weeks, newly-formed hard tissue was observed in all transplants. When porous HAP/b-TCP was used, dentin-like hard tissue was observed on the inner wall with minimum cell inclusions and odontoblast-like cells were aligned adjacent to the hard tissue. When HAP/b-TCP powders or PGA were used, bone-like hard tissues showed cell inclusions and cell alignment was not observed. Hard tissue from the HAP/b-TCP block group was positive for dentin markers, type I collagen, osteonectin, bone sialoprotein, and dentin sialoprotein (DSP; Tomomura et al., 2010). Nam et al. studied the effects of 3D CaP porous granules on the growth and odontogenic differentiation of hDPSCs. Differentiation behaviour on the 3D granules was confirmed by the increased alkaline phosphatase activity, up-regulation of dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1; Nam et al., 2011).

Bioactive glasses and glass ceramics: Bioactive glasses and glass ceramics are based on combinations of SiO₂, Na₂O, CaO, P₂O₅, MgO, Fe₂O₃, and other oxides. They are bioactive as described above and can offer good crystallization conditions (Kokubo, 1991; Sepulveda, 2002). Glass ceramics usually have between 30% and 90% crystallinity, which can result in zero porosity, high strength, high toughness, translucency or opacity, resorbability, and biocompatibility. They have been commonly used in dentistry as veneers, crowns, and bridges as well as other biomedical applications, including hard tissue implants and bone tissue engineering scaffolds (Misra et al., 2010). The use of bioactive glasses and glass

ceramics as scaffolds in tissue regeneration is limited because of difficulty of shaping, poor mechanical strength, brittleness, slow degradation rate, and high density.

El-Gendy et al. studied human dental pulp stromal cells (HDPStCs) cultured under basal or osteogenic conditions either in monolayers or on 3D bioactive glass scaffolds in vitro. Cell-scaffold constructs were also implanted intraperitoneally in nude mice. Osteoinductive conditions enhanced HDPStCs expression of osteogenic gene markers compared with basal conditions. Cultured HDPStCs on 3D scaffolds promoted osteogenic gene expression compared with monolayer culture under both basal and osteogenic conditions. In vivo, implantation of the HDPStCs 3D bioactive glass constructs showed evidence of sporadic woven bone-like spicules and calcified tissue. (El-Gendy, Yang, Newby, Boccaccini, & Kirkham, 2013).

2.3 | Composite scaffolds

The development of advanced biomaterials finds more opportunities by combining biomaterials in the form of biopolymers and bioceramics, either synthetic or natural (Pérez, Won, Knowles, & Kim, 2013). Each individual material has its own characteristic advantages and disadvantages, but composite materials, or hybrids, often show an excellent balance between the strengths and weaknesses of their individual components providing overall improved properties (Liu & Ma, 2004). For example, polymers that lack adequate stiffness can be combined with stiffer materials like ceramics to overcome their inherent weak point and make them suitable for dental tissue regeneration. At the same time, polymers fabrication is much easier compared with ceramic scaffolds, which are brittle with less versatile porous structures. Also, resorption products of CaP minerals are suitable neutralizers for acidic degradation products of polymers, thereby reducing the associated inflammation. Likewise, polymers that lack bioactive properties to induce proliferation and differentiation of stem cells into bone or dentin osteogenic cells can be modified with bioactive ceramics and glasses (Ko et al., 2010). Although there are many successful composite/hybrid materials applied in the biomedical field, those that have been explored to regenerate dental tissues are still limited.

Hybrids and composites made of cellulose–alginate, cellulose–agarose, cellulose–chitosan, chitosan–alginate, and chitosan–agarose have been tested as scaffolds for regeneration of different tissues. H. Chen et al. tested scaffolds made of a polyelectrolyte complex (PEC) of chitosan and CMC. The addition of CMC to chitosan decreases the pore diameter and increases internal porosity of the scaffold, which reinforces it and facilitates cell nutrition. Pulp cells cultured in these PEC scaffolds showed improved adhesion, spreading, cell capacity, and 3D configurations compared with pure chitosan scaffolds. Also, the expression of osteonectin and DSPP were increased. Thus, the scaffold made of the chitosan/CMC complex was a promising substrate for pulp tissue engineering (H. Chen & Fan, 2013).

Ravindran et al. have developed a 3D multilayered co-culture system. They used type I collagen and chitosan blends that were seeded with DPSC and HAT-7 dental epithelial cells to assess epithelial–mesenchymal interactions. This layered macroscale scaffold had a biomimetic structure with tuneable mechanical characteristics that supported the motility of the two cell types in all directions (Ravindran, Song, & George, 2010).

Highly porous polymer/ceramic composite scaffolds appear to be a promising substrate for tissue engineering due to its excellent mechanical properties and bioactivity. Zheng et al. tested four different types of 3D scaffolds for tooth-tissue regeneration, including PLGA and three other composites scaffolds that contained 50 wt% of PLGA combined with three different CAPs: HAP, TCP, and calcium carbonate hydroxyapatite (CDHA), respectively. Among all tested groups, PLGA/TCP maximized the differentiation of hDPSCs and dentin formation (Zheng et al., 2011).

Danilovic et al. evaluated the effects of PRP in combination with HAP as a pulp capping material. The histological analysis revealed that the healing process was characterized by dentin bridge formation, with maintaining morphology and functional integrity of dental pulp. The inflammatory reaction was scored as mild to moderate suggesting the good biocompatibility of these combined materials (Danilovic, Petrović, Marković, & Aleksic, 2008). An analogous approach consisting of composites scaffolds made of calcium silicate ceramics in combination with biodegradable polymers has been recently explored. Chiu et al. investigated the suitability of mineral trioxide aggregate/PCL (MTA/PCL) hybrid 3D scaffolds as a micro-environment for the osteogenic differentiation of HDPCs. They showed that the MTA/PCL scaffold had high porosity (70%) and uniform macropores with compressive strength of 4.5 MPa. This scaffold promoted adhesion, proliferation, and differentiation of HDPCs. Thus, this 3D-printed MTA/PCL porous composite exhibited excellent physical and chemical properties for enhanced osteogenesis differentiation (Chiu, Fang, Hsu, Lin, & Shie, 2017). Similarly, Ho et al., studied the physicochemical properties and in vitro odontogenic potential of 3D printed Biodentine/PCL composite scaffolds. Their results showed that not only did the composite scaffolds support the proliferation and differentiation of HDPCs but also they exhibited a good apatite-forming ability (Ho, Fang, Wang, Huang, & Shie, 2018). Gandolfi et al. extended this approach by introducing an extra CaP phase in the composite scaffolds and produced highly porous PLA-based scaffolds doped with calcium silicate and dicalcium phosphate dihydrate using thermally induced phase separation. These scaffolds showed biocompatibility, biointeractivity, balanced degradation by deposition/nucleation of apatite, and suitable porosity for proper cell colonization (Gandolfi et al., 2018).

3 | NEW SCAFFOLDS TO ADDRESS EXISTING CHALLENGES

Recently, Galler et al. reported for the first time a comparison of natural versus synthetic hydrogel scaffolds, with or without functionalized modifications, for in vitro and in vivo dental pulp tissue engineering (Galler et al., 2017). The natural scaffolds were made of collagen or fibrin and compared with two groups of synthetic scaffolds, that is, PEG-based scaffolds with and without cell adhesion motif and enzyme-cleavable site and two different self-assembling peptides. The use of natural materials, particularly fibrin, led to higher viability of dental pulp stem cells and pulp-like tissue formation compared with all synthetic materials. Thus, fibrin appeared to be the most promising scaffold material for dental pulp tissue engineering among the tested hydrogel scaffolds in spite of that fibrin scaffolds did not have the highest control over the material properties and were not functionalized with specific bioactive motifs. However, fibrin possesses an endogenous capability of growth factor binding and slow release (Spicer & Mikos, 2010). This effect might have contributed

to the favourable results for pulp-like tissue formation for this material in combination with dentin-derived proteins. These results question the appropriateness of past strategies for developing scaffolds made of synthetic molecules for dentin-pulp regeneration. At the same time, they provide a guide for further designs of scaffolds with increasing biomimetic features and diverse stimuli that both ECM and signalling proteins provide in vivo. Thus, smarter scaffolds designed for simulating the required biofunctional properties with spatial and temporal control are needed.

The current focus is on designing biomaterials that replicate the remodelling dynamics of the extra cellular environment via enabling specific cell–cell and cell-scaffold interactions. The three strategies we highlight here to overcome the current limitations in scaffolds' designs for enhancing dentin-pulp complex regeneration are (a) incorporating new internal modulation factors, such as customization of scaffolds' physical properties; (b) incorporation of external modulation factors, such as controlled delivery of multiple bioactive agents; and (c) conjugating stimuli responsive components (Figure 1). Following, we are presenting a series of innovative biomaterial-based approaches that have been already explored for regenerating different tissues, and we highlight them as viable alternatives for dentin-pulp complex regeneration.

3.1 | Polymeric microspheres

Polymeric microspheres have been developed as internal modulators for supporting cell growth and/or delivering drugs and GFs in tissue engineering applications. W. Huang et al. developed a scaffold by embedding poly (beta-hydroxybutyrate-co-beta-hydroxyvalerate) (PHBV) microspheres into a PLGA matrix to repair bone defects (W. Huang, Shi, Ren, Du, & Wang, 2010). PHBV is a polyhydroxyalkanoate-based polymer which is biodegradable, nontoxic, and produced naturally by bacteria. PLGA/30%PHBV scaffolds had 80% porosity, and their mechanical properties can be modulated as the higher the amount of PHBV microspheres, the higher the compressive strength of the PLGA/PHBV scaffold. Song et al. fabricated and evaluated scaffolds for a sustained-release of proteins. The scaffold was chitosan-based embedded with PLGA microspheres to maintain the long-term stability of nutrients' concentration within the scaffolds. In this design, the polymer microspheres were loaded with bovine serum albumin (BSA) to study the protein release pattern from the PLGA-shelled structure. Then, the BSA-loaded PGA microspheres were mixed with a chitosan solution to create the scaffolds (Song et al., 2013). The BSA release was sustained and cumulative with little initial burst. Liu et al. reported nano-fibrous hollow microspheres (NF-HMS) self-assembled from star-shaped poly (l-lactic acid) (SS-PLLA) and linear (PLLA) as an injectable cell carrier for cartilage regeneration. NF-HMS nanofibres have an average diameter of 160 nm, which is in the same scale of collagen fibres. Because the high porosity (often >90%) is desired for scaffolds to provide sufficient space for cell growth and ECM deposition, the open and hollow structure of these scaffolds contributed to achieve a porosity as high as 96.7%. This facilitated cell seeding, proliferation, and tissue regeneration. NF-HMS efficiently accommodated cells and promoted cartilage regeneration by increasing both quantity and quality of regenerated cartilage, compared with control microspheres (Liu, Jin, & Ma, 2011).

Potential application in regenerative dentistry—Controlled release of GFs from dentin appears to be an important mechanism of defence against injuries. Factors such as TGF- β , bone morphogenetic proteins (BMPs), PDGF, FGF, and vascular endothelial GF (VEGF) are incorporated into the dentin matrix during dentinogenesis and are retained there as “quarantined” molecules. When these molecules are re-released, they are bioactive and inductive of DPSCs recruitment, their differentiation and the secretion of mineralizable matrices (Casagrande, Cordeiro, Nor, & Nor, 2011). The concept of incorporating different forms of microspheres either to simulate the nano-fibrous architecture of ECM or to enable a higher load of GFs and slow down their release, which abrogates the initial burst phase, is a promising tool to be applied for dental tissue regeneration. This concept could be a clinically optimal solution to repair tissue defects with complex shapes if the cell carrier is injectable, as it is the case of NF-HMS.

3.2 | Interpenetrating polymer networks (IPNs)

Although protein-based gels, such as those made of fibrin, have been investigated as scaffolds for tissue engineering, their biomedical application has been limited because of their poor mechanical properties, particularly for conditions that require shape stability or bear significant load. To overcome this issue, IPN hydrogels that are made of mixtures of proteins with other polymers have been developed. The IPN hydrogels are composed of two polymers with different storage moduli, which allows for distinctive mechanical response to different mechanical stimuli. Lee and Kurisawa developed a novel IPN hydrogel composed of fibrin and hyaluronic acid-tyramine (HA-Tyr). Cell proliferation and capillary formation occurred in IPN hydrogels were higher than on control fibrin scaffolds. In these IPN scaffolds, when the fibrin network in the gels was completely degraded and contracted upon interaction with cells, the shape of the IPN hydrogels was preserved due to the structural support provided by the non-degraded HA-Tyr network (Lee & Kurisawa, 2013).

Potential application in regenerative dentistry—Restoring the dentino-pulpal defect is a longstanding problem in restorative dentistry. There are many limitations of dental biomaterials used to induce reactionary dentin to seal the exposed pulp chamber or those used as scaffolds for dentin-pulp complex regeneration. Eventually, the reparative or the regenerative process might not be completed successfully. Based on the internal modulation concept, it could be a promising solution to introduce a bifunctional scaffold to the dental field; a stable defect filler coronally and a dentinogenesis promoter pulpally.

3.3 | Polyelectrolyte coated polymeric scaffolds

Shah et al. explored a bone regeneration strategy using a polyelectrolyte multilayer coating on PLGA using the layer-by-layer technique. The coating was made of alternating layers of BMP-2 and PDGF-BB. This promoted elution of GFs over adjusted time scales to regenerate bone tissues by endogenous cells. Also, this approach offered simultaneous delivering of multiple GFs to the site of the wound based on electrostatic interactions between the polymer multilayers and GF. The number of layers and type of molecules that composed the coating can be easily customized and enables a cell-free regenerative therapy. Mature and mechanically-sound local bone bridged the defect as early as 2 weeks after implantation. (Shah et al., 2014).

Potential application in regenerative dentistry—The hybrid dental tissues of endodontium are complex and engineering these tissues presents many unique design challenges. In addition, multiple GFs are involved to trigger the regenerative cascade including progenitor cells recruiting factors, odontoblast and angiogenesis differentiating factors, and ultimately mineral nucleation factors. Such an approach could be of prime value to deliver a diverse pull of GFs in a sequentially-controlled process and over physiologically meaningful periods providing a new design for external modulation of tissue regeneration. Besides, a synthetic and cell-free approach for precise dental tissue repair has higher potential to be more rapidly translated to the clinical arena than cell-based tissue engineering approaches.

3.4 | Cell adhesive-nonadhesive scaffolds

Hydrogel systems can direct cell performance by providing cell-adhesive or nonadhesive micro-environments. Hydrogels made of ECM molecules, such as collagen, have fibrous microstructures and are suitable for cell adhesion, growth, and migration. This characteristic is unlike alginate hydrogels, which are nonadhesive and prevent migration. Dixon et al. developed an alginate/collagen hydrogel customized to promote stem cell self-renewal with a micro-environmental switch to direct differentiation. These hydrogels could be “switched”; that is, the alginate component can be ionically de-cross-linked and efficiently removed. The switching of the hydrogel-based material enabled the removal of the cell self-renewal component (alginate) and switching to a micro-environment that promoted differentiation (collagen; Figure 2). Adjusting the timing of this switch can preferentially direct the stem cells differentiation. For instance, ectoderm requires early differentiation (early switch), whereas mesoderm/endoderm requires late differentiation (late switch; Dixon et al., 2014).

Potential application in regenerative dentistry—This strategy addresses the role of the micro-environment, provided by the scaffold, on fate choices of stem cells. It demonstrates that the micro-environment can be controlled over time and thus tailored to control different cell responses representing a model of stimuli responsive and internally modulated scaffolds. As dentin and dental pulp are formed from ectomesenchyme, which is derived from ectoderm, “early switch” of this hybrid scaffold could promote the differentiation of cells with odontoblastic lineage that enhances the regeneration of affected tissues.

3.5 | Spatially-controlled stimuli-responsive scaffolds

Xu et al. (2015) have developed a novel ECM-mimicking hydrogel scaffold for human induced pluripotent stem cells derived mesenchymal stem cells (hiPS-MSCs) differentiation into fibroblasts. The hydrogel had a fibrinogen backbone with polyethylene glycol (PEG) diacrylate (DA) UV-crosslinkable groups. These two components were infiltrated into electrospun PCL fibres together with connectiveTGF (CTGF). Upon exposure to UV light, cross-linking between PEG and the fibrogenic component is initiated (Figure 3). This novel scaffold with biomimetic nanostructures was thus capable to supply spatial allocation of stem cells as well as anchorage and storage of GFs to direct stem cells differentiation. Thus, a combination of the three main strategies presented in (Figure 1) were included in this work (Xu et al., 2015).

Potential application in regenerative dentistry—The combination of PCL as a nanostructured biocompatible polymer, fibrinogen which can biochemically interact with damaged tissues, in addition to the appropriate GF for reparative dentinogenesis could represent a promising injectable and spatially controlled scaffold for progenitor dental pulp stem cells commitment and differentiation into dentino-pulpal tissues.

3.6 | Multifunctional recombinant polymer scaffolds

We have recently presented a new biomimetic scaffold made of elastin-like recombinant polymeric electrospun nanofibres that incorporate a statherin-derived peptide (St-ELR) with ability to induce intrafibrillar biomineralization (Lan, Li, Heo, Aksan, & Aparicio, 2015; Figure 4). In doing so, we modulated the rigidity of the scaffold as well as introduced biochemical signalling with the incorporation of HAP after biomineralization. The versatility in the sequence design and post-modification of these recombinant polymers allowed us to conjugate a short antimicrobial peptide, GL13K, to provide anti-infection properties to the scaffold (X. Chen, Hirt, Li, Gorr, & Aparicio, 2014). The study indicated that biomimetic mineralized ELR scaffolds supported the proliferation and odontogenic differentiation of hDPSCs (Lan, Li, Rodriguez-Cabello, & Aparicio, 2017). The peptide functionalized st-ELR membrane exerted antimicrobial ability against *Streptococcus mutans* and *Streptococcus gordonii* bacteria.

Potential application in regenerative dentistry—Our research has initially shed light on the tailored design of recombinant multifunctional polymers for regenerating dentin tissue. This recapitulates structure, physical, and chemical properties of ECMs in mineralized tissues. In addition, this research provides a new approach to modify the scaffold with diverse bioactive molecules to obtain multiple functions, while maintaining good interaction with native tissues.

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FIGURE 1.

Strategies for developing new smart tissue engineering scaffolds for dentine-pulp complex regeneration (The authors would like to thank the “Pérez et al., 2013” for permission to reuse this figure). See text for details regarding each of the three main strategies. ECM: extracellular matrix; GFs: growth factors; SAP: self-assembled peptide [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2.

Alginate/collagen hydrogel customized to promote stem cell self-renewal with a micro-environmental switch to direct differentiation. (a) Alginate serves as structural modulator to prevent the adhesive and fibrous network created by collagen. Upon switching (chelation of Ca²⁺ ions), alginate is removed and collagen fibres are generated forming an adhesive micro-environment. (c) Quantitation of alginate and collagen before and after switching. (d) Spectroscopy measuring collagen fibre character during cross-linking and switching of hydrogels. Alginate prevents complete collagen network formation until chelation and washout (The authors would like to thank the “Dixon et al., 2014” for permission to reuse this figure) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3.

Schematic diagram of preparation of PFP-C nanocomposites and their fibrogenic capacity on hiPS-MSCs. (1) A PF solution with CTGF was added on the top of a PCL mesh. (2) UV light exposure to crosslink PF. (3) A free-standing PFP-C composite was formed. (4) hiPS-MSCs were seeded on the composite. (5) Fibrogenesis process synergetically promoted by the adhesive motif on PFP and the signalling induction of CTGF. PF: Poly(ethylene glycol)-Fibrinogen; PCL: Poly(ϵ -caprolactone); PFP: integrated PCL fibers and PF hydrogel into one 3D scaffold; CTGF: connective tissue growth factor; PFP-C: the CTGF loaded fiber/hydrogel composite; (hiPS-MSCs): human induced pluripotent stem cells derived mesenchymal stem cells. (The authors would like to thank the “Xu et al., 2015” for permission to reuse this figure) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 4. Electrospun fibrillar scaffolds made of elastin-like recombinant polymers. Scaffolds with a statherin-derived peptide sequence and conjugated antimicrobial GL13K peptides before (a) and after (b) intrafibrillar mineralization

TABLE 1

Biomaterials-based scaffolds for regeneration of the dentin-pulp complex

Biomaterials	Advantages	Limitations	References
Natural and naturally-derived polymeric scaffolds			
Polysaccharides	Biocompatibility low cost Ease of processing	Batch-to-batch variations Poor mechanical properties	
Alginate	Degrades in vivo by enzymolysis Mildness of gelation conditions Low immunogenicity Promotes healing Large diversity	Low mechanical stiffness Uncontrolled in vivo degradation rate	Fujiwara et al., 2006; Kumabe et al., 2006
Chitosan	Biodegradable Antimicrobial Possesses tissue healing effects Osteoinductive Has the ability to bind to growth factors, glycosaminoglycans and DNA Easily processed into membranes, gels, nanofibres, beads, scaffolds and sponges	Can prevent the absorption of needed vitamins and minerals Could be dangerous to those who are allergic to shellfish	N. R. Kim et al., 2009; Ravindran et al., 2010, composite (chitosan/collagen I)
Cellulose	High water-holding capacity High crystallinity Fibrillar network Ease of mouldability High tensile strength	Potential immunoresponse Biodegradation, in human, is limited or absent owing to the lack of specific microbial or fungal enzymes	H. Chen & Fan, 2013, composite (carboxymethyl cellulose/chitosan)
Extracellular matrix components	Dynamic environment Provides the chemical signals to guide the cellular behavior Amenable to be selectively degraded and remodelled. Nano-scale biological materials Controlled growth factors release	Difficult to process and sterilize Batch-to-batch variations	
Hyaluronic acid	Injectable Low immunogenic potential	Poor mechanical strength Rapid in vivo degradation rate	Inuyama et al., 2010
Collagen	Osteoblastic differentiation stimulant Allogeneic collagen, e.g. bovine collagen, has excellent biocompatibility and low immunogenicity in humans Allows for easy placement of cells and growth factors Allows for replacement with natural tissues after undergoing degradation	Difficult to customize for specific applications. Immune reaction could be a concern Modest physical strength	Sumita et al., 2006; Prescott et al., 2008
Gelatin	Biodegradable Low antigenicity Low cost and it contains motifs that modulate cell adhesion High number of accessible functional groups for modification	Mechanical properties of gelatin gels are very sensitive to temperature variation and time Difficult to purify and sterilize	Ishimatsu et al., 2009
Proteins/peptides	Dynamic environment Provides the chemical signals to guide the cellular behavior Amenable to be selectively degraded and remodelled. Potential for multifunctionality		
Fibrin	Fibrin hydrogels are injectable Reproducible and can be moulded into 3D shapes Low Cost Autologous sources-no undesirable immunogenic reactions	Undergoes rapid shrinkage and degradation. Has low mechanical stiffness.	Yang et al., 2012; Danilovic et al., 2008, composite (platelet rich plasma/hydroxyapatite), Galler et al., 2017

Biomaterials	Advantages	Limitations	References
Silk protein	Good cell adhesion properties Adjustable Physical characteristics by the concentration of fibrinogen/thrombin		
	Biodegradable Non-immunogenic Support cell attachment and proliferation Robust mechanical strength Slow controlled degradation Can be prepared in a range of material format (films, hydrogels and microspheres)	A special process is required to remove the outer irritant sericin coating of the silk	Xu et al., 2008; W. Zhang et al., 2011
	Hydrogel systems Biodegradable Cellular interaction stimulant Tailored toward specific applications	Hard to precisely control peptide assemblies Expensive materials Complex design parameters	Galler et al., 2008; Galler et al., 2012
Synthetically-engineered polymeric and ceramic scaffolds			
	Biocompatible Mild inflammatory Cheap and reproducible They possess tailorable mechanical properties and degradation rate	They lack the physiological chemical information that is physiologically found in the ECM Accumulation of acidic degradation products is a concern	Mooney et al., 1996, (PGA) Bohl et al., 1998, (PGA) Buurma et al., 1999, (PGA) Cordeiro et al., 2008, (PLA) El-Backly et al., 2008, (PLGA) Sakai et al., 2010, (PLA) G. T. Huang, Yamaza, et al., 2010, (PLGA)
	Biodegradable, degradation products present in the metabolic pathways Conductive for seeding of stem cells		
Bio-ceramic scaffolds			
	Calcium phosphates Hydroxyapatite (HAP) Tricalcium phosphate (TCP) Biphasic calcium phosphate (HA/TCP)	Brittleness, limited applications as a replacement of highly load-bearing area	Tomomura et al., 2010; Nam et al., 2011; Zheng et al., 2011, composite (TCP/PLGA)
	Bioactive glasses and glass ceramics	Difficulty of shaping-high density Poor mechanical strength Brittleness Slow degradation rate	El-Gendy et al., 2013