Scaffolds for Dental Pulp Tissue Engineering

K.M. Galler[1]*, R.N. D'Souza[2], J.D. Hartgerink[3], and G. Schmalz[1]

[1]University of Regensburg, Department of Restorative Dentistry and Periodontology, Regensburg, Germany; [2]Baylor College of Dentistry, Department of Biomedical Sciences, Texas A&M, Dallas, USA; and [3]Rice University, Departments of Chemistry and Biomedical Engineering, Houston, TX, USA; *corresponding author, kerstin.galler@klinik.uni-regensburg.de

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ABSTRACT

For tissue engineering strategies, the choice of an appropriate scaffold is the first and certainly a crucial step. A vast variety of biomaterials is available: natural or synthetic polymers, extracellular matrix, self-assembling systems, hydrogels, or bioceramics. Each material offers a unique chemistry, composition and structure, degradation profile, and possibility for modification. The role of the scaffold has changed from passive carrier toward a bioactive matrix, which can induce a desired cellular behavior. Tailor-made materials for specific applications can be created. Recent approaches to generate dental pulp rely on established materials, such as collagen, polyester, chitosan, or hydroxyapatite. Results after transplantation show soft connective tissue formation and newly generated dentin. For dentin-pulp-complex engineering, aspects including vascularization, cell-matrix interactions, growth-factor incorporation, matrix degradation, mineralization, and contamination control should be considered. Self-assembling peptide hydrogels are an example of a smart material that can be modified to create customized matrices. Rational design of the peptide sequence allows for control of material stiffness, induction of mineral nucleation, or introduction of antibacterial activity. Cellular responses can be evoked by the incorporation of cell adhesion motifs, enzyme-cleavable sites, and suitable growth factors. The combination of inductive scaffold materials with stem cells might optimize the approaches for dentin-pulp complex regeneration.

Over the past two decades, tissue engineering and regenerative medicine have become increasingly important areas of research. With a combination of biomaterials, competent (stem) cells, and inductive growth and differentiation factors, the goal is to improve or, ideally, fully restore the functions of diseased tissues or organs. What seemed like science fiction only 20 years ago is within the realm of possibility today, and various tissue engineering products are already being applied in clinical practice. Spectacular developments include the generation of induced pluripotent stem cells (Takahashi and Yamanaka, 2006), a beating heart in a petri dish (Jakab *et al*., 2008), the first successful transplantation of a tissue-engineered trachea (Macchiarini *et al*., 2008), or, in the field of dentistry, the first bioengineered tooth germ, which developed and erupted in the oral cavity as a functional replacement (Ikeda *et al*., 2009). Advances in tissue engineering are translating into medical practice and changing therapeutic strategies. The area of material science has contributed considerably to this process. With the development of more versatile and sophisticated biomaterials, scaffolds have transitioned from bioinert passive cell carriers and mere delivery vehicles to bioactive and instructive matrices, which can be controlled in all aspects of material behavior. Although dentistry is one of the disciplines which have long capitalized on the use of biomaterials, these serve mainly to replace lost tissues and restore their function. However, tissue engineering approaches in dentistry are evolving. In the fields of periodontology and oral surgery, regenerative strategies have already been implemented in daily practice. Commercially available products for bone and periodontal tissue regeneration are available to clinicians, and have improved treatment outcomes and success rates (Costello *et al*., 2010; Villar and Cochran, 2010). More recently, engineering of dental pulp and dentin with pulp-derived stem cells has made considerable progress (Cordeiro *et al*., 2008; Huang *et al*., 2010; Sakai *et al*., 2010). With a wide range of biomaterials choices, the question is how far we can optimize our strategies for dentin-pulp complex engineering with the help of novel and smart biomaterials, which are tunable and tailor-made for this specific approach.

BIOMATERIALS FOR DENTAL PULP ENGINEERING

For most regenerative strategies, an organic scaffold is used to provide a surface on which cells may adhere, grow, and spatially organize. Various classes of biomaterials are available to the tissue engineer (Table 1).

An ideal scaffold should facilitate the attachment, migration, proliferation, and three-dimensional spatial organization of the cell population required for structural and functional replacement of the target tissue. Biocompatibility is of utmost importance to prevent adverse tissue reactions. Since the host cells will, in any case, interact with the scaffold, biodegradability should be tun-

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Key Words

scaffolds, dental tissue engineering, dental stem cells, hydrogel, self-assembling peptides, regenerative medicine.

Table 1. Biomaterials for Tissue Engineering

Biomaterial	Source	Advantages	Limitations
NATURAL POLYMERS			
Polysaccharides	Plant, Animal	Derived from renewable sources	Number of biologically derived polymers is limited
Alginate	Sea Algae	Large diversity	
Dextran	Bacteria	Unique but complex structures	Difficult to process
Chitosan	Crustaceans	Functional groups, tailorable chemistry	Properties may differ
Cellulose	Plant cell walls		Undesirable immunoresponse
Starch	Crops	Specific recognition domains	Pathogen transmission
Hyluronan	ECM	Intrinsic biodegradability	
		Composition of hybrid materials	
Extracellular Matrix	Tissue-specific	Temporary controlled growth factor release	Difficult to process and sterilize
		Favorable environment for constructive tissue remodeling	Batch variations
		Processing into many forms possible	
SYNTHETIC POLYMERS			
Polyester		Minimal foreign body reaction	Environment unlike ECM
PLA: Poly(lactic) acid		Tailorable mechanical properties and degradation rate, wettability, and protein adsorption	Accumulation of acidic degradation products
PGA: Poly(glycolic) acid			
PLGA: Copolymer of PLA and PGA			
PCL: Poly(ε-caprolactone)		Degradation products present in the metabolic pathways	
Poly(urethanes)			
Poly (ether ester)		Functional groups to attract cells or bind growth factors	
PEG: poly(ethylene glycol)			
PBT: poly(butylene terephthalate)		Cheap and reproducible production	
		Easily processed into any shape	
HYDROGELS			
Natural materials		High biocompatibility	Disadvantages of the individual material source
Collagen		Tissue-like water content	
Fibrin		Viscoelastic properties similar to those of soft connective tissue	Due to low mechanical stiffness not suitable for certain applications, *e.g.*, bone grafts
Proteoglycans, HA			
Chitosan		Efficient transport of nutrients and waste	
Alginate			
Synthetic materials		Uniform cell encapsulation	
PEG: Poly(ethylene glycol)		Injection and gelation *in situ*	
SAP: Self-assembling peptides		Chemical or physical cross-linking	
		Modifications with biofunctional molecules or growth factors possible	
BIOCERAMICS			
Calcium Phosphates		Biocompatible	Brittle
Hydroxyapatite		Excellent bone-bonding properties	Subject to fatigue
Tricalcium phosphate		Biodegradable	Decrease of mechanical strength under humid conditions
Biphasic calcium phosphate (HA/TCP)		Osteoconductive	
Bioactive Glasses = silica-based glasses		Variable rates of degradation	

able, to facilitate constructive remodeling, which is characterized by scaffold degradation, cellular infiltration, vascularization, differentiation and spatial organization of the cells, and, eventually, replacement of the scaffold by the appropriate tissues.

The two categories of materials that are most commonly used in tissue engineering are synthetic polymers such as poly(lactic) acid (PLA) and poly(glycolic) acid (PGA) (Vacanti *et al*., 1998; Gloria *et al*., 2010), and matrices derived from biological sources such as reconstituted collagen (Glowacki and Mizuno, 2007). Table 2 provides an overview of biomaterials that have been utilized particularly for dental pulp engineering. Early studies utilized human dental pulp cells on PGA fibers, collagen I, and alginate, where a pulp-like tissue was observed with the PGA scaffolds after 45 to 60 days of *in vitro*cell culture (Mooney *et al*., 1996; Bohl *et al*., 1998). After isolation and *in vitro* characterization of dental pulp stem cells from deciduous teeth and third molars, the cells were mixed with hydroxyapatite/tricalcium phosphate (HA/TCP), and the formation of dentin, bone, and dentin-pulp-like complexes was observed (Gronthos *et al*., 2000; Miura *et al*., 2003). A comparison of different materials (collagen

Table 2. Biomaterials Utilized for Dental Pulp Tissue Engineering

Material	Engineering Approach	Result	Reference
PGA, collagen I, alginate	Pulp fibroblasts seeded onto different materials, cell culture *in vitro*	Pulp-like tissue after 45 to 60 days on PGA	Mooney *et al.*, 1996 Bohl *et al.*, 1998
HA/TCP	Stem cells from dental pulp (SHED, DPSC) mixed with HA/TCP powder transplanted into nude mice	Generation of dentin or bone (SHED) and dentin-pulp-like complexes (DPSC)	Gronthos *et al.*, 2000 Miura *et al.*, 2003
Collagens I and III, chitosan, gelatin	Human dental pulp cells seeded into different materials for comparison *in vitro*	Adhesion and proliferation: Col I > Col III > Gelatin >> Chitosan ALP Activity: Col I > Col III > Gelatin >> Chitosan Mineralization: Col I > Col III > Gelatin Oc, Dspp, and Dmp-1 expression on collagen	Kim *et al.*, 2009
Collagen I with Dmp-1	Collagen scaffolds laden with Dmp-1 and dental pulp stem cells were placed in dentin disks with a simulated furcal perforation and transplanted subcutaneously into nude mice	Formation and organization of new pulp tissue	Prescott *et al.*, 2008
PLA	SHED seeded onto PLA scaffolds into tooth slices, subcutaneous transplantation into nude mice	Formation of vascularized soft connective, pulp-like tissue and new tubular dentin	Cordeiro *et al.*, 2008 Sakai *et al.*, 2010
PLGA	SCAP and DPSC seeded onto PLGA into root canals sealed with MTA on one side, subcutaneous implantation into nude mice for 3-4 months	Formation of a pulp-like tissue, deposition of dentin along the root canal wall	Huang *et al.*, 2010

I and III, alginate, and chitosan) rendered best results in terms of proliferation and mineralization activity on type I collagen. Highly promising results were achieved by Nör's group (Cordeiro *et al*., 2008), which demonstrated the formation of a vascularized pulp-like tissue, odontoblast-like cells, and newly generated dentin after seeding SHED cells onto PLA in dentin disks. Similarly, Huang *et al*. observed soft tissue and deposition of new dentin after transplantation of stem cells from apical papilla (SCAP) onto PGLA in an empty root canal space (Huang *et al*., 2010). In summary, collagen I and the synthetic polymers showed the most favorable results among the materials studied for this particular application. In terms of biocompatibility and degradation, all the above-described materials exhibited satisfactory results. Synthetic polyester such as PLA, PGA, and their co-polymers are non-toxic and biocompatible, they degrade by hydrolysis, and have gained FDA approval for numerous applications (Chan and Mooney, 2008). Collagen is biocompatible and degradable by enzymes, but natural polymers are often difficult to process and are afflicted with the risk of transmitting animal-associated pathogens or provoking an immunoresponse. Alginate, a polysaccharide derived from red algae, offers a mild cell encapsulation process, since it can be cross-linked *via* Ca^{2+}. However, it degrades in a rather uncontrolled manner *via* dissolution, since the material is sensitive to calcium chelating compounds (Boontheekul *et al*., 2005). Chitosan is derived from chitin, a polysaccharide found in crustaceans. Because of its good biocompatibility and degradability *via* naturally occurring enzymes, it has been used for numerous tissue engineering applications (Jiang *et al*., 2008).

However, neither described category has all the structure and properties of an ideal material, which should most closely resemble the cells' physiological environment: natural extracellular matrix (ECM). The ECM acts as a structural support, but its role goes far beyond this. The ECM is a nanostructured environment that provides the chemical signals 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 cells living within it. Polymers like PLA have the advantage of being biodegradable, biocompatible, and inexpensive and easy to prepare. However, they lack the chemical information that is physiologically found in the ECM. In contrast, collagen offers the chemical and structural information of the ECM but is difficult to customize for specific applications. Because of its biological origin, purity and immune reaction can be of concern. An ideal scaffold should combine the best properties of each of these groups of biomaterials. These

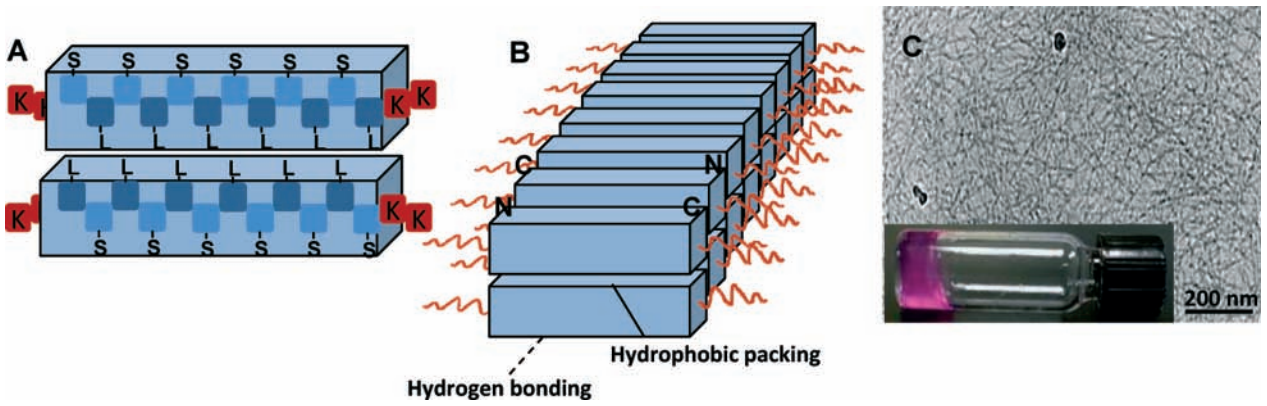

Figure. Schematic of multidomain peptide self-assembly. **(A)** The peptide monomers are made of a central block of alternating hydrophilic (S = serine) and hydrophobic (L = leucine) residues. **(B)** Dimers form because of the tendency of the hydrophobic amino acids to shield from the water. Positively charged residues (K = lysine) provide water solubility and aid in cross-linking and stabilization of the nanofibers after the addition of negatively charged ions, such as phosphate. The dimers string together, forming a nanofiber, which is stabilized by hydrogen bonding along the fiber axis. N: N-terminus. C: C-terminus. **(C)** The TEM image shows the nanofibrous network, macroscopically, and gelation is observed.

would be structurally similar to ECM at the nanoscale, be able to present complex molecular information to the cells, and be easy to modify for specific applications. To address these deficiencies, novel synthetic matrices are being developed for tissue engineering. Among these, peptide-based nanofibers are particularly promising because of their ease of synthesis, chemical diversity, and high control over various aspects of material behavior (Hartgerink *et al*., 2002; Zhang, 2003; Silva *et al*., 2004). Regarding dentin-pulp-complex engineering, the scaffold should allow us to address the particular challenges of this approach, including contamination control in the root canal, vascularization and innervation of a long and narrow space, the incorporation of growth and differentiation factors relevant to odontoblast differentiation, the support of mineral formation, and the possibility for creation of acellular matrices capable of recruiting resident stem cells in the respective tissues.

SELF-ASSEMBLING PEPTIDES

Self-assembling peptides (SAP; Fig.) are an excellent model of a material which can be modified and customized, and thus address the various requirements for a specific tissue-engineering approach. They are usually synthesized at 15 to 25 amino acids in length, which, based on their design, undergo self-assembly, generate nanofibrous networks, entrap water, and thus form hydrogels. Different concepts for self-assembling peptides exist; the multidomain peptide (MDP) system discussed here was developed in Dr. Hartgerink's laboratory. It features an ABA block motif, in which the central B block is made of alternating hydrophilic and hydrophobic amino acid residues, which provide the driving force for self-assembly. In water, they assemble on opposing sides of the peptide backbone, and dimers form because of the tendency of the hydrophilic residues to shield from the water. These dimers string together and form sandwich-like β-sheet nanofibers, 6 nm wide and 2 nm high, where hydrogen bonding occurs along the fiber axis (Dong *et al*., 2007). The flanking region is made up of charged residues, which make the molecules water-soluble and offer the possibility of cross-linking *via* oppositely charged ions. Since the peptide chains are made of naturally occurring amino acids, the resulting materials are biocompatible and can be designed to be biodegradable.

Hydrogel systems in general offer advantages such as viscoelastic properties similar to those of soft connective tissues, fast diffusion of nutrients and metabolites, and the possibility of homogenous cell encapsulation. The use of multidomain peptides is furthermore advantageous because of an automated synthesis process, high control over the material properties, a wide range of chemical functionalities, and the possibility for tailoring toward specific applications. Single amino acids serve as building blocks which can be used in a modular fashion, and the peptide sequence determines the properties of the resulting material. Bioactive motifs can already be incorporated into these systems during the synthesis process. Further options for customization will be discussed in the sections below.

BIODEGRADABILITY

The organization of MDP molecules into β-sheet aggregates with poor water solubility and a fiber assembly process reminiscent of amyloid raises concerns that this material cannot be degraded *in vivo* and might not be suitable as a tissue engineering scaffold. This is underscored by the devastating effects of amyloid aggregates in the brain, causing Alzheimer's disease and dementia, or other organs such as the heart, kidney, and vascular system (Stefani, 2010). If susceptibility to proteolytic degradation is programmed into these β-sheet-forming peptides, MDPs can be adapted to requirements for biological applications. A hexapeptide containing the MMP-2 consensus cleavage motif LRG was successfully incorporated into the central block without altering the length of the peptide, gelation process, or viscoelastic properties. The resulting peptide was confirmed to

be degraded by MMP-2 *in vitro*, and cell culture studies demonstrated that the cells started migrating into the hydrogel matrix if the cleavage site were present (Galler *et al*., 2010).

CELL-MATRIX INTERACTIONS

The first step of cell-matrix interaction, cell adhesion, can be mediated by various short peptide motifs, which mimic ligands on molecules abundant in natural ECM. It is particularly attractive to incorporate these motifs into self-assembling peptides, because this can be done as part of the synthesis process. Sequences derived from fibronectin include the integrin-binding tripeptide RGD (Ruoslahti, 1996), REDV (Massia and Hubbell, 1992), PHSRN (Aota *et al*., 1994), or KNEED (Wong *et al*., 2002). Similar motifs can be found in laminin (Yamada and Kleinman, 1992) and collagen (Bhatnagar *et al*., 1997). Other highly interesting sequences include short heparin-binding sequences, which can be utilized to link growth factors to the matrix. Heparin, a highly negatively charged glycosaminoglycan, can bind growth factors in the ECM, protect them from rapid degradation, and release them slowly in response to cell-mediated matrix degradation. Consensus heparin-binding sequences have been described as XBBXBX or XBBBXXBX (where X is a hydrophobic amino acid and B a basic amino acid residue) (Cardin and Weintraub, 1989). With this indirect mechanism of binding, various growth factors can be incorporated into scaffolding systems. FGF-2 or TGFβ 1 might be particularly interesting for the work with dental stem cells, since the former mainly stimulates cell proliferation, while the latter affects cell morphology and differentiation toward an odontoblast-like phenotype 100 (He *et al*., 2008). Heparin-mediated incorporation or growth factors can furthermore be utilized to address another highly important aspect in tissue engineering, namely, the supply of implanted cells with nutrients through new blood vessel formation and connection to the existing vascular network. Stimulation of vasculogenesis *via* heparin-bound vascular endothelial growth factor (VEGF) in self-assembling peptides has been demonstrated in *in vivo* applications (Rajangam *et al*., 2006, 2008).

VISCOELASTIC PROPERTIES

In living tissues, the elastic moduli span several orders of magnitude, ranging from 100 Pa in the brain to 950 kPa in cartilage or tendon (Levental *et al*., 2007). *In vitro* experiments show that cell adhesion, morphology, and gene expression profiles change on chemically equivalent surfaces with different rigidities (Nemir and West, 2010). In general, cells tend to migrate from softer to stiffer environments (Wells, 2008). Whereas they appear most motile at intermediate stiffness, increased matrix moduli generally stimulate cellular differentiation, where the optimum has to be established individually for each cell type. Having a library of MDP's with a range of matrix moduli would be advantageous, since this might provide another means of controlling cell behavior. Previous work in Dr. Hartgerink's laboratory demonstrated how the mechanical properties of MDP hydrogels can be varied through the modification of the peptides' chemical functionality (Aulisa *et al*., 2009). Relatively minor changes in fiber surface chemistry (changing amino acid residues in the central block motif and/or the flanking region) resulted in changes in matrix rigidities. Highest moduli were achieved by the induction of covalent bond formation *via* cysteine disulfide bonds, which resulted in a 60-fold increase in stiffness. Fine-tuning is furthermore possible by altering the peptide concentration, which can be adjusted between 0.1% and 4% by weight.

Apart from hydrogel stiffness, important aspects for biomedical applications are shear thinning behavior and shear recovery. For the material to be loaded into a syringe and injected through a needle, these characteristics would be highly desirable. Several of the MDP systems offer this feature. Oscillatory rheometry tests confirmed that, after the induction of sheer, the hydrogels undergo sheer recovery, where the storage modulus, a measure for gel stiffness, recovers to nearly 100% of the initial value in less than a minute, which makes these materials ideal candidates for biomedical applications requiring syringe injection.

BIOMINERALIZATION

During the synthesis of dentin and enamel, an organic matrix precedes the mineral produced by ameloblasts and odontoblasts. Non-collagenous proteins play a key role in the mineralization process, where negatively charged surfaces and phosphorylated serine residues attract Ca^{2+}, initiate crystal growth, and control the orientation and elongation of the hydroxyapatite crystals. The main players are called SIBLINGS (small integrin binding ligand, N-linked glycoprotein), which include bone sialoprotein (BSP), osteopontin (OPN), dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), and matrix extracellular phosphoprotein (MEPE). These organic components, commonly found in the matrix of mineralizing tissues, share the presence of the integrin-binding peptide RGD to mediate cell adhesion; they contain multiple serine-rich domains in β-sheet conformation for phosphorylation and display a high density of negative charge to initiate crystal nucleation (George *et al*., 1993; Tye *et al*., 2003). Short peptide sequences derived from DMP-1 have been identified, which display Ca-induced self-assembly into β-sheet structures and provoke hydroxyapatite crystal growth (He *et al*., 2003). Features of these mineralization-inducing peptide motifs can be programmed into scaffolds. Self-assembling peptides offer the advantage that mineralization domains such as phosphorylated serines and acidic domains can be incorporated into the peptide monomers during synthesis. Groundbreaking work demonstrated that peptide-amphiphile molecules can be functionalized by incorporation of phosphorylated serine residues, which facilitates Ca^{2+}-binding and HA crystal nucleation and growth along the fiber long axis (Hartgerink *et al*., 2001). Our previous work demonstrated mineral nucleation along the nanofibers of self-assembling peptides in the presence of dental stem cells (Galler *et al*., 2008). Utilizing these motifs for dental tissue engineering might facilitate the generation of the mineralized component of the dentin-pulp complex.

CONTAMINATION CONTROL

Tissue engineering approaches to regenerate dental pulp will always involve contamination control, since the causes of the loss of dentin and dental pulp, such as caries or trauma, involve the bacterial contamination of these tissues. Whereas local disinfection is important, antibacterial activity can also be programmed into the scaffold material. It is known that certain low-molecular-weight peptides show antibacterial activity (Brennan *et al*., 2006). High-throughput approaches allow for the screening of large numbers of appropriate peptide sequences to identify additional motifs with antibacterial activity. Peptide hydrogels can incorporate antimicrobial activity, for example, *via* lysine-rich surfaces, which facilitate electrostatic interaction of the peptide with the negatively charged bacterial surface, leading to disruption of the bacterial membrane (Salick *et al*., 2007). An additional means of contamination control might contribute to the elimination of bacteria in the root canal system, a requirement for successful new-tissue generation.

CONCLUSION AND FUTURE PERSPECTIVE

Self-assembling peptide systems are a promising class of biomaterials for tissue engineering, since they allow for a 'bottom-up' approach of generating ECM-like materials, which offer high control at the molecular level and produce injectable materials ideally suited for small defects. They are an example of a tunable matrix, where features such as (1) matrix modulus, (2) sheer recovery, (3) cell adhesion motifs, (4) enzyme-cleavable sites for cell-mediated degradation, (5) controlled release of bioactive molecules, (6) mineral nucleation, and (7) antibacterial activity can be incorporated into the scaffold, thus making it a versatile, tailor-made matrix which could be highly useful for dentin-pulp engineering. With the recent results from transplantation experiments, optimized scaffolding systems might contribute toward the development of therapeutic strategies for regenerative endodontic approaches in the near future.

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