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Title: Scaffold-based and scaffold-free strategies in dental pulp regeneration

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Abstract

Regenerative dentistry has come a long way from pulp capping to pulp regeneration research, which aims to regenerate the pulp-dentin complex and restore its functions compromised by pulp injury and/or inflammation. Due to unique anatomical limitations of the tooth structure, engineering a suitable microenvironment that facilitates angio-/vasculogenesis and innervation is a challenging task. Cell-based tissue engineering approaches have shown a great potential in achieving this goal. Biomedical approaches in creating a regenerative microenvironment are mainly represented by either scaffold-based or scaffold-free strategies. The scaffold-based strategy mainly relies on the use of biomaterials to create a structural base that supports cells throughout the process of tissue formation. The scaffold could be a classical 3D construct with interconnected pores, a hydrogel with cells embedded in it or a combination of these two. Scaffold-free approach has been considered a bottom–up strategy that employs cell sheets, spheroids, or tissue strands as building blocks. The outcome of this strategy relies on the capacity of these building blocks to secrete favorable extracellular matrix and to fuse into larger tissue constructs. Both the scaffold-free and scaffold-based systems are required as complimentary, rather than competing, approaches for pulp regeneration. A combined synergetic strategy, through which multicellular building-blocks could be integrated with robust 3D scaffolds, might represent an optimal solution to circumvent some of the major drawbacks of current methods in pulp regeneration while concurrently fostering their advantages.

Clinical relevance

The long-term goal of pulp regeneration is to regenerate the dentin-pulp complex and restore its functions compromised by pulp injury and/or inflammation. Engineering the vasculature and innervation are two important aspects in achieving this goal of pulp regeneration and to maintain pulp homeostasis. Tissue engineering approaches in pulp regeneration are represented by two somewhat opposing strategies, scaffold-based and scaffold-free.

Introduction

Recent advances in biotechnology have revolutionized the research fields involved in biologically based regenerative therapies in dentistry. It is expected that regenerative medicine would offer many benefits in terms of long-term survival rates and prognosis.

The long-term goal of pulp regeneration research is to regenerate the dentin-pulp complex and restore its functions compromised by pulp injury and/or inflammation. Since the isolation and characterization of dental pulp stem cells, it has been recognized as a promising cell source in pulp regeneration strategies (1). Subsequent studies on cell-based pulp-tissue engineering have shown a great potential in pulp regeneration research; several animal studies demonstrated that DPSC constructs can give rise to dentin-pulp like tissues upon transplantation *in vivo* (2-6). However, the regenerative outcome is highly dependent on number of factors, including the post-implantation cell survival, level of inflammation at the site and the presence of proper stimuli in the surrounding microenvironment to promote lineage-specific differentiation. Due to lack of oxygen and nutrients at the implantation site, stem cell survival is compromised once introduced *in vivo* (7). It was shown that post implantation cell survival could drop to less than 5% within the first 10 days (8). Further, the root canals with necrotic pulp often have an inflammatory microenvironment, which has a negative effect on stem cell survival as pro-inflammatory cytokines diminish stem cell proliferation/self-renewal and promote cell death (8, 9). It is still questionable which level of inflammation would allow pulp regeneration to proceed. Without proper stimuli from the surrounding microenvironment, the ability of stem cells to participate in tissue regeneration is compromised. Therefore, in order to achieve complete pulp regeneration in a controlled and predictable manner, engineering of an artificial microenvironment capable of directing stem cell response is critical. In achieving this aim, it is important to consider the cellular, structural, and signaling cues that modulate stem cell participation in tissue maintenance, regeneration, and repair.

In addition, engineering the vasculature and innervation are two important aspects in achieving the goal of pulp regeneration and to maintain pulp homeostasis. Vasculature in the pulp tissue plays a critical role in nutrition and oxygen supply, functions as a conduit for the transport of metabolic waste, and regulates inflammation (7). Due to the unique anatomy of the tooth structure, the pulp space which is surrounded by a hard dentin, receives a limited blood supply through the apical foramen. Furthermore, when transplanted *in vivo*, tissue constructs depend solely on the oxygen supply through nearest capillary or by diffusion up to 200 μm (7). Therefore, in order to prevent apoptosis of distant cells in greater size tissue constructs, timely formation of a capillary network is required. Nerve fibers contribute to angiogenesis, extravasation of immune cells to regulate inflammation, pulp homeostasis, and pulp defense mechanisms (10). Therefore, engineering a suitable microenvironment that facilitates angio-/vasculogenesis and innervation is necessary in pulp regeneration approaches.

Functional cell-containing constructs are developed in this regard to restore damaged structures as well as cells of the dental pulp. Recently, biomedical approaches in tissue engineering are mainly represented by two somewhat opposing strategies, scaffold-based and scaffold-free.

The scaffold-based strategy mainly relies on the use of biomaterials to create a structural base that supports cells throughout the process of tissue formation. The scaffold could be a classical 3D construct with interconnected pores, a hydrogel with embedded cells or a combination of these two (Figure 1) (2, 3, 5, 6, 11). The use of a decellularized extracellular matrix (ECM) of a tissue or an organ is another promising trend (12). The scaffolding strategy is very versatile in terms of mechanical properties and degradation profile of the construct, as well as the potential to incorporate growth factors. In addition, a wide range of possibilities are being

offered in rapidly evolving 3D printing technologies including the use of bio-inspired composites, multi-material constructs and shape-morphing systems (13). The use of cell-free scaffolds is another approach with the aim of inducing host cells homing into the scaffolds, which is not further discussed in this paper.

In contrast to the scaffolding approach, in scaffold-free approach, the cells create their own microenvironment through self-assembly of monodispersed cells into 3D tissue, which permits true interactions between different types of cells without any influence from an artificial material (Figure 1). Scaffold-free approach has been considered a bottom–up strategy that employs cell sheets (14, 15), spheroids (16), or tissue strands (17) as building blocks (Figure 1). The outcome of this strategy relies on the natural capacity of these building blocks to combine and form larger tissue constructs. In comparison to the scaffold-based approach, the initial cell density is significantly higher in scaffold-free strategy. Thus, cell proliferation and migration are not limiting factors, reducing the time necessary for tissue formation dramatically. Scaffold-free approach therefore, can successfully overcome some of the major challenges related with scaffolding methods such as failure to mimic natural extracellular matrix (ECM), lack of the intercellular cross talk, and difficulty of prevascularization (18, 19).

As described, scaffolds that are capable of simulating the natural microenvironment, allow cell-cell, cell-ECM and cell-soluble factor interactions. On the other hand, pulp regeneration via scaffold-free approaches allows direct cellular interactions without any interference from an exogenous material and creates the necessary microenvironment in order to adapt to specific needs. In this review, we aim to discuss scaffold-based and scaffold-free strategies investigated in pulp regeneration and some future perspectives in this regard.

Scaffold-based approach in dental pulp regeneration

The progress in the area of material science has contributed immensely in the current improvements of scaffolds used for tissue engineering purposes. The development of more multipurpose and advanced biomaterials lead to a transition in the scaffolds from biocompatible cell transporters and simple delivery vehicles to bio-functional and guiding matrices. Furthermore, with the advancements in technology, all aspects of material behavior could be controlled and customized for the purpose of use. In the fields of periodontology and oral surgery, commercially available products for bone and periodontal tissue regeneration have already been used in clinical practice and thus have improved treatment outcomes and success rates (20, 21). Accordingly, the field of pulp-dentin complex regeneration with pulp-derived stem cells has progressed continuously (2, 3, 22). Currently, with a wide range of available scaffold materials, the challenge is to optimize and functionalize the chosen matrix for dentin-pulp complex engineering.

Biomaterials in scaffold-based approach

Scaffolds are often used to provide a carrier surface, on which the cells may adhere, proliferate, and spatially organize. The aim of choosing a specific biomaterial for scaffold is to fabricate a specific microenvironment that can mimic natural cell-cell, cell-ECM and cell-soluble factor interactions in pulp-dentin complex. Therefore, it is important to discuss how different materials used as scaffolds in various pulp regeneration studies have achieved this objective.

Various classes of biomaterials have been used and table 1 provides an up to date overview of biomaterials that have been utilized for dental pulp engineering (Table 1). An ideal scaffold for pulp-dentin regeneration should facilitate the attachment, migration, proliferation, three-dimensional spatial organization and differentiation into odontogenic, vasculogenic and neurogenic lineages of the stem/progenitor cells of interest. Additionally, biocompatibility of

the material is of the utmost importance in order to prevent any adverse reactions by host tissue. Another critical aspect is being able to facilitate constructive remodeling with a tunable biodegradability to match the regeneration rate. With the scaffold degradation, a cascade of tissue reactions take place including, cellular infiltration, vascularization, differentiation, spatial organization and replacement of the scaffold by the target tissues.

Polyglycolic acid and polylactic acid Polyglycolic acid (PGA) and polylactic acid (PLA), among various synthetic polymer scaffolds proposed for dental tissue engineering, are biodegradable polyesters that can be derived from a range of renewable sources (23, 24). Scaffolds fabricated from PGA have demonstrated their ability to facilitate adhesion and proliferation of pulpal fibroblasts, dental pulp progenitor cells and cells from *ex vivo* human pulp tissues (23-25). Through rabbit and mouse xenograft models, it has been shown that copolymers of PGA and PLA seeded with dental pulp progenitor cells can give rise to pulp-like tissue (2, 4, 26). Moreover, seeding SHED cells onto PLA in dentin disks demonstrated formation of a vascularized pulp-like tissue, odontoblast-like cells, and new dentin (3). Comparably, Huang et al. reported constructs of stem cells from apical papilla (SCAP) and PGLA inserted into an empty root canal space and transplanted *in vivo* can results in pulp-like soft tissue and deposition of continuous layer new dentin (2). However, synthetic polymers can induce either acute or chronic inflammatory response. Furthermore, the hydrolytically degraded byproducts may cause local pH decrease jeopardizing the clinical application (27).

Polyethylene glycol

Polyethylene glycol is another polymer used in tissue engineering approaches, including pulp regeneration (28-30). Dental pulp progenitor cells attached to electro-spun polyethylene glycol scaffolds have been transduced to form 3-dimensional tissue structures (31).

In addition, these synthetic polymer scaffolds have been used to deliver a variety of agents, such as anti-inflammatory agents, growth factors and adhesive proteins (32-36). Apart from providing support for cell growth and proliferation, such scaffolds could also control pulpitis and facilitate pulpal repair. The superior handling properties and relative ease of production in synthetic polymer scaffolds give them a better perspective in endodontic regeneration. Yet, they bear little resemblance to the native extracellular environment of the dental pulp. Natural scaffolds constitutive of ECM components thus, have emerged with a better resemblance to microenvironment.

Natural scaffolds

Collagen is a natural scaffold, therefore, is biocompatible and degradable by enzymes. On the downside, natural polymers are often hard to manipulate and are stricken with the risk of triggering an immune response and transmitting pathogens. Collagen hydrogels have been broadly used as scaffolds to encapsulate dental pulp stem/progenitor cells with or without growth factors and anti-inflammatory molecules to regenerate pulp-like tissues in various animal models (5, 37-39).

Alginate, another natural agent, is a polysaccharide derived from red algae, which offers a mild gelation process, since it can be cross-linked via Ca2+. However, its degradation is difficult to control and dissolution is easily triggered by calcium chelating compounds (40). Chitosan is derived from chitin, a polysaccharide found in crustaceans. It has been used for various tissue regeneration purposes as chitosan demonstrates biocompatibility and degradability via naturally occurring enzymes (41). Although, there is a growing interest in using biomimetic scaffolds composed of ‘‘natural’’ substances, compared to synthetic polymer scaffolds, these are not as mechanically resilient.

Host derived scaffolds

Induction of bleeding and formation of an intracanal blood clot is a current procedure used in regenerative endodontics to provide a scaffold for pulp-dentin regeneration (42). In immature teeth with open apices, induced bleeding results in the delivery of stem cells from apical papilla (SCAP) into the root canal space through the apical foramen. This approach eliminates the need to inject foreign stem cells. These qualities, in addition to the low cost, clinical simplicity, short setting time and cervical sealability with Tricalcium silicate-based materials provide an attractive treatment option for both patients and dental practitioners. Furthermore, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have been widely used in regenerative dentistry. Recent findings suggest PRF to have a greater regeneration potential when compared with traditional PRP (43). In addition, pulp-derived ECM utilized in three-dimensional injectable scaffold has shown potential in regenerative therapy (44). In fact, decellularized human dental pulp itself has been shown to support the proliferation and differentiation of SCAP (12).

Other trends in scaffolds

The biomaterials listed above have their own strengths and weaknesses (Figure 1). Thus, there is a current trend to develop scaffolds by combining complex biomaterials. For instance, 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. Further, the resorption products of Calcium phosphate minerals in ceramics 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 producing cells can be modified with bioactive ceramics and glasses (45). 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.

Self-Assembling Peptides

Self-assembling peptides being amenable to be modified and customized, could address numerous requirements of dental pulp tissue-engineering approaches (46). Since the peptide chains are made of naturally occurring amino acids, the resulting materials are biocompatible and can be designed to be biodegradable (46). Peptide hydrogel systems provide benefits such as viscoelastic properties comparable to those of soft connective tissues like dental pulp, fast diffusion of nutrients and metabolites, and the potential of homogenous cell encapsulation (47, 48).

PuraMatrix, a commercially available peptide hydrogel, has been used in dental pulp regeneration studies. PuraMatrix by itself was unable to form dental pulp unless it was combined with DPSCs (6, 11). When DPSCs and HUVECs were encapsulated in PuraMatrix, HUVECs were able to prevascularize the cellular constructs and to give rise to vascularized pulp-like tissues upon transplantation in a tooth-root model *in vivo* (6, 11). PuraMatrix provided a microenvironment that supported cell survival, cell-cell interactions, cell migration and capillary network formation in the absence of exogenous growth factors, which ultimately contributed to successful dental pulp regeneration (11).

Scaffold-free approaches in pulp regeneration

The use of prefabricated scaffold-free multicellular units such as cell sheets, spheroids, and, more recently, tissue strands as building blocks has emerged as a unique approach in pulp regeneration. Self-assembly is the fundamental characteristic in scaffold-free approach in tissue

engineering (49). The scaffold-free cellular constructs being able to produce their own ECM are adaptive in nature to the target environment and can form large cohesive tissue constructs. Additionally, their capacity to secrete growth and differentiation factors, induce innervation and vascularization are essential attributes that facilitates their use in pulp regeneration.

Cell sheets

Although different methods have been used for producing cell sheets and spheroids in the past, cell sheets are now commonly prepared by culturing a monolayer of cells in culture dishes coated with a thermoresponsive polymer (50). It has also been reported that 3D tissue constructs with prevascular networks can be produced *in vitro* by stacking sheets prepared from endothelial cells and fibroblasts (51). Furthermore, substrates that are patterned in a way to culture sheets of cells aligning along a certain direction are introduced where production of anisotropic tissue constructs is needed (52).

Syed-Picard et al. reported engineering of self-assembled three-dimensional (3D) scaffold-less tissues from human dental pulp cells (DPCs) (15). They have fabricated cell sheets that self-assembled into cylindrical 3D tissue constructs (15). The cells in these constructs, without any support from exogenous materials, were able to generate their own microenvironment. When these cylindrical 3D tissue constructs were inserted into a human tooth root and transplanted in a mouse model, dental pulp-like tissues were regenerated. The newly formed tissue was vascular, capable of forming dentin-/ bone-like tissue, and containing odontoblast-like cells along the dentin surface, as confirmed by DSP expression. Hence, it was evident that cell-sheet engineered 3D scaffold-less tissue constructs have the potential to be used in pulp regenerative therapies. In fact, the *in vitro* and *in vivo* results of this study have consistently shown that the scaffold-less tissue constructs are highly cellular, solid tissues that express dentin proteins on the periphery and pulp properties in the core, which resembles those of the dentin-pulp complex (53).

Furthermore, another study has described a cell-sheet-based 3D cell pellet cultivation system for SCAPs (14). This pellet system has presented several unique biological characteristics, including high cell utility efficiency, even cell distribution, satisfactory size and good handling properties, particularly with regard to the abundance ECM components, which are vital for the regeneration of pulp/dentine-like tissue (14). These cell pellets, up on *in vivo* transplantation, have given rise to homogenous dental pulp-like tissue with well-established vascularity (14). Continuous layers of newly deposited dentine-like tissue and odontoblast-like cells, which express DSPP, ALP and BSP, have been found next to the existing dentin (14).

Cell spheroids

Multicellular spheroids are arguably the most popular building blocks in scaffold-free tissue engineering, mostly due to its convenience of handling (54). There is a wide range of fabrication techniques of cell spheroids from numerous antiadhesive cell-culture plates to microfluidic and fully automated hanging-drop cultures (55). Most importantly, it has been shown that spheroids display enhanced angiogenic and regenerative capacity, which can be attributed to the strong cell–cell crosstalk within the spheroids and their close resemblance to physiological conditions of complex 3D tissue architecture (56). Due to their high vasculogenic and angiogenic potential, spheroids can be specifically used for regenerating tissues where vascularization is critical (56). The shape of the cell spheroids further facilitates their use not only as building-blocks for bottom–up tissue self-assembly but also for engineering of tissue in the setting of bioprinting and automated tissue assembly (57).

We developed a scaffold-free 3D microtissue spheroids of dental pulp stem cells (DPSCs) using a non-adhesive micro-mold. In this microtissue model, we characterized the interactions between DPSCs and HUVECs in 3D, focusing on cell viability, prevascularization, and differentiation capacities. We determined the cellular properties of this self-assembling constructs and investigated the potential of these spheroids for angiogenesis and pulp-like tissue regeneration *in vivo* (16). The regenerated dental pulp-like tissue was vascular and contained odontoblast-like cells along the dentin surface, as confirmed by nestin and DSP expression (16).

The self-assembly of DPSCs and HUVECs into spheroids allowed them to secrete their own ECM and facilitated 3D spatial arrangement of HUVECs into a vascular-like network. Furthermore, the DPSC-HUVEC interactions within the spheroids not only led to prevascularization and enhanced vasculature following transplantation *in vivo* but also attenuated the ECM deposition, resulting a more stable microenvironment for co-cultures. Our results demonstrated that cells of different types can be self-assembled into microtissues and promote development of their own ECM-containing microenvironment without the support of a secondary artificial material (16).

Our *in vitro* analysis also showed that DPSCs and HUVECs in 3D microtissue spheroids synergistically act in osteo/odontogenic differentiation and angiogenesis (58). Furthermore, the results showed that both of these processes are likely to be regulated by a combination of factors, such as concentration of angiogenic growth factors, ECM deposition, and remodeling, all of which are in turn influenced by the presence of DPSCs and HUVECs (58). These findings provide insight into the complex intercellular cross talk occurring between DPSCs and ECs in the context of angiogenesis and pulp regeneration (58). The findings of our study also highlight the significance of developing a 3D microenvironment that supports cell-cell interactions, which can in turn contribute toward an optimal atmosphere for successful pulp regeneration strategies (58).

Tissue strands

Tissue strands represent the latest addition to the scaffold-free building blocks. The tissue strands are fabricated by injecting cell suspensions into a tubular alginate capsule, which is later removed by a sodium citrate solution (59). Multiple cell types can be arranged into tissue strands (59). With the use of computer-aided design (CAD)-based technologies, the exact location of each individual building block within a complex 3D tissue construct can be decided (60). However, accomplishing satisfactory mechanical properties in scaffold-free tissue strands is still a critical task (61).

Itoh et al. more recently have evaluated and reported the feasibility of using scaffold-free rod-shaped tissue strands of DPSCs for pulp regeneration therapy (17). They have established a method for fabricating rod-shaped cell constructs composed of DPSCs using thermoresponsive hydrogel and assessed their ability to self-organize through *in vitro* odontoblastic differentiation (17). This method allowed preparation of DPSC constructs in a variety of sizes and shapes through computed tomography image design. Therefore, the technology established in this study shows promise for achieving tailor-made dental pulp regeneration therapy that can be adjusted to the needs of each pulp-less tooth (17).

Current trends in scaffolding

An ideal material for scaffold should closely resemble the cells' physiological microenvironment, particularly, ECM. None of the materials described have all the structure and properties of an ideal material. The ECM acts as a structural support, but its role goes far

beyond that. The ECM is a nanostructured environment that provides the physical and chemical signals required to modulate cellular behavior and reinforce a particular phenotype. Furthermore, the ECM is a dynamic environment and can be selectively degraded and remodeled by the incorporated cells.

In dentin-pulp-complex engineering, the scaffold should be able to address number of challenges specific to this approach. For example, microbial contamination control in the root canal, vascularization and innervation of a long and narrow space surrounded with hard dentin, the incorporation of growth and differentiation factors relevant to odontoblast differentiation, the support of mineralized tissue formation, and the potential of creating acellular matrices capable of recruiting resident stem cells in the respective tissues. Therefore, functionalization of a candidate scaffold material to address above mentioned requirements may be necessary prior to application in pulp regeneration.

Combination of hydrogels with robust porous scaffolds is a novel trend that aim to fabricate mechanically stable constructs with a highly biomimetic environment that can mimic natural ECM (62). This approach can overcome the issues of low and inhomogeneous cell distribution in conventional porous scaffolds and low mechanical strength of hydrogels, while still utilizing the favorable cell migration and spreading properties of hydrogels. Furthermore, sequential multimodal bioprinting of hydrogels, continuous multimaterial bioprinting and coaxial extrusion of cell-containing hydrogels are novel methods trending in tissue engineering strategies, which aim to create relatively thick vascularized tissue constructs (63). In these strategies, one main advantage is the ability to predetermine the specific locations for different cells and materials within the engineered construct. However, where the hydrogels are used, their composition needs to be adjusted for the optimal function of different cell types.

Future perspectives: Synergy of scaffold-based and scaffold-free approaches

In the scaffold-free approach, number of cell types can be combined to create the complex architecture of tissues and organs by utilizing the feature of controlled assembly of heterogeneous building blocks. However, the scaffold-free approach also comes with shortcomings such as inferior mechanical properties of individual building blocks. This could lead to possible cell damage during manipulation. Another drawback is the time it takes for the initial fusion of the building blocks to obtain a cohesive construct.

Despite the vast quantity of new knowledge and unquestionable advancement achieved during the past few decades, pulp regeneration still faces plenty of challenges. Clinical translation encounters considerable hurdles, especially in the aspects of post-implantation cell survival, angiogenesis and neurogenesis. To achieve these breakthroughs in pulp regeneration research, it is critical to pursue novel unconventional strategies. A combined synergetic strategy of scaffold-based and scaffold-free approaches might represent an optimal solution to circumvent some of the major drawbacks of current methods in pulp regeneration, while concurrently fostering their advantages (Figure 1- Schematic diagram) (62). The integration of multicellular building-blocks with robust 3D scaffolds would not only protect them from mechanical damage but also provides means for additional functionalization of the pulp constructs and delivery of growth factors. Furthermore, in a synergetic approach, despite the use of scaffolds, cell proliferation and migration would not be decisive factors due to the high cell density of spheroids and therefore, the time necessary for pulp-tissue formation could be reduced significantly.

Fabrication of microscaffolds containing individual cell spheroids is a recently reported technique that demonstrates the potential of synergistic strategy of scaffold-based and scaffold-

free methods in tissue engineering. In this technique, cell spheroids are produced directly within each microscaffold, which are subsequently assembled into a cohesive 3D tissue construct. Microscaffolds enable rapid bottom–up assembly of tissue constructs with better mechanical stability. Such a modular process is highly advantageous with respect to scalability (61). Furthermore, with alteration of the microscaffold properties, the system can be optimized for different cell and tissue types. This specific approach is a form of bio-assembly, as discussed in recent publications on biofabrications (63). However, implementing this emerging synergetic strategy in pulp regeneration possess number of challenges. Firstly, all microscaffolds reported so far were produced from non-biodegradable materials, therefore, these materials cannot be used in pulp regeneration. However, there are recently reported biomaterials such as cross-linkable gelatins developed for two-photon polymerization technique, which could be suitable for this purpose (64). Secondly, assembling of microscaffolds inside the small and tapering pulp chamber may require additional advance techniques. However, microscaffold is not the only option available to combine scaffold-based and scaffold-free approaches.

Combination of cell spheroids with 3D biodegradable scaffolds is a practical alternative. Manual placement of spheroids into the scaffold pores may create issues in technique sensitivity. However, this is already solvable with currently available technologies, such as utilization of various robotic systems capable of automated manipulation and placement of spheroids (60, 65). Combining such systems with a 3D printer that can produce robust macroporous scaffolds is rather straightforward.

Taken together, both the scaffold-free and scaffold-based systems are required as complimentary, rather than competing, approaches for pulp regeneration. The varying levels of pulp inflammation and tooth maturation require more than one approach to provide relief for all different conditions. Some cases are better candidates for scaffold-free cell systems whereas others could be better treated with scaffolds loaded with cells, cell-spheroids or/and growth factors. The future will see more advancements in these two tracks, therefore, we expect that those developments will strongly boost the progress in pulp regeneration and help to develop relevant clinical solutions.

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Table 1: Properties of biomaterials used in scaffold-based approach in pulp regeneration

Biomaterials	Favorable Properties	Limitations
Polymers- Synthetic Polyester PLA: Poly(lactic) acid PGA: Poly(glycolic) acid PLGA: Copolymer of PLA and PGA PCL: Poly(ε-caprolactone) Poly (urethanes) Poly (ether ester) PEG: poly(ethylene glycol) PBT: poly(butylene terephthalate)	• Higher mechanical strength • Porosity can be altered according to specific need • Lower immunogenicity • Adjustable mechanical properties • Ability to arrange in to different shapes • Lower degradation rate • Wettability tailored according to the need • Can modify with functional groups to attract cells or growth factors • Cost-effective • User-Friendly	• Local pH decrease • Induce Inflammatory host responses • Degraded by hydrolysis • Toxic byproducts
Polymers- Natural Polysaccharides Chitin Cyclodextrins Alginate Dextran Chitosan Cellulose Starch Hyluronan	• Better biodegradability • Produced by a biological process • Occur naturally • Variety in options • Unique structural properties • Composition can be adjusted as hybrid materials	• Low strength • Inconsistent with seeding cells • Difficult to control the size of the pores • May require chemical modifications • High water solubility
Bioceramics	• Biocompatible • High osteoconductivity • Tailorable resorption • Good angiogenic ability	• Brittle • Low degradation rate Nonhomogeneous particle size and shape Difficult porosity control

Host-derived scaffolds Decelullarized extracellular matrix Platelet-rich plasma Platelet-rich fibrin	• Provide favorable environment for tissue growth • Allow controlled growth factor release • Adaptable in to specific shapes	• Special equipment and reagents are required • Comparatively high cost
Hydrogels- Natural Collagen Fibrin Hyaluronic acid (Proteoglycans) Chitosan Alginate Gelatin Dextran/Dextran sulfate	• High biocompatibility • Viscoelasticity similar to connective tissue • Efficient transport of nutrients and elimination of waste • Potential to self-assembly • Allows cell-cell interactions	• Mechanically weak • Undergoes rapid degradation • Undergoes contraction
Hydrogels- Synthetic PEG: Poly(ethylene glycol) SAP: Self-assembling peptides Polymethacrylamide Polyamides	• Allow uniform dispersion of cells • Injectable and allows gelation in situ • Higher cross-linking ability • Modifiable with bi-functional molecules or growth factors • Transformability to smart hydrogels which response according to the microenvironment	• Slow gelation • Requires UV light which may cause cell death

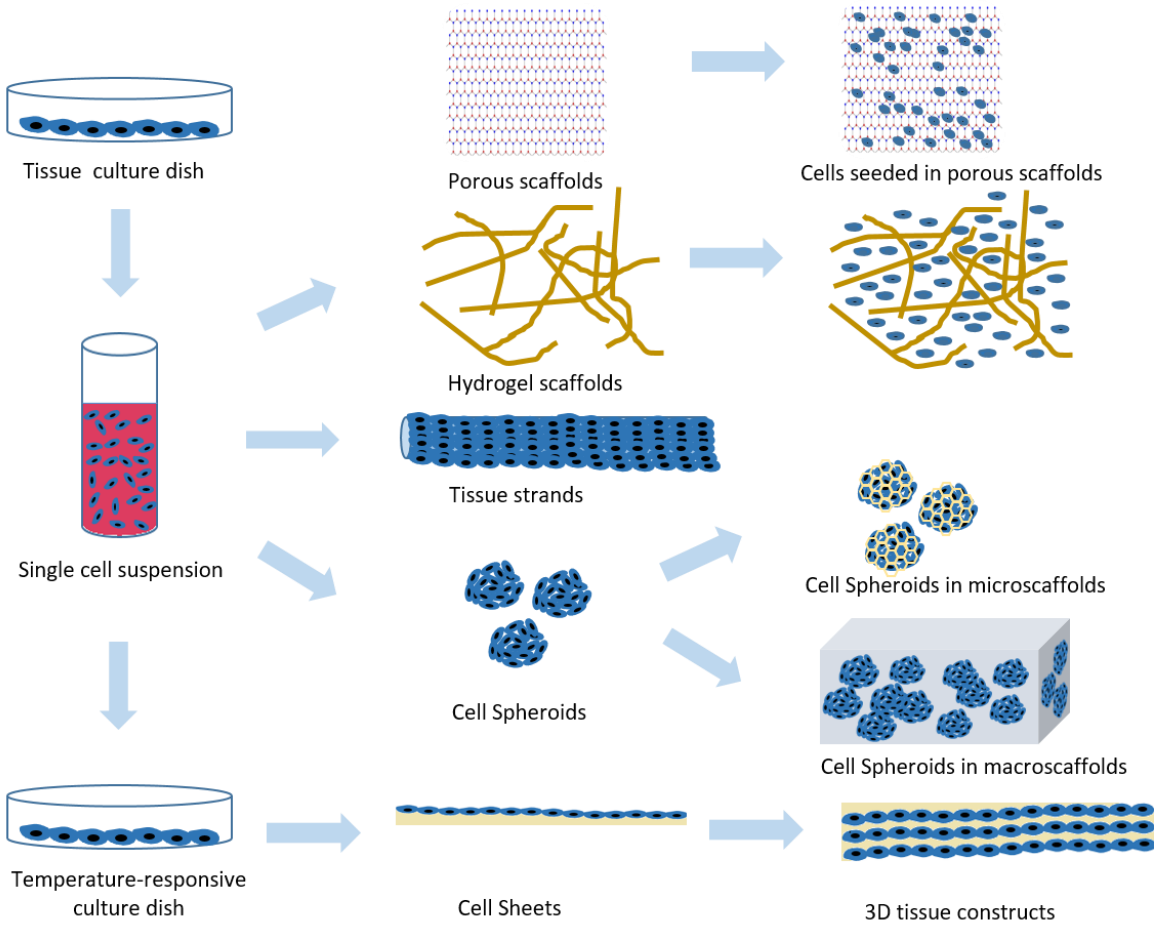

Figure 1: Scaffold-based, scaffold-free and synergetic strategies in cell-based tissue engineering