REVIEW ARTICLE

Scaffolds for dental pulp tissue regeneration: A review

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Abstract

A key of success in tissue regeneration is the use of a suitable scaffold either to carry specialized cells *ex vivo* or to orchestrate and differentiate the homing of endogenous cells *in vivo*. This review aims to elucidate the materials that have been studied for dental pulp tissue regeneration/engineering and summarize their properties, advantages, and disadvantages. PubMed databases were searched for engineering, pulp regeneration, endodontics, and stem cells) without time restrictions. The search was restricted to articles published in English language. When necessary, additional searches for the structure, properties and history of the specific scaffold materials were achieved. Data from clinical, *in vivo* and *in vitro* studies were extracted, classified and reviewed. By providing an overview of possible scaffolds for pulp tissue regeneration, we aim to improve the understanding of the requirements of the clinical application of regenerative endodontics.

Introduction

Pulp tissue regeneration may present an ideal alternative treatment to traditional root canal therapy. The present concept of pulp tissue regeneration includes two possible approaches.[1] The first is revascularization, where a new pulp tissue is expected to grow into the root canals from the remaining tissues exist apically in the root canal.[2] The second includes the replacement of the diseased pulp with a healthy tissue that is able to revitalize the tooth and restore dentin formation process. The stem cell therapy, gene therapy, three-dimensional (3D) cell printing, scaffold implantation, and pulp implantation are suggested for this approach.[3]

In tissue engineering, the selection of a suitable scaffold is critical. Scaffolds can be identified as biocompatible structures that support cells growth and provide a suitable environment for tissue formation. Good scaffolds should allow cell attachment, proliferation, migration, differentiation, and provide mechanical support for the extracellular matrix generation.[4,5] Ideally, scaffolds must be biodegradable as a native tissue and should degrade in a controlled manner which is consistent with the formation of the new tissue.[6,7] Conductivity, suitable porosity, sterilizability, and economic cost are other properties to be considered for scaffolds.[8,9]

Scaffolds can be classified as artificial (synthetic) or natural. Natural scaffolds are usually more biocompatible and have the advantage of providing specific cell interactions.[10] However, they have the shortcomings of difficulty in obtaining in large amounts, the large batch to batch variations, limited design ability and the lack of good mechanical properties. In contrast, synthetic-derived scaffolds have good reproducible mechanical properties and controlled degradation time, but they lack the presence of cellular signals required for tissue engineering.[10]

Scaffold for Pulp Tissue Regeneration

Scaffold for revascularization of immature permanent teeth

Root canal revascularization procedure aims to restore the blood supply of necrotic pulp tissues of permanent immature teeth.[2] Some researchers indicate revascularization as a regenerative approaches[3] others considered pulp regeneration is incomplete if restricted only to revascularization and should include other significant events such as alignment of odontoblasts on

the dentin surface, and generation of the different types of dental pulp nerve fibers (i.e., nociceptive, sympathetic, and parasympathetic)[11] The revascularization technique depends on the induction of bleeding through the open apical foramen intra the chemically cleaned canal. The canal dentin and the blood clot[2] provide scaffolds in the root canal revascularization. More recently, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are suggested as further possible scaffolds.[12] Table 1 shows materials used for dental pulp revascularization by different researchers.

Blood clot

The utilization of a blood clot to regenerate dental pulp tissues was first practiced by Ostby and resulted in a growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals.[13] In 1974, Myers and Fountain[14] succeeded to generate 0.1-1.0 mm of soft connective tissues into the root canal using blood clots. Later on, successful clinical landmark cases of pulp tissue revascularization were reported.[2,15] Currently, there are growing shreds of evidence of the success of blood clot revascularization procedure for pulp tissue regeneration in immature teeth. Since it is believed that tissues are not able to grow into empty spaces with the absence of suitable scaffolds,[16] it can be suggested that blood clots yield good scaffolds to fill intracanal spaces and aid the growth of new tissues.[17]

The blood clot consists of fibrin matrix that traps cells necessary for tissue regeneration.[1] It also provides a suitable pathway for cells from the periapical area including macrophages and fibroblasts to migrate into the root canal and enhance the new tissue growth.[17,18] The rich content of growth factors allows the blood clot to play an important role in cell differentiation[18,19] and thus, promotion of tissue regeneration.[20] These growth factors include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and platelet-derived epithelial growth factor, known also as vascular permeability factor.[21,22]

The limitation of the blood clot as a scaffold for pulp regeneration comes from the fact that the composition of a clot is variable. The concentrations of cells trapped in a clot might differ leading to unpredictable outcomes. Therefore, using PRP as a controlled scaffold to replace the blood clot in tissue regeneration was suggested.[12,23,24]

PRP and PRF

The PRP was introduced to dentistry world in 1997 by Whitman.[25] Since then it was widely used to promote wound healing after oral maxillofacial, implant, and endodontic surgery.[23,25-29] Because it is rich with important growth factors, PRP has been nominated as a scaffold for pulp tissue regeneration.[12,30,31] The revascularization of pulp-like tissue in necrotic immature teeth using PRP was reported.[32,33] It was suggested that PRP is able to attract stem cells from surrounding periapical tissues.[12] When PRP is combined with dental pulp cells, increased vital tissue regeneration was observed in root canals of dogs' immature teeth.[34]

Some of the growth factors and cytokines found in platelets are transforming growth factor-β (TGF-β) 1 and 2, PDGF, VEGF, epidermal growth factor, fibroblast growth factor, insulin-like growth factor-2 (IGF-2), IGF-1, keratinocyte growth factor, interleukin-8, and connective tissue growth factor.[35,36] These factors have the ability to enhance wound healing and stimulate matrix remodeling and angiogenesis.[37] In addition, they have a role in controlling local inflammatory response and promoting cell proliferation and differentiation during osteogenesis.[38]

To prepare PRP, blood is extracted, collected with anticoagulant and immediately centrifuged for a variable time which is completed within an hour. By first centrifugation process, blood is separated into three layers, the bottom is red blood cells, the second is a buffy coat layer and the supernatant layer which is acellular plasma known as platelet-poor plasma.

Table 1: Materials used for dental pulp revascularization by different researchers

Materials	Researcher	Results
Blood clot	Ostby, 1961[13]	Growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals
	Myers and Fountain, 1974[14]	Generation of soft connective tissues into the root canal
	Banchs and Trope, 2004[2]	Successful revascularization of immature permanent teeth with apical periodontitis
	Iwaya *et al*., 2001[15]	Successful revascularization of an immature permanent tooth with apical periodontitis and sinus tract
	Thibodeau and Trope, 2007[17]	Successful pulp revascularization of a necrotic infected immature permanent tooth associated with swelling
	Ding *et al*., 2009[31]	Exhibit complete root development, with a positive response to pulp testing
Platelet-rich plasma	Torabinejad and Turman, 2011[12]	Regeneration of vital tissues in a tooth with necrotic pulp and a periapical lesion
	Bezgin *et al*., 2014[32]	Concentrated PRP resulted in narrowing of the apical foramen and convergence of the apical walls in the treated teeth
Platelet-rich fibrin	Jadhav *et al*., 2015[33]	Periapical healing, apical closure, root lengthening and dentinal wall thickening Revascularization of an immature, non-vital permanent tooth with apical periodontitis
	Fakhr Tabatabayi *et al*., 2015[43]	Resolution of periapical lesion, root development and apical closure of necrotic immature tooth

The following steps differ between protocols but always aim to collect the buffy coat layer and discard the other layers.

Clinically, the ability of PRP to create vital tissue in the root canal is relatively faster.[12] PRP clots into the root canal within 5 min.[12] It is faster than blood which needs about 15 min to clot.[2,17] In addition, PRP is easier to apply, harder, and more suitable for the subsequent placement of mineral trioxide aggregate and permanent restorations.[12] Unlike blood clot procedure, anesthesia is not necessary for PRP application since periapical bleeding is not indicated. PRP has an additional value in patients where bleeding into root canal cannot be established.[31] However, blood extraction from young patients and the need of additional equipment are the main disadvantages of PRP procedure.

Currently, PRP is referred as a first-generation platelet concentrate, and the PRF is known as a second-generation platelet concentrate.[39] PRF was developed first by Choukroun *et al.* (2001)[39] It has the benefit of slow release of growth factors for a prolonged period of 7-14 days. Hence, it is superior to PRP which shows fast release growth factors in 7-14 h.[40] Successful pulp revitalization cases using PRF were reported.[41-43]

Dentin

The root canal space is wholly enclosed by acellular dentin matrix rich with growth factors.[44,45] Some of them are Growth hormone,[46] IGF-1 and -2,[47-49] bone morphogenetic protein-2 (BMP-2), -4 and -6,[50] and TGF-β-1, -2 and -3.[45,51,52] When release from dentin matrix, these growth factors play a key role in regulation the inflammatory response, tissue healing and regeneration and odontoblast differentiation.[45,47,53] The release of growth factors can be enhanced if dentin is treated with chelating agents such as an ethylene diamine tetraacetic acid (EDTA). Although it was reported that removal of the whole smear layer from the root canal walls is with a less value for cell attachment,[54] it is believed that dentin treated with EDTA releases some growth factors necessary to stimulate dentin regeneration such as TGF-β.[45,55] EDTA-treated dentin was able to induce the regeneration of complete dentin tissues *in vivo*.[45,56]

Possible scaffolds for pulp tissue regeneration [Table 2]

Synthetic polymers

Synthetic biodegradable polymers, such as polyglycolic acid (PGA), polylactic acid (PLA) and poly-lactic-coglycolide, have been initially approved by the Food and Drug Administration (FDA) as drug delivery systems.[57,58] The application of these polymers as matrices for cell transplantation was the first suggested by Vacanti *et al.*[59] Their biocompatibility and a broad range of reproducibility make them attractive for tissue engineering studies.[60-63] Polymers scaffold shape, porosity, mechanical properties, pores diameter, and degradation time can be successfully controlled in the preparation techniques.[64,65] Degradation of synthetic polymers are generally occurred by simple hydrolysis.[66] Table 3 shows some synthetic polymers used previously for dental pulp regeneration.

The first clinical use of PGA was as an absorbable suture.[67] It was also used in head and neck surgery as an implant for bone regeneration, cartilage repair,[68] cartilage reconstruction,[69] and as a potential membrane for periodontal therapy.[70] PGA is considered an immunologically inert material although migration of inflammatory monocytes were observed following PGA implant.[71] No infection or reaction of a foreign body were observed following using PGA pins to fix displaced elbow fractures in children.[72]

The first attempt for pulp tissue engineering *in vitro* was achieved using PGA with human pulpal fibroblasts. A new tissue-like construct with similar cellularity as in normal pulp tissue could be observed.[73] When compared to alginate and a Type I collagen hydrogel, PGA was more conducive for cell proliferation.[62] PGA scaffold enhanced the growth of new blood vessels and the odontogenic differentiation of human fibroblasts when cultured on it.[74] However, collagen sponge scaffold was found to be superior to PGA *in vitro* and *in vivo* for tooth-tissue engineering purposes using porcine dental pulp cells.[75]

Similarly, the poly-L-lactic acid (PLLA) polymer is a widely-used, FDA-approved biodegradable polymer.[76,77] PLLA scaffold was able to produce tissue similar in architecture and cellularity to dental pulp tissue when transplanted with human dermal microvascular endothelial cells,[78] or stem cells in human exfoliated deciduous teeth (SHED)[79] into immunodeficient

Table 2: Possible scaffolds for dental pulp regeneration

Classification	Scaffold
Synthetic polymers	PGA
	PLA
	PLLA
	PLGA
	OPLA
	PGA/PLLA
Bioactive ceramics	HA
	β-TCP
	BCP
BG	Silicate bioactive glass
	borate and borosilicate glass
Naturally derived scaffold	Fibrin
	Collagen
	HYA sponge
	Amniotic membrane
	Polysaccharides (chitin, chitosan, cellulose, alginate, agar, pectins, dextran and glycosaminoglycans)

PGA: Polyglycolic acid, PLA: Poly-lactic acid, PLLA: Poly-L-lactic acid, PLGA: Poly-lactic-coglycolide, OPLA: Open-cell polylactic acid, HA: Hydroxyapatite, β-TCP: Beta-tricalcium phosphate, BCP: Biphasic calcium phosphate, HYA: Hyaluronic acid, BG: Bioactive glass

Table 3: Synthetic polymers used for dental pulp regeneration by different researchers

Scaffold	Researcher	Results
PGA	Mooney *et al*., 1996[73]	A new tissue-like construct with similar cellularity as in normal pulp tissue
	Buurma *et al*., 1999[74]	Growth of new blood vessels and odontogenic differentiation of human fibroblasts
	Bohl *et al*., 1998[62]	Growth of dental pulp-like tissues with a very high cell density and significant collagen deposition
PLLA	Nör *et al*., 2001[78]	Growth of tissue similar in architecture and cellularity to dental pulp tissue
	Sakai *et al*., 2004[79]	Differentiate of stem cells seeded on PLLA into angiogenic endothelial cells and odontoblasts capable of generating tubular dentin
OPLA	Gotlieb *et al*., 2008[82]	Stem cells seeded on OPLA and transplanted into cleaned and shaped canals of human extracted teeth were able to attach to the root canal dentin
PGA/PLLA	Young *et al*., 2002[80]	Regeneration of a tooth crown contained enamel, dentin and a well-defined pulp chamber
	Duailibi *et al*., 2008[81]	Engineering of tooth crowns, containing dentin, enamel, pulp, and periodontal ligament tissues

mice. A PGA/PLLA scaffold was also successful for the regeneration of a tooth crown contained enamel, dentin and a well-defined pulp chamber using cells isolated from tooth buds and transplanted into rat omentum.[80] When similar constructs were transplanted into rat jaws, periodontal ligament tissues could be observed around the generated crown.[81] Finally, the synthetic open-cell PLA (OPLA) is another promising polymer for dental pulp regeneration. SHED seeded on OPLA and transplanted into cleaned, and shaped canals of human extracted teeth were able to attach to the root canal dentin.[82]

Bioactive ceramics

Calcium phosphate ceramics such as hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP) are totally biocompatible and bioactive crystallized materials.[83,84] These bioactive ceramics were widely used as bone graft materials and showed a great ability to form a strong direct bond with the host bone.[85] Cells cultured on the porous form of ceramics could attach, proliferate, and expressed dentin sialophosphoprotein, which is a dentin marker.[86]

HA [(Ca10 (PO4)6(OH)2] has been suggested as an effective scaffold for regeneration of dentin and dentin-pulp complex.[87-90] HA is a non-biodegradable ceramic while β-TCP [β-TCP Ca3(PO4)2] is considered a biodegradable.[91] However, the mechanical properties of TCP are inferior to those of HA. BCP has been developed from HA and TCP to display the advantages of the both ceramics.[92,93] BCP was widely investigated as a possible scaffold for pulp and dentin tissue regeneration. When pulp-derived cells were mixed with HA or HA/TCP and transplanted subcutaneously in nude mice, bone and dentin-like mineralized tissues were generated.[92,94,95] Moreover, the formation of dentin bridge after pulp capping in pig teeth model was stimulated using pulp cells seeded on HA/TCP scaffold.[96]

Another combination of a polymer [poly (D,L-lactide-co-glycolide)] and a ceramic (β-TCP) was also tested.[97] The resulted scaffold showed excellent mechanical and physicochemical properties and was ideal when seeded with osteoblast-like cells for 3D bone regeneration. One of the good advantages of this scaffold was that the non-porous scaffold would become porous in the *in vivo* conditions after implantation.[97] However, to the best of our knowledge, the advantages of the combination of a polymer and a ceramic for pulp tissue regeneration have not been tested yet.

Bioactive glass (BG)

BG is a group of synthesized surface reactive biomaterials that have an amorphous structure and high mechanical strength.[84] It has been investigated extensively for the use as implant materials in the human body to repair and replace diseased or damaged bone. BG is able to support osteogenesis[98] and its ability to support pro-angiogenesis has been reported recently.[99] This suggests the application of BG in soft tissue repairing and engineering.[84] Silicate BG (known by its commercial name: Bioglass) has been traditionally used in BG researches.[100] However, for tissue engineering purposes, new BGs based on borate and borosilicate compositions have been suggested,[101-103] the biocompatibility and controllable degradation rate of these new glass scaffolds have been reported.[104,105] When degrades, BG will be converted into an HA-like substance that is able to bond to soft and hard tissues. The degradation also releases ions that contribute in osteogenesis and angiogenesis.[106,107] It is well recognized that odontogenesis pathway is very similar to osteogenesis pathway and that odontogenesis and angiogenesis are essential for the successful generation of the dentin-pulp complex. Taken together, all these facts suggest that using BG as a scaffold for dentin and dental pulp engineering might be promising.

Naturally derived scaffold

The biological properties of natural extracellular matrix (ECM) actively contribute in regeneration processes of damaged tissues. This makes ECM attractive as scaffolds for tissue engineering.[108] Some examples of natural ECM scaffolds are Fibrin,[109] collagen,[110] hyaluronic acid (HYA) sponge,[111] amniotic membrane (AM),[112] and many types of polysaccharides.[113] Table 4 shows some naturally derived scaffold used for dental pulp regeneration in previous studies.

Fibrin was first used in 1909 to induce hemostasis.[114] Subsequently, fibrin sealants, also known as

Table 4: Naturally derived scaffold used for dental pulp regeneration by different researchers

Naturally derived scaffold	Researcher	Results
Collagen	Zhang *et al.*, 2006[86]	Collagen scaffold allowed stem cells attachment and proliferation, and stimulated the odontogenic differentiation of *in vitro* but not *in vivo*
	Gotlieb *et al.*, 2008[82]	Stem cells seeded on collagen and transferred into prepared root canals attached to canal walls
	Inuyama *et al.*, 2010[111]	*In vitro*, dental pulp cells adhered to collagen scaffold *In vivo*, dental pulp proliferation and vessel invasion were observed
HAM	Alshehadat *et al.*, 2014[132]	HAM provided a suitable environment for stem cell attachment, proliferation and odontogenic differentiation
Glycol chitin-based hydrogel	Park *et al.*, 2013[138]	Promoted the odontogenic differentiation of human dental pulp cells
Chitosan (monomer)	Matsunaga *et al.*, 2006[142]	Increased the ALP activity and BMP2 gene expression significantly (*in vitro*). Promoted the proliferation of pulp fibroblasts and induces mineralization by odontoblast-like cells (*in vivo*)
Alginate	Dobie *et al.*, 2002[153]	Induced the differentiation of pulp cells into odontoblast-like cells and secreted tubular dentin matrix
	Kumabe *et al.*, 2006[154]	Induced the differentiation of pulp cells into functional odontoblast-like cells
Agar	Kikuchi *et al.*, 1996[157]	Induced the differentiation of dental mesenchymal cells into functional odontoblast-like cells
	Kikuchi *et al.*, 1996[158]	Induced the differentiation of dental mesenchymal cells into tubular matrix-forming cells
HYA	Sasaki and Kawamata-Kido, 1995[171]	Stimulated the formation of reparative dentin after dental pulp capping by inducing the differentiation of mesenchymal cells of the amputated pulp
	Inuyama *et al.*, 2010[111]	*In vitro*, cells adhered to HYA scaffold. *In vivo*, cell-rich reorganizing new tissue was observed near the HYA scaffold

HAM: Human amniotic membrane, HYA: Hyaluronic acid, ALP: Alkaline phosphatase, BMP2: Bone morphogenetic protein-2

"fibrin tissue adhesives" or "fibrin glues," were developed and used to seal tissues together and to control bleeding.[115] The fibrin-rich network was placed at the site of tissue defects after trauma and participated effectively in the regeneration process by attracting different cell types including the inflammatory cells.[116] 3D fibrin gel was useful for tissue engineering as it allowed the homogenous growth of fibroblasts and the production of confluent collagen.[109] In addition, native fibrin-based matrices provided a good substrate for endothelial cells and thus, could be considered for angiogenesis.[117] Since successful angiogenesis is critical for the success connective tissue engineering,[118] using fibrin-based scaffold might help in pulp tissue regeneration and engineering. However, additional studies are required to confirm this suggestion.

Although the pulpal extracellular matrix consists mainly of fibrillar collagen (Type I and Type III) which is similar in composition to other soft tissue collagen,[119] However, the two types did not provide a suitable environment for dental pulp cell growth.[120] The spongeous collagen scaffold allowed stem cells attachment and proliferation, and stimulated the cells to express odontoblast markers *in vitro*, but the *in vivo* evaluation experiment showed that the scaffold gave rise to form connective tissues more than dentin-like tissues.[86] In an interesting study,[82] SHED were cultured on a collagen scaffold and transplanted into cleaned and shaped canals of human extracted teeth placed in Dulbecco's Modified Eagle's Medium culture medium. The SEM observation showed that SHED had attached to canal walls which indicated that using collagen scaffold as a stem cell carries might be promising. However, additional *in vivo* studies for the behavior and fate of cells are still required.

AM is the innermost layer of the placenta. It consists of a single epithelial layer, a thick basement membrane and a stromal which has no blood vessels or nerves. AM was used successfully for a long time as a dressing in wound areas,[121] to treat ocular disorders and diseases, and for ocular surface reconstruction.[122] In oral and maxillofacial surgery, AM was nominated for guided bone regeneration,[123] to accelerate the gingival epithelialization and vascularization in the gingival wound,[124] to close the oronasal fistula,[125] and to prevent ankylosis of temporomandibular joint when used as interposition graft material.[126]

The high biocompatibility[127] of human AM (HAM), in addition to its low immunogenicity[128] and the presence of several growth factors[129] recommend it to be used as a tissue engineering scaffold.[112] HAM was found to support the chondrocyte attachment and proliferation,[130] to get mineralized in osteogenic media and thud, has the potential for bone tissue regeneration.[131]

The structure of HAM meets with dental pulp tissue in several points. First, both of them are soft connective tissues. Second, the presence of monolayer cells in the peripheral zone

of both of them, i.e., epithelial cells in HAM and odontoblasts in pulp tissue. This gives a notable distinguished similarity. HAM was evaluated as a natural scaffold for dental pulp tissue regeneration *in vitro* in our Dental School. The preliminary results[132] showed that both sides of denuded HAM, i.e. basement and stroma, provided a suitable environment for SHED attachment, proliferation, and spread. SHED cultured on HAM also showed high expression of some of the odontoblast specific markers with a low level of inflammatory gene expressions.

Polysaccharides are long carbohydrate molecules that present promising materials for tissue engineering due to their availability, biocompatibility and good biological properties.[133] Some examples of polysaccharides used in tissue engineering are chitin, chitosan, cellulose, alginate, agar, pectins, dextran and glycosaminoglycans. Polysaccharides have been applied in various forms and have been tested for engineering of a lot of tissues including blood vessels, bone, cartilage, neural tissue and others.[134-137]

Chitin is a long-chain polymer of N-acetylglucosamine found in cell walls of fungi, shells of crabs and shrimps, and exoskeletons of insects. Studies on chitin as a tissue engineering material are rare. However, in a recent study, a biodegradable porous glycol chitin-based thermoresponsive hydrogel scaffold was found to promote the odontogenic differentiation of human dental pulp cells, and thus it may provide a promising material for pulp and dentin regeneration.[138]

Unlike chitin, chitosan, which is the deacetylated form of chitin, has been widely tested as a tissue engineering material. Chitosan has been used as a root canal dressing in teeth with periapical lesions because of its antibacterial properties.[139] Other pharmacological effects such as antihypertensive, wound healing promotion and serum cholesterol lowering were reported.[140,141] Chitosan has been suggested as a scaffold for dental pulp regeneration. In cell culture works, treated the culture media with chitosan monomer (D-glucosamine hydrochloride) resulted in a significant increase of alkaline phosphatase activity and BMP-2 gene expression of osteoblasts. When used as a direct pulp capping in rats, the chitosan promoted the proliferation of pulp fibroblasts and induced mineralization by odontoblast-like cells.[142]

Cellulose is a polysaccharide consists of a linear chain of β (1→4) linked D-glucose units. It is found in the cellular walls of green plants and can be synthesized by fungi and some bacteria. Bacterial cellulose is preferable for tissue engineering because it is more biocompatible and has better mechanical properties.[133] Cellulose scaffold can be used in the form of porous membranes,[143] electrospun porous microfibrous 3D scaffolds[144] and microporous scaffolds.[145] Cellulose scaffolds have an angiogenic effect[146] and provided a suitable matrix for a human saphenous vein to attach and proliferate.[147] When combined with chitosan, microfibrous cellulose acetate scaffolds improved the formation of capillary tube-like structures *in vitro*.[148] Therefore, cellulose scaffolds are promising for dental pulp revascularization and regeneration.

Alginate is a biocompatible natural polysaccharide with relatively poor mechanical properties.[149] It has been applied for tissue regeneration researches in the form of injectable hydrogels,[150] porous scaffolds,[151] and electrospun nanofibrous scaffolds.[152] The stiffness and mechanical strength of alginate could be improved by increasing calcium content and cross-linking density.[149] For dentin-pulp regeneration, alginate coated with TGF-β or treated with acid were able to induce the pulp cells differentiated into odontoblast-like cells and secreted tubular dentin matrix.[153] Similar findings were reported when subcultured human dental pulp cells in an alginate scaffold were transplanted subcutaneously into the backs of nude mice.[154]

Agar is a jelly-like substance discovered in the 17th in Japan. It is derived from the polysaccharide agarose, which forms the supporting structure in the cell walls of certain species of algae and released on boiling.[155] Agar has been used in dentistry as an impression material and as a solid substrate to contain culture media in which microorganisms can grow.[156] The differentiation of dental mesenchymal cells into functional odontoblast-like cells was observed on the agar surface.[157] Similar finding was reported when the culture media was treated with EDTA-soluble dentin components, TGF-β1, BMP-2 combined with heparin or fibronectin, or the activin-binding protein follistatin.[47,158]

Dextran is a complex polysaccharide used as an antithrombotic (antiplatelet) to reduce blood viscosity, or as a volume expander in hypovolemia.[159] It is synthesized from sucrose by certain lactic acid bacteria. In tissue regeneration researches, heparan-like polymers prepared from dextran, named RGTA, were found to stimulate bone regeneration in different models of bone defects. This is mainly because they have the ability to promote the differentiation of bone-forming alone or in association with heparin-binding growth factors.[160] Properties of dextrans as tissue scaffolds were developed when gelatin-based biomaterials were added to form hydrogels. These hydrogels can release their cargo of proteins in a controlled manner and may provide effective levels of growth factors during all stages of tissue regeneration.[161,162] A hydrogel scaffold fabricated from dextran (Dex-GMA)/gelatin and loaded with BMP growth factors was able to enhance the periodontal tissue regeneration in a dog model.[77]

HYA is one of the major glycosaminoglycans in the extracellular matrix[163,164] of the new developed dentin and pulp tissues.[165] HYA sponge is a good potential scaffold for tissue engineering because it can be modified structurally and chemically for a wide range of applications.[166-170] HYA stimulates the formation of reparative dentin after dental pulp capping by inducing the differentiation of mesenchymal cells of the amputated pulp.[171] The biocompatible structure and biodegradation rate of HYA have suggested it as a suitable scaffold for blood vessel proliferation and dental pulp regeneration.[111] Interestingly, HYA was added to β-TCP and used as a graft material after periradicular surgery in a dog model. However, this combination did not lead to extra improvement in the bone

tissue healing.[172] To the best of our knowledge, the effects of the 3D HYA sponge on the regeneration of dental pulp tissue has not been detected yet.

Conclusion

Several scaffolds have been found to be attractive for dental pulp regeneration. Traditional inert scaffolds that are merely passive cell carriers have shifted into sophisticated and inductive matrices with controlled material behavior. Each type of the scaffolds has its own properties which may match one proposed regenerative procedure more than the others. Optimal scaffolds with novel biomimetic properties are still needed to provide an adequate environment with appropriate morphogenic signaling for responsive stem/progenitor cells that will develop into a complete functional dental pulp. It is still challenging to fully understand these properties and realize how they can be integrated to the clinical procedures of the dental pulp regeneration. We hope that this review article will provoke novel ideas of using specific scaffold in a specific regenerative technique that will yield the desired pulp-dentin complex.

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References

1. Bansal R, Bansal R. Regenerative endodontics: A state of the art. Indian J Dent Res 2011;22:122-31.
2. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 2004;30:196-200.
3. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. J Endod 2007;33:377-90.
4. Goldberg M, Septier D, Bourd K, Menashi S. Role of matrix proteins in signalling and in dentin and enamel mineralisation. Comptes Rendus Palevol 2004;3:573-81.
5. Du C, Moradian-Oldak J. Tooth regeneration: Challenges and opportunities for biomedical material research. Biomed Mater 2006;1:R10-7.
6. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J 2001;10 Suppl 2:S96-101.
7. Kretlow JD, Klouda L, Mikos AG. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007;59:263-73.
8. Slaughter A, Evans LK. Culturally sensitive oral health educational materials for older African Americans. J Health Care Poor Underserved 2007;18:868-86.
9. Yen AH, Sharpe PT. Stem cells and tooth tissue engineering. Cell Tissue Res 2008;331:359-72.
10. Kim BS, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol 1998;16:224-30.
11. Spångberg LS. The Emperor's new cloth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:643-4.
12. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report. J Endod 2011;37:265-8.
13. Ostby BN. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol Scand 1961;19:324-53.
14. Myers WC, Fountain SB. Dental pulp regeneration aided by blood and blood substitutes after experimentally induced periapical infection. Oral Surg Oral Med Oral Pathol 1974;37:441-50.
15. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001;17:185-7.
16. Torneck CD. Reaction of rat connective tissue to polyethylene tube implants. I. Oral Surg Oral Med Oral Pathol 1966;21:379-87.
17. Thibodeau B, Trope M. Pulp revascularization of a necrotic infected immature permanent tooth: Case report and review of the literature. Pediatr Dent 2007;29:47-50.
18. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery. I. The incisional wound. J Endod 1991;17:425-35.
19. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489-96.
20. Saber SE. Tissue engineering in endodontics. J Oral Sci 2009;51:495-507.
21. Seppä H, Grotendorst G, Seppä S, Schiffmann E, Martin GR. Platelet-derived growth factor in chemotactic for fibroblasts. J Cell Biol 1982;92:584-8.
22. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: Implications for measurement of circulating VEGF levels in clinical disease. Clin Sci (Lond) 1998;94:395-404.
23. Wiltfang J, Schlegel KA, Schultze-Mosgau S, Nkenke E, Zimmermann R, Kessler P. Sinus floor augmentation with beta-tricalciumphosphate (beta-TCP): Does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Implants Res 2003;14:213-8.
24. Llames SG, Del Rio M, Larcher F, García E, García M, Escamez MJ, *et al.* Human plasma as a dermal scaffold for the generation of a completely autologous bioengineered skin. Transplantation 2004;77:350-5.
25. Freymiller EG, Aghaloo TL. Platelet-rich plasma: Ready or not? J Oral Maxillofac Surg 2004;62:484-8.
26. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The *in vitro* effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res 2006;17:212-9.
27. Anitua E. Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999;14:529-35.
28. Demiralp B, Keçeli HG, Muhtarogullar M, Serper A, Demiralp B, Eratalay K. Treatment of periapical inflammatory lesion with the combination of platelet-rich plasma and tricalcium phosphate: A case report. J Endod 2004;30:796-800.
29. Ito K, Yamada Y, Nagasaka T, Baba S, Ueda M. Osteogenic potential of injectable tissue-engineered bone: A comparison among autogenous bone, bone substitute (Bio-Oss®), platelet-

rich plasma, and tissue-engineered bone with respect to their mechanical properties and histological findings. J Biomed Mater Res Part A 2005;73:63-72.
30. Hargreaves KM, Giesler T, Henry M, Wang Y. Regeneration potential of the young permanent tooth: What does the future hold? J Endod 2008;34 7 Suppl:S51-6.
31. Ding RY, Cheung GS, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: A clinical study. J Endod 2009;35:745-9.
32. Bezgin T, Yilmaz AD, Celik BN, Sönmez H. Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. Int Endod J 2014;47:41-9.
33. Jadhav GR, Shah D, Raghvendra SS. Autologus Platelet Rich Fibrin aided Revascularization of an immature, non-vital permanent tooth with apical periodontitis: A case report. J Nat Sci Biol Med 2015;6:224-5.
34. Zhu W, Zhu X, Huang GT, Cheung GS, Dissanayaka WL, Zhang C. Regeneration of dental pulp tissue in immature teeth with apical periodontitis using platelet-rich plasma and dental pulp cells. Int Endod J 2013;46:962-70.
35. Yu W, Wang J, Yin J. Platelet-rich plasma: A promising product for treatment of peripheral nerve regeneration after nerve injury. Int J Neurosci 2011;121:176-80.
36. Borrione P, Gianfrancesco AD, Pereira MT, Pigozzi F. Platelet-rich plasma in muscle healing. Am J Phys Med Rehabil 2010;89:854-61.
37. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158-67.
38. Hiremath H, Gada N, Kini Y, Kulkarni S, Yakub SS, Metgud S. Single-step apical barrier placement in immature teeth using mineral trioxide aggregate and management of periapical inflammatory lesion using platelet-rich plasma and hydroxyapatite. J Endod 2008;34:1020-4.
39. Keswani D, Pandey RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: A case report. Int Endod J 2013;46:1096-104.
40. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts *in vitro*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:707-13.
41. Shivashankar VY, Johns DA, Vidyanath S, Kumar MR. Platelet Rich Fibrin in the revitalization of tooth with necrotic pulp and open apex. J Conserv Dent 2012;15:395-8.
42. Mishra N, Narang I, Mittal N. Platelet-rich fibrin-mediated revitalization of immature necrotic tooth. Contemp Clin Dent 2013;4:412-5.
43. Fakhr Tabatabayi S, Bakhtiar H, Vatanpour M, Honarvar M. Apical closure in a necrotic immature tooth by revascularization therapy using platelet-rich fibrin: A case report. J Res Dent Sci 2015;11:242-6.
44. Tziafas D, Lambrianidis T, Beltes P. Inductive effect of native dentin on the dentinogenic potential of adult dog teeth. J Endod 1993;19:116-22.
45. Guo W, He Y, Zhang X, Lu W, Wang C, Yu H, *et al.* The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. Biomaterials 2009;30:6708-23.
46. Zhang CZ, Li H, Young WG, Bartold PM, Chen C, Waters MJ. Evidence for a local action of growth hormone in embryonic tooth development in the rat. Growth Factors 1997;14:131-43.
47. Bègue-Kirn C, Smith AJ, Loriot M, Kupferle C, Ruch JV, Lesot H. Comparative analysis of TGF beta s, BMPs, IGF1, msxs, fibronectin, osteonectin and bone sialoprotein gene expression during normal and *in vitro*-induced odontoblast differentiation. Int J Dev Biol 1994;38:405-20.
48. Cassidy N, Fahey M, Prime SS, Smith AJ. Comparative analysis of transforming growth factor-beta isoforms 1-3 in human and rabbit dentine matrices. Arch Oral Biol 1997;42:219-23.
49. Joseph BK, Savage NW, Daley TJ, Young WG. *In situ* hybridization evidence for a paracrine/autocrine role for insulin-like growth factor-I in tooth development. Growth Factors 1996;13:11-7.
50. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell 1993;75:45-58.
51. Thesleff I, Vaahtokari A. The role of growth factors in determination and differentiation of the odontoblastic cell lineage. Proc Finn Dent Soc 1992;88 Suppl 1:357-68.
52. D'Souza RN, Happonen RP, Ritter NM, Butler WT. Temporal and spatial patterns of transforming growth factor-beta 1 expression in developing rat molars. Arch Oral Biol 1990;35:957-65.
53. Smith AJ, Matthews JB, Hall RC. Transforming growth factor-beta1 (TGF-beta1) in dentine matrix. Ligand activation and receptor expression. Eur J Oral Sci 1998;106 Suppl 1:179-84.
54. Ring KC, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. J Endod 2008;34:1474-9.
55. Smith AJ, Murray PE, Sloan AJ, Matthews JB, Zhao S. Trans-dentinal stimulation of tertiary dentinogenesis. Adv Dent Res 2001;15:51-4.
56. Li Y, Lü X, Sun X, Bai S, Li S, Shi J. Odontoblast-like cell differentiation and dentin formation induced with TGF-ß1. Arch Oral Biol 2011;56:1221-9.
57. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 2000;21:2475-90.
58. Mauth C, Huwig A, Graf-Hausner U, Roulet J. Restorative applications for dental pulp therapy. Top Tissue Eng 2007;3:1-32.
59. Vacanti JP, Morse MA, Saltzman WM, Domb AJ, Perez-Atayde A, Langer R. Selective cell transplantation using bioabsorbable artificial polymers as matrices. J Pediatr Surg 1988;23:3-9.
60. Atala A, Mooney DJ. Synthetic Biodegradable Polymer Scaffolds. Berlin: Springer; 1997.
61. Nakashima M. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. Cytokine Growth Factor Rev 2005;16:369-76.
62. Bohl KS, Shon J, Rutherford B, Mooney DJ. Role of synthetic extracellular matrix in development of engineered dental pulp. J Biomater Sci Polym Ed 1998;9:749-64.
63. Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006;27:3413-31.
64. Mikos AG, Bao Y, Cima LG, Ingber DE, Vacanti JP, Langer R. Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation. J Biomed Mater Res 1993;27:183-9.
65. Ma PX, Zhang R. Microtubular architecture of biodegradable

polymer scaffolds. J Biomed Mater Res 2001;56:469-77.
66. Bakhtiari L, Rezaie HR, Hosseinalipour SM, Shokrgozar MA. Investigation of biphasic calcium phosphate/gelatin nanocomposite scaffolds as a bone tissue engineering. Ceram Int 2010;36:2421-6.
67. Frazza EJ, Schmitt EE. A new absorbable suture. J Biomed Mater Res 1971;5:43-58.
68. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000;21:2529-43.
69. Rotter N, Aigner J, Naumann A, Planck H, Hammer C, Burmester G, *et al.* Cartilage reconstruction in head and neck surgery: Comparison of resorbable polymer scaffolds for tissue engineering of human septal cartilage. J Biomed Mater Res 1998;42:347-56.
70. Berahim Z, Moharamzadeh K, Rawlinson A, Jowett AK. Biologic interaction of three-dimensional periodontal fibroblast spheroids with collagen-based and synthetic membranes. J Periodontol 2011;82:790-7.
71. Santavirta S, Konttinen YT, Saito T, Grönblad M, Partio E, Kemppinen P, *et al.* Immune response to polyglycolic acid implants. J Bone Joint Surg Br 1990;72:597-600.
72. Hope PG, Williamson DM, Coates CJ, Cole WG. Biodegradable pin fixation of elbow fractures in children. A randomised trial. J Bone Joint Surg Br 1991;73:965-8.
73. Mooney DJ, Powell C, Piana J, Rutherford B. Engineering dental pulp-like tissue *in vitro*. Biotechnol Prog 1996;12:865-8.
74. Buurma B, Gu K, Rutherford RB. Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur J Oral Sci 1999;107:282-9.
75. Tonomura A, Mizuno D, Hisada A, Kuno N, Ando Y, Sumita Y, *et al.* Differential effect of scaffold shape on dentin regeneration. Ann Biomed Eng 2010;38:1664-71.
76. Allen KD, Athanasiou KA. Scaffold and growth factor selection in temporomandibular joint disc engineering. J Dent Res 2008;87:180-5.
77. Chen DC, Avansino JR, Agopian VG, Hoagland VD, Woolman JD, Pan S, *et al.* Comparison of polyester scaffolds for bioengineered intestinal mucosa. Cells Tissues Organs 2006;184:154-65.
78. Nör JE, Peters MC, Christensen JB, Sutorik MM, Linn S, Khan MK, *et al.* Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest 2001;81:453-63.
79. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S, *et al.* SHED differentiate into functional odontoblasts and endothelium. J Dent Res 2010;89:791-6.
80. Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J Dent Res 2002;81:695-700.
81. Duailibi SE, Duailibi MT, Zhang W, Asrican R, Vacanti JP, Yelick PC. Bioengineered dental tissues grown in the rat jaw. J Dent Res 2008;87:745-50.
82. Gotlieb EL, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. J Am Dent Assoc 2008;139:457-65.
83. Denissen H, Montanari C, Martinetti R, van Lingen A, van den Hooff A. Alveolar bone response to submerged bisphosphonate-complexed hydroxyapatite implants. J Periodontol 2000;71:279-86.
84. Rahaman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, *et al.* Bioactive glass in tissue engineering. Acta Biomater 2011;7:2355-73.
85. Daculsi G, Weiss P, Bouler JM, Gauthier O, Millot F, Aguado E. Biphasic calcium phosphate/hydrosoluble polymer composites: A new concept for bone and dental substitution biomaterials. Bone 1999;25 2 Suppl:59S-61.
86. Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA. The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials 2006;27:5658-68.
87. Hayakawa S, Li Y, Tsuru K, Osaka A, Fujii E, Kawabata K. Preparation of nanometer-scale rod array of hydroxyapatite crystal. Acta Biomater 2009;5:2152-60.
88. Yoshikawa M, Tsuji N, Shimomura Y, Hayashi H, Ohgushi H, editors. Effects of laminin for osteogenesis in porous hydroxyapatite. Macromol Symp 2007;253:172-8.
89. Ando Y, Honda MJ, Ohshima H, Tonomura A, Ohara T, Itaya T, *et al.* The induction of dentin bridge-like structures by constructs of subcultured dental pulp-derived cells and porous HA/TCP in porcine teeth. Nagoya J Med Sci 2009;71:51-62.
90. Mastrangelo F, Nargi E, Carone L, Dolci M, Caciagli F, Ciccarelli R, *et al.* Tridimensional response of human dental follicular stem cells onto a synthetic hydroxyapatite scaffold. J Health Sci Tokyo 2008;54:154.
91. Li B, Chen X, Guo B, Wang X, Fan H, Zhang X. Fabrication and cellular biocompatibility of porous carbonated biphasic calcium phosphate ceramics with a nanostructure. Acta Biomater 2009;5:134-43.
92. Daculsi G, LeGeros R. Tricalcium phosphate/hydroxyapatite biophasic calcium phosphates. Handbook of Bioceramics and their Clinical Applications. London: Woodhead Publishing Ltd.; 2008. p. 395.
93. Nilen RW, Richter PW. The thermal stability of hydroxyapatite in biphasic calcium phosphate ceramics. J Mater Sci Mater Med 2008;19:1693-702.
94. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, *et al.* Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531-5.
95. Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, *et al.* Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 2003;82:976-81.
96. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, *et al.* Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 2006;1:e79.
97. Letic-Gavrilovic A, Todorovic L, Abe K. Oral tissue engineering of complex tooth structures on biodegradable DLPLG/beta-TCP scaffolds. Adv Exp Med Biol 2004;553:267-81.
98. Rahaman MN, Brown RF, Bal BS, Day DE. Bioactive glasses for nonbearing applications in total joint replacement. Semin Arthroplasty 2006;17:102-12.
99. Gorustovich AA, Roether JA, Boccaccini AR. Effect of bioactive glasses on angiogenesis: A review of *in vitro* and *in vivo* evidences. Tissue Eng Part B Rev 2010;16:199-207.
100. Kim HW, Kim HE, Knowles JC. Production and potential of bioactive glass nanofibers as a next-generation biomaterial. Adv Funct Mater 2006;16:1529-35.
101. Conzone SD, Day DE. Preparation and properties of porous microspheres made from borate glass. J Biomed Mater Res A 2009;88:531-42.

102. Jung SB, Day DE. Conversion kinetics of silicate, borosilicate, and borate bioactive glasses to hydroxyapatite. Phys Chem Glasses Eur J Glass Sci Technol Part B Eur J Glass Sci Technol Part B 2009;50:85-8.
103. Liu X, Pan H, Fu H, Fu Q, Rahaman MN, Huang W. Conversion of borate-based glass scaffold to hydroxyapatite in a dilute phosphate solution. Biomed Mater 2010;5:15005.
104. Fu Q, Rahaman MN, Fu H, Liu X. Silicate, borosilicate, and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and *in vitro* degradation. J Biomed Mater Res A 2010;95:164-71.
105. Bi L, Rahaman MN, Day DE, Brown Z, Samujh C, Liu X, *et al.* Effect of bioactive borate glass microstructure on bone regeneration, angiogenesis, and hydroxyapatite conversion in a rat calvarial defect model. Acta Biomater 2013;9:8015-26.
106. Hench LL, Polak JM. Third-generation biomedical materials. Science 2002;295:1014-7.
107. Hu GF. Copper stimulates proliferation of human endothelial cells under culture. J Cell Biochem 1998;69:326-35.
108. Rosso F, Marino G, Giordano A, Barbarisi M, Parmeggiani D, Barbarisi A. Smart materials as scaffolds for tissue engineering. J Cell Physiol 2005;203:465-70.
109. Ye Q, Zünd G, Benedikt P, Jockenhoevel S, Hoerstrup SP, Sakyama S, *et al.* Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. Eur J Cardiothorac Surg 2000;17:587-91.
110. Hudon V, Berthod F, Black AF, Damour O, Germain L, Auger FA. A tissue-engineered endothelialized dermis to study the modulation of angiogenic and angiostatic molecules on capillary-like tube formation *in vitro*. Br J Dermatol 2003;148:1094-104.
111. Inuyama Y, Kitamura C, Nishihara T, Morotomi T, Nagayoshi M, Tabata Y, *et al.* Effects of hyaluronic acid sponge as a scaffold on odontoblastic cell line and amputated dental pulp. J Biomed Mater Res B Appl Biomater 2010;92:120-8.
112. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater 2008;15:88-99.
113. Bacáková L, Novotná K, Parízek M. Polysaccharides as cell carriers for tissue engineering: The use of cellulose in vascular wall reconstruction. Physiol Res 2014;63 Suppl 1:S29-47.
114. Galanakis I, Vasdev N, Soomro N. A review of current hemostatic agents and tissue sealants used in laparoscopic partial nephrectomy. Rev Urol 2011;13:131-8.
115. Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF. A review on endogenous regenerative technology in periodontal regenerative medicine. Biomaterials 2010;31:7892-927.
116. Clark R. The Molecular and Cellular Biology of Wound Repair. New York: Springer Science & Business Media; 1996.
117. Hall H, Baechi T, Hubbell JA. Molecular properties of fibrin-based matrices for promotion of angiogenesis *in vitro*. Microvasc Res 2001;62:315-26.
118. Jabbarzadeh E, Starnes T, Khan YM, Jiang T, Wirtel AJ, Deng M, *et al.* Induction of angiogenesis in tissue-engineered scaffolds designed for bone repair: A combined gene therapy-cell transplantation approach. Proc Natl Acad Sci U S A 2008;105:11099-104.
119. Summitt JB, dos Santos J. Fundamentals of Operative Dentistry: A Contemporary Approach. Hanover Park, IL: Quintessence Publication; 2006.
120. Huang GT, Sonoyama W, Chen J, Park SH. *In vitro* characterization of human dental pulp cells: Various isolation methods and culturing environments. Cell Tissue Res 2006;324:225-36.
121. Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, Hsi BL. Human amnion as an adjunct in wound healing. Lancet 1980;1:1156-8.
122. Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. J Pharmacol Sci 2007;105:215-28.
123. Gomes MF, dos Anjos MJ, Nogueira TO, Guimarães SA. Histologic evaluation of the osteoinductive property of autogenous demineralized dentin matrix on surgical bone defects in rabbit skulls using human amniotic membrane for guided bone regeneration. Int J Oral Maxillofac Implants 2001;16:563-71.
124. Rinastiti M, Harijadi, Santoso AL, Sosroseno W. Histological evaluation of rabbit gingival wound healing transplanted with human amniotic membrane. Int J Oral Maxillofac Surg 2006;35:247-51.
125. Kesting MR, Loeffelbein DJ, Classen M, Slotta-Huspenina J, Hasler RJ, Jacobsen F, *et al.* Repair of oronasal fistulas with human amniotic membrane in minipigs. Br J Oral Maxillofac Surg 2010;48:131-5.
126. Tuncel U, Ozgenel GY. Use of human amniotic membrane as an interpositional material in treatment of temporomandibular joint ankylosis. J Oral Maxillofac Surg 2011;69:e58-66.
127. Nakamura T, Sekiyama E, Takaoka M, Bentley AJ, Yokoi N, Fullwood NJ, *et al.* The use of trehalose-treated freeze-dried amniotic membrane for ocular surface reconstruction. Biomaterials 2008;29:3729-37.
128. Houlihan JM, Biro PA, Harper HM, Jenkinson HJ, Holmes CH. The human amnion is a site of MHC class Ib expression: Evidence for the expression of HLA-E and HLA-G. J Immunol 1995;154:5665-74.
129. Wolbank S, Hildner F, Redl H, van Griensven M, Gabriel C, Hennerbichler S. Impact of human amniotic membrane preparation on release of angiogenic factors. J Tissue Eng Regen Med 2009;3:651-4.
130. Boo L, Sofiah S, Selvaratnam L, Tai C, Belinda P, Kamarul T. A preliminary study of human amniotic membrane as a potential chondrocyte carrier. Malays Orthop J 2009;3:16-23.
131. Lindenmair A, Wolbank S, Stadler G, Meinl A, Peterbauer-Scherb A, Eibl J, *et al.* Osteogenic differentiation of intact human amniotic membrane. Biomaterials 2010;31:8659-65.
132. Alshehadat SA. Interaction of amniotic membrane with deciduous teeth stem cells as potential scaffold for dental pulp tissue engineering. Ph.D., Thesis, Universiti Sains Malaysia School of Dental Sciences, Malaysia; 2014.
133. Petersen N, Gatenholm P. Bacterial cellulose-based materials and medical devices: Current state and perspectives. Appl Microbiol Biotechnol 2011;91:1277-86.
134. Shi Q, Li Y, Sun J, Zhang H, Chen L, Chen B, *et al.* The osteogenesis of bacterial cellulose scaffold loaded with bone morphogenetic protein-2. Biomaterials 2012;33:6644-9.
135. Andersson J, Stenhamre H, Bäckdahl H, Gatenholm P. Behavior of human chondrocytes in engineered porous

bacterial cellulose scaffolds. J Biomed Mater Res A 2010;94:1124-32.
136. Banerjee A, Arha M, Choudhary S, Ashton RS, Bhatia SR, Schaffer DV, *et al.* The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells. Biomaterials 2009;30:4695-9.
137. Du F, Wang H, Zhao W, Li D, Kong D, Yang J, *et al.* Gradient nanofibrous chitosan/poly ?-caprolactone scaffolds as extracellular microenvironments for vascular tissue engineering. Biomaterials 2012;33:762-70.
138. Park SJ, Li Z, Hwang IN, Huh KM, Min KS. Glycol chitin-based thermoresponsive hydrogel scaffold supplemented with enamel matrix derivative promotes odontogenic differentiation of human dental pulp cells. J Endod 2013;39:1001-7.
139. Ikeda T, Yanagiguchi K, Viloria I, Hayashi Y. Relationship between lysozyme activity and clinical symptoms following the application of chitin/chitosan in endodontic treatment. Chitosan Per, OS: From Dietary Supplement to Drug Carrier Grottammare. Alaska, US: Atec; 2000. p. 275-92.
140. Asaoka K. Chitin-Chitosan-the Choice Food Supplement for Over 10,000 Physicians in Japan: A Complete Analysis of its Clinical Applications to Various Diseases. New York: Vantage Press; 1996.
141. Koide S. Chitin-chitosan: Properties, benefits and risks. Nutr Res 1998;18:1091-101.
142. Matsunaga T, Yanagiguchi K, Yamada S, Ohara N, Ikeda T, Hayashi Y. Chitosan monomer promotes tissue regeneration on dental pulp wounds. J Biomed Mater Res A 2006;76:711-20.
143. Sato Y, Terashima M, Kagiwada N, Tun T, Inagaki M, Kakuta T, *et al.* Evaluation of proliferation and functional differentiation of LLC-PK1 cells on porous polymer membranes for the development of a bioartificial renal tubule device. Tissue Eng 2005;11:1506-15.
144. Han D, Gouma PI. Electrospun bioscaffolds that mimic the topology of extracellular matrix. Nanomedicine 2006;2:37-41.
145. Bodin A, Bharadwaj S, Wu S, Gatenholm P, Atala A, Zhang Y. Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials 2010;31:8889-901.
146. Esguerra M, Fink H, Laschke MW, Jeppsson A, Delbro D, Gatenholm P, *et al.* Intravital fluorescent microscopic evaluation of bacterial cellulose as scaffold for vascular grafts. J Biomed Mater Res A 2010;93:140-9.
147. Bäckdahl H, Esguerra M, Delbro D, Risberg B, Gatenholm P. Engineering microporosity in bacterial cellulose scaffolds. J Tissue Eng Regen Med 2008;2:320-30.
148. Rubenstein D, Han D, Goldgraben S, El-Gendi H, Gouma PI, Frame MD. Bioassay chamber for angiogenesis with perfused explanted arteries and electrospun scaffolding. Microcirculation 2007;14:723-37.
149. Sakai S, Kawakami K. Synthesis and characterization of both ionically and enzymatically cross-linkable alginate. Acta Biomater 2007;3:495-501.
150. Hatch A, Hansmann G, Murthy SK. Engineered alginate hydrogels for effective microfluidic capture and release of endothelial progenitor cells from whole blood. Langmuir 2011;27:4257-64.
151. Yamamoto M, James D, Li H, Butler J, Rafii S, Rabbany S. Generation of stable co-cultures of vascular cells in a honeycomb alginate scaffold. Tissue Eng Part A 2010;16:299-308.
152. Hajiali H, Shahgasempour S, Naimi-Jamal MR, Peirovi H. Electrospun PGA/gelatin nanofibrous scaffolds and their potential application in vascular tissue engineering. Int J Nanomedicine 2011;6:2133-41.
153. Dobie K, Smith G, Sloan AJ, Smith AJ. Effects of alginate hydrogels and TGF-beta 1 on human dental pulp repair *in vitro*. Connect Tissue Res 2002;43:387-90.
154. Kumabe S, Nakatsuka M, Kim GS, Jue SS, Aikawa F, Shin JW, *et al.* Human dental pulp cell culture and cell transplantation with an alginate scaffold. Okajimas Folia Anat Jpn 2006;82:147-55.
155. Davidson A. The Oxford Companion to Food. New York: Oxford University Press; 2014.
156. Mac Faddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore, London: Williams & Wilkins; 1985.
157. Kikuchi H, Sawada T, Yanagisawa T. Effects of a functional agar surface on *in vitro* dentinogenesis induced in proteolytically isolated, agar-coated dental papillae in rat mandibular incisors. Arch Oral Biol 1996;41:871-83.
158. Kikuchi H, Amano H, Yamada S. Putative role of basement membrane for dentinogenesis in the mesenchyme of murine dental papillae *in vitro*. Cell Tissue Res 2001;303:93-107.
159. Lewis SL, Dirksen SR, Heitkemper MM, Bucher L. Medical-Surgical Nursing: Assessment and Management of Clinical Problems, Single Volume. New York: Elsevier Health Sciences; 2014.
160. Blanquaert F, Barritault D, Caruelle JP. Effects of heparan-like polymers associated with growth factors on osteoblast proliferation and phenotype expression. J Biomed Mater Res 1999;44:63-72.
161. Bessa PC, Balmayor ER, Azevedo HS, Nürnberger S, Casal M, van Griensven M, *et al.* Silk fibroin microparticles as carriers for delivery of human recombinant BMPs. Physical characterization and drug release. J Tissue Eng Regen Med 2010;4:349-55.
162. Chen FM, Wu ZF, Sun HH, Wu H, Xin SN, Wang QT, *et al.* Release of bioactive BMP from dextran-derived microspheres: A novel delivery concept. Int J Pharm 2006;307:23-32.
163. Dowthwaite GP, Edwards JC, Pitsillides AA. An essential role for the interaction between hyaluronan and hyaluronan binding proteins during joint development. J Histochem Cytochem 1998;46:641-51.
164. Day AJ, Sheehan JK. Hyaluronan: Polysaccharide chaos to protein organisation. Curr Opin Struct Biol 2001;11:617-22.
165. Felszeghy S, Hyttinen M, Tammi R, Tammi M, Módis L. Quantitative image analysis of hyaluronan expression in human tooth germs. Eur J Oral Sci 2000;108:320-6.
166. Angele P, Kujat R, Nerlich M, Yoo J, Goldberg V, Johnstone B. Engineering of osteochondral tissue with bone marrow mesenchymal progenitor cells in a derivatized hyaluronan-gelatin composite sponge. Tissue Eng 1999;5:545-54.
167. Ramamurthi A, Vesely I. Smooth muscle cell adhesion on crosslinked hyaluronan gels. J Biomed Mater Res 2002;60:195-205.
168. Liu Y, Shu XZ, Gray SD, Prestwich GD. Disulfide-crosslinked hyaluronan-gelatin sponge: Growth of fibrous tissue *in vivo*. J Biomed Mater Res A 2004;68:142-9.

169. Kim HD, Valentini RF. Retention and activity of BMP-2 in hyaluronic acid-based scaffolds *in vitro*. J Biomed Mater Res 2002;59:573-84.
170. Peattie RA, Nayate AP, Firpo MA, Shelby J, Fisher RJ, Prestwich GD. Stimulation of *in vivo* angiogenesis by cytokine-loaded hyaluronic acid hydrogel implants. Biomaterials 2004;25:2789-98.
171. Sasaki T, Kawamata-Kido H. Providing an environment for reparative dentine induction in amputated rat molar pulp by high molecular-weight hyaluronic acid. Arch Oral Biol 1995;40:209-19.
172. Segari WA, Radwan DA, El Hamid MA. The effect of adding hyaluronic acid to calcium phosphate on periapical tissue healing following periradicular surgery in dogs. Tanta Dent J 2014;11:122-9.

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