Scaffolds for Dentin–Pulp Complex Regeneration

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Regenerative dentistry aims to regenerate the pulp–dentin complex and restore those of its functions that have become compromised by pulp injury and/or inflammation. Scaffold-based techniques are a regeneration strategy that replicate a biological environment by utilizing a suitable scaffold, which is considered crucial for the successful regeneration of dental pulp.

Keywords: dental pulp ; guided tissue regeneration ; tissue scaffolds ; biocompatible materials

1. Natural and Natural-Derived Polymeric Scaffolds

Natural and natural-derived polymeric scaffolds, namely peptides and polysaccharides, are derived from natural sources and can form hydrogels that possess high water absorbing capacity [1]. They are similar to the native cellular milieu, are highly biocompatible, naturally available and inexpensive. However, due to their biologic nature, these scaffolds present a number of significant limitations, such as batch-to-batch variation and poor mechanical performance [2]. Nonetheless, in many cases, the benefits of using these scaffolds outweigh the drawbacks, rendering them appropriate for regenerating the dentin–pulp complex.

1.1. Polysaccharides

Polysaccharides or polycarbohydrates are long chain polymeric carbohydrates composed of monosaccharide units, bounded by glycosidic linkages, which can be hydrolyzed in monosaccharide or oligosaccharides. The most commonly used polysaccharides for dental-pulp regeneration are alginate, cellulose and chitosan.

Alginate

Alginate is naturally derived from the cell walls of brown algae (Phaephyceae) or certain bacteria, such as Pseudomonas and Azotobacter. It consists of a linear copolymer of homopolymeric blocks of (1→4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues, that can be arranged in consecutive single monomers or alternating M- and G-residues, known to form hydrogels [3][4][5]. The gelling properties of this biopolymer are related to interactions with ions such as calcium (via ionic cross-linking), or to low environmental pH value [6]. Physical and chemical properties of alginate are influenced by the M/G ratio as well as by its structural organization [7]. Thus, by increasing calcium levels, the cross-linking density is higher and the alginate gains mechanical strength [8]. Alginates are biocompatible, have low immunogenicity, and exhibit large diversity [9]. However, they also have low mechanical strength and uncontrolled rates of biodegradation [10]. Nonetheless, due to their almost temperature-independent gel state in the presence of multivalent cations, they are suitable for cell immobilization and can be used as hydrogels for various biomedical applications. Alginate hydrogels can be used in dentin–pulp complex regeneration. By adding growth factors such as transforming growth factor beta (TGFβ) or by acid-treating these hydrogels, it is possible to observe differentiation of odontoblast-like cells and regular tubular dentin secretion when applied to cultured human tooth slices [11]. Fujiwara et al. studied the use of alginate as a scaffold in a subcultured rat dental-pulp-derived cells transplantation into nude mice. They found that if beta-glycerophosphate is present, the mRNA of the dentin sialophosphoprotein gene was expressed, as well as alkaline phosphatase, an early marker of odontoblast differentiation. This work showed that subcultured rat dental-pulp-derived cells seeded in an alginate scaffold can differentiate into odontoblast-like cells and can induce calcification [12].

Chitosan

Chitosan is a natural linear polysaccharide, composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine [13]. Derived from chitin present in fungi, or in the exoskeleton of marine crustaceans and insects [14][15], chitosan is biocompatible, biodegradable and possesses antimicrobial and regenerative properties [16]. An advantage of this scaffold is the possibility for it to bind to growth factors, and also to DNA and glycosaminoglycans [15]. However, controversial results have been re-ported on the use of chitosan as a scaffold for dental pulp stem cells (DPSCs) growth

and differentiation. For example, Kim et al. compared the growth and differentiation properties of these cells in three different natural scaffolds: collagen, gelatine and chitosan. Proliferation and differentiation of DPSCs was not appropriately supported in chitosan when compared with gelatine and collagen [17]. However, Feng et al. have reported the successful use of a 3D porous chitosan scaffold on the support, growth and differentiation of DPSCs to nerve cells [18].

Cellulose

Cellulose is the most abundant organic linear polysaccharide. Composed of several hundred to many thousands of glucose units connected by β-1,4-glucosidic linkage [19], it is present on cell walls of green plants, in algae and oomycetes. Cellulose presents excellent biocompatibility, is non-toxic and low cost [20]. Based on these characteristics, the use of cellulose-based hydrogels for biomedical applications has gained attention. However, its poor mechanical properties have limited its application on hard tissue regeneration [21].

1.2. Extracellular Matrix Derived

The extracellular matrix (ECM) is a complex network that provides structural and biochemical (i.e., signaling response) support to the surrounding cells [22]. The ECM is mainly composed of extracellular macromolecules that include structural proteins (e.g., collagen and elastin), specialized proteins (e.g., fibrillin, fibronectin and laminin) and glycosaminoglycans (GAGs), hyaluronic acid (HA) and minerals [23]. ECM-based components are produced by resident cells and secreted to surrounding medium via exocytosis [24]. ECM composition can be manipulated for the construction of different types of polymeric and composite scaffolds [16]. ECM scaffolds have gained attention in tissue engineering and regenerative medicine due to their capacity to incorporate and release growth factors. However, batch-to-batch variation and difficulties in processing and sterilizing these compounds present some disadvantages [25].

Hyaluronic Acid (HA)

HA is an anionic, non-sulphated GAG, and is present on the extracellular matrix of connective, epithelial and neural tissue. HA is a polymer of disaccharides, composed of D-glucuronic acid and N-acetyl-D-glucosamine [26]. When applied to exposed pulp, HA has been shown to stimulate the production of reparative dentin, aiding in the repair of damaged teeth. HA can be applied in the form of a 3D sponge to create an optimal environment for blood vessel proliferation and stem cell differentiation. This enables the growth of new tissue and the regeneration of damaged tissue, promoting dental pulp revitalization [27]. Scaffolds of HA have important roles for tissue regeneration (cell proliferation and migration), inflammatory response and its degradation products include pro-angiogenic factors. HA hydrogels are biocompatible and display low immunogenic potential [26], but present poor mechanical properties and in vivo degradation kinetics need further improvements to allow complete repopulation of the root canal space by vital tissue [27][28]. In 2010, Inuyama et al. analyzed the behavior of HA sponges seeded with a dental pulp cell line as a scaffold for dental pulp regeneration. In this study, the authors reported a cell-rich reorganizing tissue in the amputated dental pulp region, suggesting that HA sponges are indeed a suitable scaffold for pulp regeneration [29]. Moreover, Silva et al. have investigated HA hydrogels incorporated with cellulose nanocrystals (CNCs) and reinforced with platelet lysate. The incorporation of CNCs remarkably enhanced the stability and mechanical properties of HA hydrogels. It was found that resistance against hydrolytic and enzymatic degradation, the ability to recruit cells, and proangiogenic activity were significantly enhanced by this combination [30].

Collagen

Collagen is the most abundant structural protein of the extracellular matrix in mammalian connective tissues and presents the closest viscoelastic properties to real pulp tissue [31]. Collagen is composed of amino acids sequences, typically glycine-X-Y, where X and Y are frequently proline or hydroxyproline, that together form a triple helix. Collagen has multiple applications in medicine, such as cardiac applications, bone grafts or tissue regeneration. Collagen can be extracted from several animal/human sources, such as bone, cartilage, tendon, ligament or skin [32]. Due to its origin, collagen displays low immunogenicity. Collagen is permeable and presents a porous structure, it is also biocompatible and biodegradable [33]. Collagen is involved in regulating cell morphology, adhesion, migration and differentiation [34]. All of these characteristics make this natural polymer a promising biomaterial and scaffold for tissue engineering. However, poor mechanical strength and poor structural stability upon hydration are some disadvantages that can compromise its application [35]. Cross-linking of collagen scaffolds and blending collagen with other materials, such as inorganic materials or natural/synthetic polymers, may provide improvements to achieve better mechanical strength [35][36]. Sumita et al. analyzed the performance of collagen sponge as a 3D scaffold for tooth-tissue engineering. This in vivo study showed that collagen sponge allowed a more reliable tooth generation when compared with a polyglycolic acid fiber mesh scaffold [37]. Additionally, Prescott et al., evaluated the regeneration of dentin pulp-like tissue using DPSCs seeded in a collagen

scaffold, with dentin matrix protein 1 (DMP1), when implanted in mice. The study concluded that this triad was sufficient to generate an organized matrix formation of pulp-like tissue [38].

Gelatin

Gelatin is composed of peptides and proteins produced by a partial hydrolysis of collagen. Its composition is similar to its parent collagen's origin. Gelatin is classified as a hydrogel and can be used in food applications, cosmetics, as a carrier, and in cell culture to promote adhesion, among other uses. One particular application is its use in hydrogel synthesis for tissue engineering. Its biocompatibility, low antigenicity, wide availability and low cost are some of the advantages of this natural scaffold [16]; however, gelatin is very sensitive to temperature alterations and degradation over time [39], which may compromise its mechanical properties. Gelatin hydrogels play an important role on cell attachment and provide access to several functional groups for biochemical modification, resulting in a high efficacy scaffold with bio-affinity and improved mechanical properties [40]. For example, Ishimatsu et al. observed dentin regeneration and the formation of dentinal bridges on the surface of regenerated dental pulp, when using controlled release of fibroblast growth factor 2 (FGF2) from gelatin hydrogels [41]. Additionally, Londero et al. performed a histologic analysis of the influence of a gelatin-based scaffold (Gelfoam) in the repair of immature dog teeth subjected to regenerative endodontic treatment, leading to the conclusion that Gelfoam improved tooth repair when combined with blood clot [42].

1.3. Proteins and Peptides

Protein and peptide scaffolds are an emerging topic in tissue engineering, due to their versatile structure, composition, and the possibility that they might produce recombinant forms [43]. In addition to biocompatibility and biodegradation, another major advantage of peptides is the possible refinement of their structures via molecular manipulations, allowing the creation of a new modified peptide with specific biological, physical and chemical properties [44].

Fibrin

Fibrin is a fibrous non-globular protein, involved in blood coagulation. It is formed by the enzymatic activity of the protease thrombin on protein plasma fibrinogen, causing its polymerization. This scaffold offers advantages in terms of biocompatibility, immunogenicity, cell adhesion, cell proliferation, cell differentiation, biodegradability and cost when compared with other scaffolds [16][45]. Fibrin hydrogels can be obtained via a patient's own blood, representing an available, reproducible, autologous scaffold, with no immunologic risk. This hydrogel can also be injected and molded to acquire desirable 3D forms [45]. It is degraded by proteases (e.g., plasmin) and metalloproteinases, allowing scaffold redesign and resorption [46]. As with other natural scaffolds, fibrin gels present poor mechanical properties, being susceptible to contraction/compaction [47] and premature degradation [48]. However, these properties can be improved with optimization of the polymerization conditions (pH, calcium/fibrinogen/thrombin concentrations) [49] when fibrin gels are combined with other natural or synthetic polymers, such as HA or calcium phosphate ceramics [46], or when fibrinolysis is controlled with aprotinin, α-aminocaproic acid or tranexamic acid [50][51][52]. Autologous fibrin-rich platelet concentrates and fibrin hydrogels have been applied in dental-pulp regeneration with promising results [53][54][55].

Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF)

Platelet-rich Plasma (PRP) and platelet-rich fibrin (PRF) are autologous bioactive platelet concentrates prepared ex vivo by centrifugation of a patient's own blood. These platelet concentrates (PC's) have been applied in several fields of medicine, such as dentistry, plastic surgery and sports medicine. The use of PC's is based on improving the healing process and tissue regeneration via the release of biologically active substances (e.g., growth factors) from platelet granules, namely platelet-derived growth factor (PDGF), TGFβ, insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and epithelial cell growth factor (ECGF) [56][57]. PRP is collected with anticoagulant and is prepared in a simple two-spin centrifugation after which three layers may be collected: (1) the platelet-poor plasma (least dense), representing 45% of the sample; (2) red blood cells (middle layer), representing 40% of the sample; and (3) the PRP (denser layer), representing 15% of the sample. PRP is then mixed with a coagulating agent, such as calcium chloride and/or topical bovine thrombin, to initiate the coagulation process [58][59]. A PRP blood clot generally contains 4% red blood cells, 95% platelets and 1% white blood cells. In PRP, the platelet concentration is five times superior to normal platelet count [60], which increases the amount of growth factors bound to the developing fibrin network or to the extracellular matrix, thus creating a chemotactic gradient for stem cell recruitment, the promotion of tissue healing and regeneration [59]. Despite the advantages of PRP, there is a lack of standardization in PRP preparation protocol, range of storage time of different platelet concentrations and usage of different polymerization strategies [58][59]. Several studies refer to the benefits of using PRP, namely in the treatment of periodontal defects [61], in endodontic regenerative treatment [62][63] and in bone regeneration [64][65].

2. Synthetic-Engineered Polymeric and Ceramic Scaffolds

2.1. Synthetic Polymeric Scaffolds

Synthetic polymers constitute the largest biodegradable polymer group. Produced under controlled conditions, synthetic polymeric scaffolds display predictable and reproducible properties (e.g., mechanical characteristics, viscosity, porosity, biodegradation). These have the advantage of being tunable (i.e., in terms of their physical and chemical properties) and can be designed to release growth factors or other bioactive molecules. They can be produced in large quantities, may be more economical than natural scaffolds and present a longer shelf life [66]. The major disadvantage of these scaffold groups is the limited bioactivity due to their hydrophobic structure [67]. Among the most popular synthetic polymers used in tissue engineering are polylactic acid (PLA), polyglycolic acid (PGA) and polylactide-co-glycolide (PLGA) [68]. PLA and PGA are, respectively, polymers produced by lactic acid and glycolic acid condensation. Their main benefit for medical applications resides in the fact that their degradation products are natural metabolites, normally excreted in urine [69]. However, concerns about PLA/PGA biocompatibility have been raised due the accumulation of the degradation products [70]. PLGA is a copolymer composed of two different monomers, the cyclic dimers of glycolic acid and lactic acid. The ratio between glycolic and lactic acid leads to the formation of different forms of PLGA, with different degradation times [16]. These synthetic polymeric scaffolds have been used in dentistry for dental-pulp tissue regeneration and shown to be amenable to the seeding of DSCs. Early studies by Mooney et al. have described the formation of new pulp-like tissues when dental pulp stem cells (DPSCs) were seeded onto fabricated PGA fibers [71]. Later, Kuang et al. produced biocompatible and biodegradable PLA-based scaffolds and assessed their regulatory role in dentin-pulp complex regeneration. The PLA-based scaffolds considerably promoted the proliferation and odontogenic differentiation of DPSCs ameliorating the expression of ALP, osteocalcin, bone sialoprotein, collagen 1, and dentin sialophosphoprotein genes in an in vitro experiment. Moreover, histological analysis demonstrated superior dentin-like tissue formation in vivo [72].

PLGA scaffolds were also shown to lead to increased proliferation and adhesion of DPSCs under simulated microgravity —a procedure which also enhances MSC growth [73]. Recently, using SHED, PLA scaffolds demonstrated both a good biocompatibility and the ability to induce mineralization [74]. The copolymers of PGAs and PLAs have been sown with dental pulp progenitor cells and have been shown in rabbit and mouse xenograft models to produce pulp-like tissue [75].

2.2. Bioactive Ceramic Scaffolds

Bioactive ceramics include calcium phosphate ceramics, bioactive glasses and glass ceramics. These are biocompatible inorganic non-metallic materials that have been widely used in tissue engineering and regeneration, namely bone implants and dentistry [76]. These compounds are known for their good resistance, although the major limitations include brittleness, poor mechanical properties (fracture strength and reliability), low resilience and high density [66].

2.3. Calcium Phosphates (CaP)

Synthetic calcium phosphate (CaP) materials like hydroxyapatite (HAP), tricalcium phosphate (TCP) and biphasic calcium phosphate (HAP/TCP) are frequently used as bone grafting materials due to their similarity to the bone mineral phase [77]. These materials are biocompatible, have low immunogenicity, present good properties of resorption and are osteoconductive [78][79]. Properties of CaP ceramics, such as porosity, degradation and ion release, affect the bioactivity of these scaffolds, namely adhesion, proliferation and differentiation of cells [79][80]. High degradation, together with calcium and phosphorus ion release regulate osteoblast and osteoclast activity, promoting the formation of bone minerals in the surface of CaP scaffolds [81][82][83][84]. The bioactivity properties vary according to the physical and chemical characteristics of the CaP material [81]. To improve their advantages and complement their limitations, CaP have been combined or mixed with other materials.

2.4. Hydroxyapatite (HAP)

HAP is a naturally occurring mineral form of calcium apatite, with a Ca/P ratio of 1.67, constituting 70% *w/w* of human bones [85][86]. Synthetic HAP is produced as a dense material that mimics the mineral composition of bone; however, porous HAP has been used in clinical applications, namely for bone regeneration. HAP scaffold characteristics, such as porosity and pore size, influence its performance [87]. HAP is the most stable calcium phosphate, presenting low solubility in physiological medium, and its surface can function as a nucleating site for bone mineral formation [80][88]. Over the years, studies in vitro and in vivo have demonstrated good biocompatibility, bioactivity, and osteoconductivity for HAP [89] [90][91]. However, the brittleness of this scaffold has limited its application when high loads are present.

Nevertheless, HAP is widely used in the coating of other materials (e.g., metal implants) [92][93] and electrospun composite scaffolds made of polycaprolactone/gelatin and nanohydroxyapatite enhanced the proliferation and odontogenic

differentiation of DPSCs [94].

2.5. Tricalcium Phosphate (TCP)

TCP is a calcium phosphate and a bone substitute material with a Ca/P ratio of 1.5. It is characterized by a high biocompatibility, good resorption properties and osteoconductivity [95]. TCP is available in two forms: α-TCP (formed at ≥1125 °C) and β-TCP (formed at 900–1100 °C). β-TCP presents higher structural stability and higher degradation rate when compared with α-TCP [81]. For these reasons, β-TCP is more widely used in bone regeneration applications [96]. In comparison to HAP, β-TCP is less stable, however it presents higher solubility and higher degradation/resorption rates during bone regeneration [97][98]. All of these characteristics have turned attention to β-TCP as a scaffold for bone regeneration [99][100].

2.6. Biphasic Calcium Phosphate (HPA/TCP or BCP)

HAP/TCP or biphasic calcium phosphates were developed to unite, at a submicron level, characteristics from both HAP and TCP—more stability from HAP and better resorption from TCP [101]. Because HAP:TCP ratios influence the ceramic performance, Arinzeh et al. compared the influence of different ratios of biphasic calcium phosphate ceramics combined with MSCs on bone formation. The authors concluded that a HA/TCP formula with higher quantities of TCP induced osteogenic differentiation and bone formation at a faster rate [102]. In 2010, Tonomura et al. tested the differential effect of scaffold shape on dentin regeneration. Their results show that, when porous HAP/β-TCP was used, dentin-like tissue with minimum cell inclusions was observed and aligned odontoblast-like cells appeared in relation to the hard tissue. In HAP/β-TCP powder and PGA groups, bone-like tissue with cell inclusions was observed with no cell alignment. Interestingly, the authors of this study were also able to conclude that the shape of the scaffold influenced the type of tissue regenerated [103].

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