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Review article

Dental pulp regeneration strategies: A review of status quo and recent advances

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ABSTRACT

Microorganisms, physical factors such as temperature or mechanical injury, and chemical factors such as free monomers from composite resin are the main causes of dental pulp diseases. Current clinical treatment methods for pulp diseases include the root canal therapy, vital pulp therapy and regenerative endodontic therapy. Regenerative endodontic therapy serves the purpose of inducing the regeneration of new functional pulp tissues through autologous revascularization or pulp tissue engineering. This article first discusses the current clinical methods and reviews strategies as well as the research outcomes regarding the pulp regeneration. Then the in vivo models, the prospects and challenges for regenerative endodontic therapy were further discussed.

1. Introduction

The dental pulp is located in the central portion of a tooth and is surrounded by dentin. It consists of nerve fibers, blood vessels, fibrous connective tissue, and plays a crucial role in oral health. The dental pulp contains nerve fibers that allow teeth to perceive various external stimuli such as temperature and mechanical injuries, thus protecting the pulp from being further affected. The vascular system within the dental pulp provides oxygen and nutrients, contributing to the maintenance of the health of tooth structures like enamel and dentin. Immune cells, such as macrophages, dendritic cells, and lymphocytes, within the dental pulp have a dual role in either promoting or delaying pulp repair and regeneration while also enhancing the tooth's resistance to infection [1]. Additionally, odontoblasts within the dental pulp can promote self-repair of dentin in response to tooth stimulation, forming reparative dentin to protect the dental pulp. Moreover, undifferentiated mesenchymal cells within the dental pulp can serve as a reservoir of cells, differentiating into fibroblasts, odontoblasts, macrophages, and more when stimulated.

Currently, standard clinical approaches for managing pulp diseases generally include root canal treatment and vital pulp therapy (Fig. 1). If a deep caries on the tooth crown is not treated in time, the bacteria inside the caries would progress into the dental pulp, causing pulp infection and inflammation. Similarly, a fracture on the tooth crown can also directly expose the dental pulp, inducing subsequent pulp infection. When teeth experience severe bacterial infection, trauma, or chronic pulpitis that render the dental pulp irreparable, root canal therapy can be employed to eliminate the infection, alleviate pain, and restore tooth function. Root canal therapy involves several steps, including root canal instrumentation, irrigation, disinfection, and tightly sealing with filling and sealant materials. Despite the widespread clinical use of root canal therapy, there are still some limitations. Due to the complexity of the root canal system, some canals may be missed, leading to incomplete bacterial clearance and an increased risk of infection recurrence, necessitating retreatment. Additionally, root canal therapy can render the tooth brittle due to the loss of pulp's blood supply, increasing the risk of tooth fracture and necessitating either extraction or restorative treatment. When teeth are mildly to moderately damaged, vital pulp therapy is often employed to preserve the dental pulp's health. Vital pulp therapy includes pulp capping and pulpotomy, which depends on the infection degree of pulp tissues [2]. When the infection hasn't affected the pulp, the pulp capping can be used to protect the pulp from

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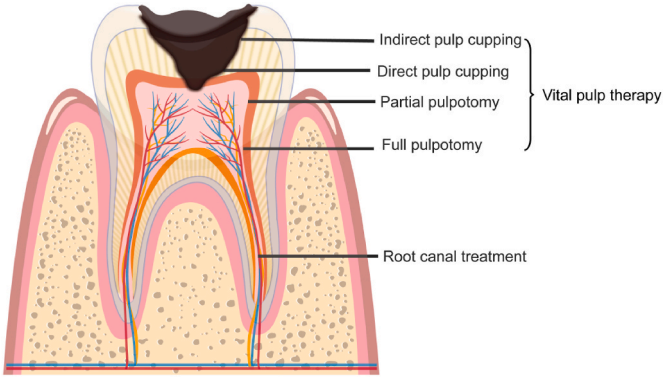

Fig. 1. Current non-surgical clinical approaches for pulp diseases.

further infection. If the pulp tissue is partly affected, pulpotomy would be considered to remove the affected tissue and preserve the healthy pulp. It can also be used in cases of dental trauma or chronic pulpitis where the pulp has mild inflammation without severe infection. Furthermore, this kind of therapy can be utilized when primary teeth exhibit mild pulp inflammation due to caries or trauma. However, for teeth with severe pulp infection, vital pulp therapy may not be sufficient to preserve the natural pulp, and root canal therapy is still required. After vital pulp therapy, as a portion of the pulp's blood supply is lost, the tooth may still become brittle, necessitating follow-up restorative treatment to protect it from further damage. Regular dental check-ups are necessary for post-treatment teeth to promptly address potential reinfection or other issues.

Recently, dental pulp engineering and regenerative medicine have garnered significant attention, leading to extensive research into various novel strategies for better pulp regeneration. In general, dental pulp regeneration includes two main approaches, i.e. pulp revascularization and pulp engineering. The primary goal of dental pulp revascularization is to induce vascularized pulp regeneration through using autologous blood or blood components to preserve the natural structure and function of the tooth. The success of the treatment may vary depending on individual circumstances and treatment techniques. The main objective and strategy for dental pulp engineering based on exogenous cell transplantation involves placing stem cells, scaffold materials, and growth factors into prepared root canals to generate new and functional dental pulp-dentine complex. Numerous in vitro and in vivo studies have shown that these techniques can regenerate dental pulp-like tissues with characteristics of nerves, blood vessels, and dentin-forming cells[3]. Dental pulp regeneration technology holds promise as an alternative to traditional root canal treatment, enabling the regeneration of functional dental pulp for better restoration of damaged teeth functionality. Compared to conventional root canal treatment, pulp regeneration can more effectively promote the natural repair process of teeth. By inducing the growth of new pulp tissue, it preserves the natural structure and morphology of the teeth, providing long-lasting normal tooth function. It may also reduce the likelihood of root fracture or root canal re-infection associated with the root canal treatment [4,5]. However, there are still challenges related to the stem cell-based pulp regeneration, such as complexity of clinical procedures, the cell sourcing, graft rejection, ethics issues, and potential treatment complications [6]. To avoid these issues, cell-free therapies, such as utilizing extracellular vesicles, decellularized extracellular matrices, and gene therapy strategies, are being investigated.

In this review, we tried to discuss the current treatment methods for pulp diseases and provide a comprehensive review of present strategies and recent advances in pulp regeneration, with a particular emphasis on functional pulp regeneration. Moreover, the obstacles and challenges in future research and clinical applications are also discussed.

2. Strategies for regenerative endodontics

2.1. Cell homing-based pulp revascularization

Pulp revascularization relies on the vitality of residual tissues within the root canal and periapical region to facilitate pulp regeneration and restore the function of the pulp-dentin complex. Treatment should involve thorough and effective disinfection while preserving the soft tissue as much as possible. This process induces bleeding in periapical tissues to form a regenerative scaffold primarily composed of blood clots and provides growth factors. Finally, the procedure concludes with a tight coronal seal [7]. Murray et al. compared the clinical efficacy of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and blood clot revascularization (BC) in the regeneration of immature permanent teeth. The results indicated that PRP and PRF were more successful in achieving apical closure, while all three methods showed similar outcomes in root lengthening, the periapical lesion healing and root canal wall thickening [8].

Pulp revascularization technique is now a relatively mature dental pulp regeneration treatment which has been applied in clinical practice as a cell homing procedure [9]. Recently, cell homing has demonstrated that dental pulp regeneration is possible. Its process contains that growth factors, chemotactic factors, and other signaling molecules loaded onto a scaffold to guide autologous stem cells to the damaged dental pulp area, promoting autologous stem cell proliferation and differentiation, helping the regeneration of dental pulp tissue [10]. Researchers are continually improving scaffold materials to enhance their biocompatibility, degradability, and ability to support the development of new pulp tissue. Terranova et al. [11] designed a composite membrane with a 3D conical scaffold made of nanofibers and tannic acid microparticles, coated with gelatin. This scaffold was intended to support the migration and colonization of DPSCs within the root canal. The study demonstrated the scaffold's biocompatibility and its potential for facilitating DPSCs migration and proliferation. The newly developed PRF-loaded methacrylated chitosan/methacrylated collagen hydrogel (ChitMA/ColMA) met essential criteria such as injectability, cytocompatibility, and bioactivity for promoting odontogenic differentiation. These qualities made it a promising scaffold for cell-homing approach in pulp-dentin complex regeneration [12]. An increasing amount of research is dedicated to gaining a better understanding of the role of growth factors in the cell homing process and exploring ways to optimize the types and delivery methods of these factors to more effectively promote dental pulp tissue regeneration. Recent study introduced a novel injectable alkaline hydrogel that activated endogenous TGFβ1 under alkaline conditions to facilitate *in situ* tissue regeneration. This hydrogel promoted the migration of bone marrow stem cells, facilitating the regeneration of complex tissues without the need for cell transplantation or exogenous growth factor delivery [13]. The latest research findings indicated that concentrated growth factor (CGF) exhibited a complex three-dimensional structure and contained key growth factors like Platelet-Derived Growth Factor-BB (PDGF-BB), Insulin-like Growth Factor 1(IGF-1), Transforming Growth Factor-β1 (TGF-β1), basic Fibrobast Growth Factor (bFGF), and Vascular Endothelial Growth Factor (VEGF). Concentrated growth factor significantly enhanced cell growth, migration, mineralization, and could be a promising biomaterial applied in regenerative endodontics [14]. One prospective randomized trial compared different regenerative endodontic procedures (REPs) using platelet concentrates as scaffolds for immature necrotic incisors in children. The study found that platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and platelet pellet (PP) yielded similar positive responses to sensitivity tests and apical closure rates to the induced blood clot (BC) [15]. The study also observed that PRP, PRF, and PP had a lower tendency for root canal obliteration compared to BC. Overall, these platelet concentrate-based REPs showed promise for successful treatment in young patients. Furthermore, a randomized clinical trial investigated the treatment

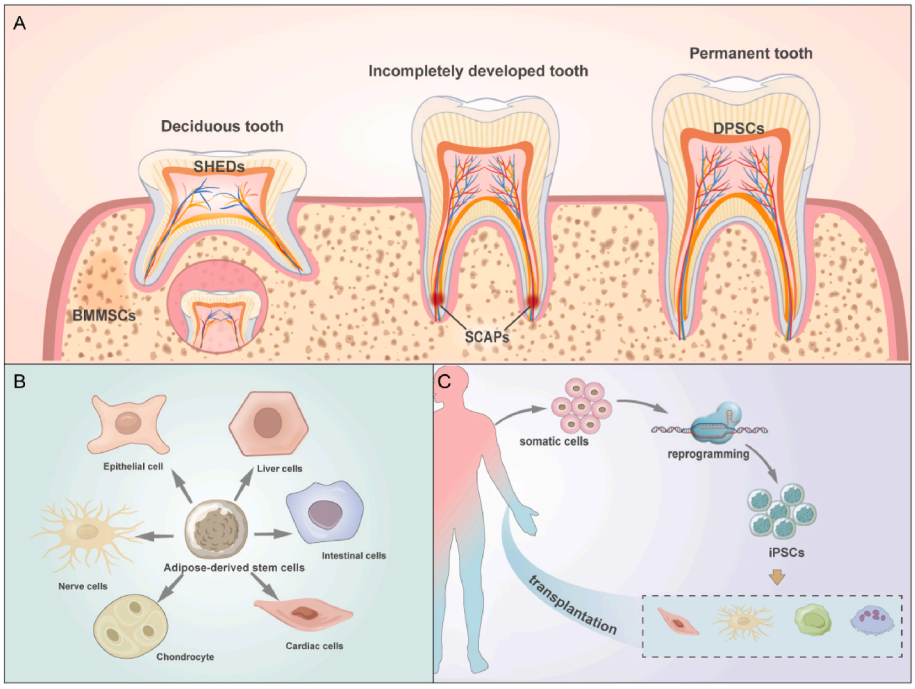

Fig. 2. Cells used for pulp regeneration.

outcomes of a cell homing-based regeneration approach compared to traditional root canal treatment for mature teeth with periapical lesions [16]. Although there was no significant difference between the two methods, REPs showed a slightly higher success rate. This was evident in higher positive responses in pulp vitality tests, reduced periapical shadow size, and lower residual bacterial presence, among other factors.

Complications in cell homing include root canal calcification and obliteration, which can affect the success of subsequent treatments [17]. Crown discoloration is another common complication observed in regenerative cases and may be related to induced bleeding or the use of chemical agents, such as Triple Antibiotic Ointment or Mineral Trioxide Aggregate in the procedure [18]. Balancing tissue growth with scaffold and signaling molecules can help mitigate these issues in the future. Maintaining an aseptic environment is crucial in regenerative endodontic procedures, but it can be challenging to balance bacterial elimination, while preserving living cells [19]. Residual bacteria can hinder pulp tissue regeneration, and the microbial complexity of oral environment poses challenges. Appropriate materials should be designed to inhibit bacterial growth and promote tissue regeneration. Recent researches predominantly concentrate on immature teeth under specific conditions, while studies on mature teeth is lacking [20]. So, the need for further investigation and broader clinical applicability are necessary.

2.2. Pulp tissue engineering

2.2.1. Cells

2.2.1.1. Dental pulp stem cells (DPSCs). Gronthos et al. first isolated DPSCs from adult human dental pulp (Fig. 2A) [21]. DPSCs are found to generate tissue resembling dental pulp, surrounding it with odontoblast-like cells and a dentin-like structure in vivo. DPSCs have self-renewal capability, multi-lineage differentiation potential, and clonogenic efficiency [22]. Classical MSC makers have been identified in DPSCs, such as CD105, CD73, CD90 [23]. They are further marked by neurovascular-associated makers, such as CD31, p75, Snail-1 and -2, and SOX-1 [24,25]. Moreover various subpopulations of DPSCs may have diverse and complementary functions in facilitating repair and regeneration within the dental pulp. Ishizaka et al. [26] transplanted dental pulp CD31(−) side population (SP) cells with tooth root subcutaneously into SCID mice and found that $CD31^{-}$ SP cells expressed higher levels of angiogenic and neurotrophic factors such as RECA1 and PGP9.5, displayed enhanced migration activity, and produced neurite outgrowth, making them a promising choice for cell therapy and regenerative applications. Additionally, the combination of DPSCs with growth factors enhances tissue regeneration more effectively. A study isolated highly angiogenic, neurogenic, and regenerative modified DPSCs (MDPSCs) subpopulation using granulocyte-colony stimulating factor (G-CSF). In vivo experiments have demonstrated that MDPSCs could promote the formation of blood vessels and dental pulp tissue [27]. The formation of regenerative dentin is crucial in the process of dental pulp regeneration. A pilot clinical study transplanted MDPSCs with G-CSF into teeth with irreversible pulpitis. Through electric pulp test and magnetic resonance imaging, the regenerated pulp exhibited good function and structure. At the same time, cone beam computed tomography (CBCT) demonstrated the formation of functional dentin [28]. These studies suggested that human MDPSCs hold promise for complete pulp regeneration in humans. In addition, DPSCs are considered more suitable for dental tissue regeneration and neurodegenerative disease applications [29].

2.2.1.2. Stem cells from human exfoliated deciduous teeth (SHEDs). SHEDs are extracted from the pulp of human deciduous primary teeth (Fig. 2A). Miura et al. [30] first discovered SHEDs and found that they were highly proliferative and capable of differentiating into various cell types, including neural cells, adipocytes, and odontoblasts [31]. Both SHED and DPSCs share a phenotypic profile of MSCs and express multiple conventional MSC markers, like CD44, CD73, CD90, CD166 [32]. In dentistry, when SHEDs are injected with Puramatrix™ or rhCollagen type I into human root canals and subsequently implanted into immunodeficient mice, SHEDs developed pulp-like tissues with functional odontoblasts capable of producing new dentin within the root canals [33]. This suggests that SHEDs, combined with appropriate scaffolds, have the potential to promote pulp-like tissue regeneration and root formation. Likewise, exosomes derived from SHED aggregates (SA-Exo), promoted angiogenesis for dental pulp regeneration in vivo [31], and in

vitro experiments demonstrated that it enhanced SHEDs endothelial differentiation and angiogenic abilities by regulating the TGF-β/SMAD2/3 signaling pathway. In addition, Xuan et al. [34] implanted autologous pulp stem cells from exfoliated deciduous teeth into necrotic immature permanent teeth and successfully regenerated dental pulp. In a clinical trial, implanting SHEDs led to the regeneration of three-dimensional pulp tissue with improved root length and reduced apical foramen width compared to traditional treatment methods. Importantly, SHEDs implantation was found to be safe during a 24-month follow-up period, suggesting its potential for effectively treating tooth injuries resulting from trauma.

2.2.1.3. Stem cells from the apical papilla (SCAPs). Sonoyama et al. [35] introduced a novel type of stem cells called Stem Cells from Apical Papilla isolated from the root apical papilla of human teeth (Fig. 2A). SCAPs express several surface markers including STRO-1, CD24, CD29, CD73, CD90, CD105, CD106, CD146, CD166. Notably, CD24 is identified as a specific marker for SCAPs. Additionally, SCAPs express a wide variety of neurogenic markers such as nestin, βIII tubulin, GAD and NeuN [36]. SCAPs display higher proliferation and mineralization potential comparing to DPSCs [37]. A recent study aimed to investigate the impact of VEGF and Nerve Growth Factor (NGF) on SCAPs during regenerative endodontic procedures. The results showed that VEGF and NGF significantly increased the expression levels of genes related to dentin and neural development in SCAPs, especially when SCAP were exposed to bacterial lipopolysaccharides. These findings suggested that NGF and VEGF have the potential to enhance dental pulp regeneration, particularly in bacterial environments [38]. Sequeira et al. [39] combined SCAPs with a platelet-rich plasma and implanted them into root segments with and without biomaterials. Results showed that when transplanted together with SCAPs, bioactive cements demonstrated excellent biocompatibility and facilitated the formation of the dentin-pulp complex and dentin bridges. Furthermore, the presence of these materials even promoted greater deposition of mineralized tissue. It suggested that SCAPs and these bioactive materials were promising for dental tissue regeneration.

2.2.1.4. Bone marrow mesenchymal stem cells (BMMSCs). Friedenstein et al. [40] first identified BMMSCs with fibroblast-like characteristics (Fig. 2A). These cells have the ability to differentiate into various tissues, including bone, cartilage, adipose tissue, tendon, and muscle. BMMSCs are characterized by positive markers including Stro-1, CD271, CD73, CD105 and CD90. Conversely, they do not express hematopoietic and endothelial markers such as CD31, CD34, and CD45 [41]. BMMSCs can differentiate into various cell types when cultured under specific conditions in vitro. These include osteogenic, chondrogenic, adipogenic and neurogenic lineages [42]. Recent study investigated the combination of rat bone marrow mesenchymal stem cells (rBMSCs) and a bioceramic material for pulp-like tissue formation. Rat incisor root fragments filled with rBMSCs and covered with iRoot BP were implanted subcutaneously for 3 months. The results showed increased blood vessel formation and the development of vascularized pulp-like tissues [43]. BMMSCs hold potential for enhancing pulp revascularization in dental treatments.

2.2.1.5. Adipose-derived stem cells (ADSCs). Zuk et al. [44] discovered a population of stem cells called processed lipoaspirate (PLA) cells, isolated from human adipose tissue. These PLA cells exhibit characteristics of mesodermal or mesenchymal origin. Additionally, they have the potential to differentiate into adipogenic, chondrogenic, myogenic, and osteogenic cells, suggesting their potential as an alternative source of multipotent stem cells to tissue regeneration (Fig. 2B). ADSCs identify several expressed proteins, including CD34, CD44, CD106, CD146, and CD166, but not STRO-1 [45]. A comparison between ADSCs and DPSCs demonstrated that both types of stem cells were successfully used to grow tooth-like structures in adult rabbits, with similar gene expression patterns and differentiation potentials. While ADSCs exhibited increased proliferation rates and demonstrated superior resistance to cellular aging, indicating their usefulness as an alternative cell source for regenerative dentistry [46]. Another study compared BMMSCs and ADSCs for dental pulp regeneration. The transplantation of ADSCs resulted in a larger amount of pulp-like tissue than BMMSCs transplantation. Additionally, ADSCs transplantation increased the area of matrix formation. This revealed that ADSCs exhibited regenerative potential, while BMMSCs showed inferior effectiveness [47].

2.2.1.6. Induced pluripotent stem cells (iPSCs). Takahashi et al. [48]first found induced pluripotent stem cells, which exhibit similar characteristics to embryonic stem cells and have the potential to differentiate into tissues from all three germ layers (Fig. 2C). By harnessing the versatile differentiation potential of iPSCs, their co-cultivation with the Hertwig's epithelial root sheath/epithelial rests of Malassez (HERS/ERM) cell line instigated epithelial differentiation through cellular interactions. Dental epithelial-like stem cell lines derived from iPSCs (EPI-iPSCs) were successfully established. Subsequent experiments indicated that these EPI-iPSCs could enhance odontogenic gene expression and promote mineralized nodule formation [49]. Recent study aimed to utilize cranial neural crest-like cells (CNCLCs) derived from iPSCs to replicate the characteristics of dental pulp tissue [50]. In a mouse model, CNCLCs combined with self-assembling peptide hydrogel successfully replicated the features of dental pulp tissue [51]. Therefore, there is an increasing need for more research focused on iPSCs to investigate their pluripotential differentiation potential for application in the field of dental pulp regeneration.

2.2.2. Scaffolds

2.2.2.1. Scaffold materials

2.2.2.1.1. Natural materials. Collagen is a versatile protein that serves as both the structural component of human and animal connective tissues and the primary constituent of the extracellular matrix. This biomaterial is often times used for tissue engineering and drug delivery system in dentistry [52–55]. Collagen can be easily combined with other biomaterials and is a polymer of interest for pulp tissue regeneration. Thant et al. [56] invented a biomaterial scaffold called AceCol, consisting of acemannan and native collagen, demonstrated improved physical and biological properties compared to collagen alone, making it a promising material for tissue regeneration.

Chitosan is a natural biopolymer derived from the shells of crustaceans such as shrimp and crabs. Chitosan supports cell adhesion, proliferation, and differentiation, making it suitable for promoting tissue regeneration. The researchers synthesized a novel calcium phosphate cement-chitosan composite scaffold incorporating metformin, which promoted the proliferation and odontogenic differentiation of DPSCs. This was evidenced by elevated expression of dentinogenic markers, robust mineral deposition, and increased alkaline phosphatase activity [57]. A study designed versatile PVA/Chitosan nanofibers with ciprofloxacin and IDR-1002, demonstrating potent anti-biofilm activity against various bacteria, such as *Enterococcus faecalis* and *Staphylococcus aureus*, and anti-inflammatory properties. In vivo, tooth fragments filled with these nanofibers promoted pulp-like tissue formation, showcasing their potential as strong candidates for antimicrobial, immunomodulatory, and regenerative approaches in dental pulp revascularization and regeneration procedures [58].

Silk is a natural protein fiber produced by silkworms, spiders, and some other insects. Silk fibroin (SF) is exceptionally biocompatible, facilitating cell attachment and growth. SF has been widely used in the field of tissue engineering [59]. A scaffold using ultrasmall superparamagnetic iron oxide (USPIO) - labeled hydroxyapatite (HA) and SF, loaded with DPSCs for regeneration was created [60]. It showed stability, low cytotoxicity, and effectively promoted dental pulp tissue

Table 1
Natural materials used for dental pulp regeneration.

Materials	Advantages	Disadvantages	Application forms
Collagen	Biocompatibility, Biodegradability, Hydrophilicity, Gel-forming Ability	Weak mechanical strength, Potential toxicity of crosslinking techniques	Nanofiber scaffolds, Hydrogels, Micro-nanoparticle
Chitosan	Bioactivity, Biocompatibility, Biodegradability, Adsorption capability, Antimicrobial activity, Anti-inflammatory property	High cost, Low storage stability	Nanofiber scaffolds, Hydrogels, Sponge, Micro-nanosphere
Silk fibroin	Biocompatibility, Biodegradability, Drug delivery, High strength and flexibility	Sensitivity to denaturation, Solubility constraints	Nanofiber scaffolds, Sponge, Hydrogel
Gelatin	Biocompatibility, Biodegradability, Gelling ability, High stability	Limited mechanical properties, Thermal sensitivity	Nanofiber scaffolds, Hydrogels, Sponge, Micro-nanosphere
Alginate	Biocompatibility, Biodegradability, Gel formation, Water retention, Bioprinting	Limited mechanical strength, Limited stability, Rigidity, Water sensitivity, Limited temperature tolerance	Nanofiber scaffolds, Hydrogels, Sponge, Micro-nanosphere
HA	Biocompatibility, Biodegradability, Gel formation, Moisture retention	Mechanical performance limitations, High cost	Nanofiber scaffolds, Sponge, Hydrogel, Micro-nanosphere
Platelet concentrate	Low immune reactivity, Rich growth factors, Reduced infection risk	Case limitation, Challenges in preparation and Storage, High cost	Nanofiber scaffolds, Hydrogels, Liquid
dECM	Biocompatibility, Structural stability, Biodegradability, Reduced infection risk, Customizability	Immunogenicity, Limited mechanical strength, High cost, Ethical concerns	Sheets, Customized Forms, 3D scaffold, Fiber or tube form

repair in mice, as seen through MRI imaging and histological analysis.

Gelatin offers excellent biocompatibility, providing the necessary support for cell growth and adhesion. Gelatin scaffolds can be fabricated into three-dimensional structures with micro and macroscopic features, mimicking various tissue and organ architectures [61] and also serve as the foundation for drug delivery systems [62]. In dentistry, researchers synthesized gelatin/fibrin scaffolds, the hybrid scaffold exhibited enhanced physicochemical and biological properties compared to scaffolds made from either material alone. It promoted the migration, proliferation, and odontogenic differentiation of DPSCs [63]. Overall, gelatin holds great promise in tissue engineering due to its versatility, biocompatibility, and degradability, making it a powerful tool for promoting tissue regeneration and repair.

Alginate is a natural polysaccharide primarily extracted from brown algae. It can serve as a carrier in drug delivery systems to control drug release rates [64]or be fabricated into various scaffold shapes for tissue engineering applications, mimicking specific tissue structures and providing a platform for cell attachment and growth [65]. Alginate gels are commonly used as bioink in bioprinting technology to precisely print scaffolds, enabling the construction of specific tissue structures. Scientists have developed a kind of bioink for 3D printing using a mixture of alginate and dentin proteins. This bioink were easy to support cell survival, enhance differentiation of SCAPs, and were essential for effectively engineering the complex structure of the pulp dentin [66]. Alginate is a crucial material in advancing research and applications in the fields of tissue engineering and regenerative medicine.

Hyaluronic acid (HA) is a naturally polysaccharide in the body, as a major component of the extracellular matrix, playing a vital role in regulating tissue injury and repair. Researchers developed injectable HA hydrogels. In vitro study suggested that the hydrogels facilitated odontoblastic differentiation of DPSCs by releasing Td and promoted vascularization through Rg1 release [67]. Similarly, Silva et al. developed an injectable hydrogel composed of Hyaluronic acid/cellulose nanocrystals/platelet lysate (HA/CNC/PL) with improved properties for tissue engineering [68]. HA scaffolds interact with other biomolecules or materials could enhance the physicochemical and biological properties. Simultaneously, as a reservoir for growth factors, they enable signaling molecules to promote stem cell migration, proliferation, and differentiation.

Platelet concentrates, including platelet-rich-plasma(PRP), platelet-rich-fibrin(PRF), and concentrated growth factor (CGF), are extracted from whole blood through centrifugation. They contain a fibrin matrix, essential cytokines and growth factors, with applications in dental and regenerative medicine [69,70]. Xu et al. co-cultured DPSCs with an increasing concentration of PRP, leading to increased cell migration, proliferation, and osteogenic differentiation. Furthermore, it was observed that this enhancement was due to autophagy (Autophagy is a complex process of intracellular degradation of senescent or malfunctioning organelles [71]) [72]. Interestingly, a study compared the regenerative effects of PRF and PRP on DPSCs. Liquid PRF demonstrated superior regenerative potential, especially in an inflammatory environment [73]. CGF was also found to enhance cell proliferation, migration, and promote dentinogenesis and angiogenesis processes, making it a potential growth factor-loaded scaffold for dentin-pulp complex healing [74].

Decellularized extracellular matrix (dECM) scaffolds have garnered interest in tissue engineering due to their biocompatibility and bioactivity. These scaffolds, created by removing immunogenic cellular components from human or animal tissues, leave the extracellular matrix framework intact. Decellularization preserves the dECM's physicochemical properties and biological functionality, making it a suitable 3D substrate for cell seeding and implantation in patients, facilitating tissue regeneration and organ restoration [75,76]. In regenerative medicine, Alqahtani et al. successfully introduced porcine dental pulp-derived ECM into a dog root canal model, which demonstrated that the decellularized ECM facilitated the expression of DSP and CD31 [77]. Analogously, a study observed organized dentin and enamel-like tissues in recell-decellularized tooth buds and natural tooth bud implants, providing a potential for bioengineering of whole teeth [78]. In addition to acellular matrices, hydrogels derived from dECM can also find applications in regenerative engineering. Researchers created hydrogel scaffolds from bone extracellular matrix (bECM) and found that bECM-derived hydrogel scaffolds significantly enhanced odontogenic gene expression and mineral deposition of DPSCs, highlighting their potential for stimulating odontogenic differentiation [79].

The advantages and disadvantages of natural materials as well as their application forms for dental pulp regeneration are summarized in Table 1.

2.2.2.1.2. Synthetic materials. Poly (α-hydroxy ester)-based scaffolds have broad applications in the field of pulp-dentine complex regeneration. Polyglycolic acid (PGA) is a highly biodegradable polymer with good mechanical properties, making it suitable for scaffold fabrication. Polylactic acid (PLA) is also biodegradable and has tunable degradation rates, allowing for controlled release of bioactive molecules. Poly lactic-co-glycolic acid (PLGA), a copolymer of PLA and PGA, combines the properties of both polymers and provides enhanced mechanical strength and degradation control [70]. A study created PGA-melanocortin peptides (PGA-α-MSH) and revealed that it had a significant impact on human pulp fibroblasts, influencing their activation and adhesion. When integrated into multilayered films, PGA-α-MSH enhanced cell adhesion and proliferation, suggesting its potential as a biomaterial for endodontic regeneration [80]. Similarly, researchers have created an innovative fibrin hydrogel incorporating clindamycin loaded PLA-nanoparticles(CLIN-PLA-NPs), which exhibited both

antibacterial properties and biocompatibility, demonstrating significant potential in dental pulp regeneration [81]. To achieve complex tooth root regeneration, Chen et al. [82] combined aligned PLGA/Gelatin electrospun sheet (APES) and dental pulp extracellular matrix (DPEM) with treated dentin matrix (TDM) respectively as scaffolds, simulating the extracellular matrix microenvironments of periodontium and dental pulp-dentin complex. In vitro studies demonstrated that APES/TDM promoted the odontogenic differentiation of DFSCs. Moreover, successful regeneration of periodontium and dental pulp-dentin complex was achieved in a miniature pig model. Polycaprolactone (PCL) is another commonly used biodegradable material in tissue engineering with wide ranging applications. Seonwoo et al. [83]created reduced graphene oxide-PCL-electrospun nanofibers (RGO-PCL NFs),which could be a useful tool for DPSC neurogenesis in neurodegenerative and neurodefective diseases. Analogously a tubular scaffold based on PCL was prepared through electrospinning technology, significantly enhancing the migration and proliferation of SCAPs when combined with fibronectin (FN) or collagen hydrogel alone or together. Additionally, it upregulated the expression of genes related to pulp regeneration [84]. In addition, polyethylene glycol (PEG) has been widely used in biology because of its non-immunogenic, non-toxic, biodegradable, and highly hydrophilic properties [85]. Han et al. engineered an injectable PLGA/PEG hydrogel, which, through controlled release of VEGF and DPSCs-exo, promptly triggered angiogenesis and maintained osteogenesis, closely resembling the natural bone formation process. Both in vitro and in vivo experiments demonstrated that this material promoted cellular vasculature and osteogenic differentiation, facilitating bone formation in a calvarial defect model [86]. PEG materials also can be used as a delivery system. For instance, Ghandforoushan et al. created a composite scaffold by embedding TGF-β1-containing nanoparticles within PEG and PLGA, it regulated DPSCs adhesion, proliferation, and differentiation, ultimately promoting their chondrogenic differentiation [87].Therefore, the use of these polymeric materials may be considered an interesting strategy for regenerative pulp therapy.

GelMA is derived from gelatin and modified by adding methacryloyl groups. This modification allows GelMA to form a gel-like material when exposed to ultraviolet light and a photoinitiator, making it useful for 3D bioprinting and creating 3D scaffolds for cell growth and tissue regeneration. GelMA's unique combination of bioactivity and tunable properties makes it a well type for engineering tissue [88]. An increasing number of studies have been focused on GelMA hydrogels. Researchers created an innovative GelMA/Platelet lysate/Laponite hydrogel microsphere system for endodontic regeneration [89]. This material exhibited excellent physicochemical properties and biocompatibility. It could keep releasing multiple growth factors effectively, thereby promoting both angiogenesis and the regeneration of pulp-like tissue. It points the way for functional dental pulp tissue regeneration.

Self-assembling peptides are short chains of amino acids that have the remarkable ability to spontaneously form into well-defined nanoscale structures or supramolecular assemblies. These peptides can assemble into various shapes, such as fibers, nanotubes, or spherical micelles, depending on their sequence and environmental conditions [90,91].Self-assembling peptides can be used to create three-dimensional scaffolds or matrices that provide a three-dimensional environment for cell growth, facilitating cell adhesion, proliferation, and differentiation in tissue engineering. A study developed a self-assembling peptide biomimetic hydrogel that mimicked the extracellular matrix. This hydrogel contained Arg-Gly-Asp peptides (RGD) and Vascular Endothelial Growth Factor (VEGF)-mimetic peptide epitopes, providing a 3D microenvironment for the proliferation and differentiation of DPSCs. In vivo experiments also demonstrated the promotion of dentin-pulp complex regeneration by the 3D self-assembling peptide scaffold [92].

Combining two or more materials to overcome the limitations of single scaffold materials is a new direction in scaffold materials development. Examples include calcium phosphate composite scaffolds,

Table 2
Important synthetic materials for pulp regeneration.

Synthetic materials	Scaffold	Functions reported	References
PGA	DGLG4-PGA-α-MSH	Through coupling with PGA, α-MSH effectively suppressed inflammation and formed DGLG4-PGA-α-MSH nanocomposite structures, efficiently fostering pulp-like tissue regeneration.	[99]
Self-assembled peptide	RAD/Dentonin hydrogel	RAD/Dentonin promoted the proliferation, migration, odontogenic differentiation, and mineralization deposition of DPSCs.	[100]
PLGA	PLGA/DEX-CD-IC	The PLGA/DEX-CD-IC electrospun fiber scaffold enabled sustained release of dexamethasone, promoting the migration, proliferation, and odontogenic differentiation of SHEDs.	[101]
PLGA	PLGA microsphere	Surface-modified PLGA microspheres enhanced DPSCs attachment, stimulated odontogenic activity, and supported the formation of 3D structures.	[102]
Composite scaffold	PCL/smBG hybrid composites	The PCL/smBG hybrid composite enhanced proliferation of DPSCs and promoted odontogenic differentiation and mineralization compared to pure bioactive glass and PCL scaffolds.	[103]
PCL	Fibronectin-PCL	Fibronectin -loaded aligned PCL nanofiber scaffolds could promote the migration, proliferation, and odontogenic differentiation of SCAPs, which was dependent on the orientation of the fibers and the concentration of fibronectin.	[104]
GelMA	GelMA microsphere	GelMA microspheres loaded with DPSCs could promote vascular and neural regeneration within the entire dental pulp, as well as the regeneration of dentin-like structures, offering the potential to become a novel material for functional whole pulp regeneration	[105]
Composite scaffold	SrCuSi4O10/ GelMA hydrogel	The SrCuSi4O10/GelMA composite hydrogel effectively eliminated oral bacteria, inhibited biofilm formation, and enhances dentine-pulp complex repair.	[106]
Composite scaffold	GelMA/PLGA microspheres	GelMA/PLGA composite microspheres loaded with simvastatin formed cryogel microspheres. Through sustained release of simvastatin, it facilitated the migration, proliferation, and differentiation of SHEDs, successfully achieving the regeneration of vascularity-enriched dental pulp-like tissue.	[107]
GelMA	GelMA microspheres	GelMA microspheres loaded with DPSCs demonstrated robust adhesion, proliferation, and secretion of extracellular	[108]

(*continued on next page*)

Table 2 (*continued*)

Synthetic materials	Scaffold	Functions reported	References
		matrix, with improved vascularized pulp-like tissue generation.	
Composite scaffold	Wnt3a-HANW@MpSi	Wnt3a-HANW@MpSi enhanced DPSCs resistance to oxidative stress, migration, odontogenic differentiation, angiogenesis and reinforced dentin-pulp complex-like tissue and blood vessel formation.	[109]
Composite scaffold	PCL/BG PCL/HA	PCL/BG scaffolds had improved mechanical properties, surface roughness, and bioactivity; PCL/HA had increased hydrophilicity and cell adhesion. Both scaffolds had promising potential for promoting DPSCs adhesion and odontogenic differentiation.	[110]
Composite scaffold	PLLA/Gel microsphere	VEGF, heparin, and Gel formed microspheres through crosslinking and were subsequently fixed inside injectable PLLA microspheres. A hierarchical microsphere system was used to facilitate controlled release of VEGF and provide a scaffold for DPSCs, successfully regenerated pulp tissues in full-length human tooth roots.	[111]
Self-assembled peptide	Self-assembling peptide hydrogels	The dentinogenic peptide, incorporating β-sheet nanofibers and a matrix extracellular phosphoglycoprotein mimic sequence, possessed excellent biological properties, facilitating the survival and proliferation of autologous stem cells and dentinogenesis	[112]

bioactive and antibacterial composites, natural-natural scaffolds, natural-synthetic scaffolds and so on [93–98]. These composites can enhance cell adhesion and tissue/cell-cell interaction, thereby promoting cell proliferation, differentiation, and tissue regeneration.

Table 2 illustrates important synthetic materials used as the scaffold material and their functions for dental pulp regeneration.

2.2.2.2. The structure of scaffolds. Scaffolds play a crucial role in tissue engineering. They enable the precise arrangement and layering of biological materials such as cells, growth factors, and scaffolds to create complex tissue structures. They are designed with excellent biocompatibility to ensure the cell attachment, growth, and differentiation on their surfaces. They can also be used as drug carriers, allowing the loading of growth factors, chemicals, or bioactive molecules and controlled release into the surrounding tissues [113], thereby promoting dental pulp regeneration. Customized scaffolds or implants can also be manufactured according to specific needs and anatomical structures. While scaffold materials possess many beneficial characteristics, they also come with certain limitations and challenges, such as the complexity of fabrication, size constraints that limit their ability to provide sufficient three-dimensional space in certain applications, and material stability issues. These factors need to be carefully assessed and addressed in specific applications.

Nanofiber materials can promote the differentiation of stem cells into specific cell types by modulating cellular signaling pathways [114]. A nanofiber matrix material could enable the controlled and slow release of dexamethasone [115]. In vitro experiments have demonstrated that this material promoted odontoblast differentiation of DPSCs through the integrin/Bone Morphogenetic Protein/mammalian target of rapamycin (integrin/BMP/mTOR) signaling pathway.

Multicellular aggregates refer to a structure formed by a group of cells coming together in three-dimensional space. They achieve various biological functions through cell-cell interactions [116]. Cell aggregates are typically a form of organoid, and used to mimic the structure and function of specific tissues and organs [117]. Itoh et al. successfully created scaffold- and growth factor-free three-dimensional DPSC aggregates using a thermoresponsive hydrogel and this constructs were able to generate pulp-like tissues rich in blood vessels in vivo [118]. Cell aggregates can eliminate the need for scaffold materials, reducing the risk of inflammation and infection. Additionally, they allow for the customization of shapes to accommodate individual root canal morphologies, offering broad prospects for dental pulp regeneration (Fig. 3A) [119].

Hydrogels play a multifaceted role by providing cell support, drug delivery capabilities, scaffold stability, and biocompatibility, among other functions. A recent study introduced an injectable hyaluronic acid/cellulose nanocrystals/platelet lysate hydrogel (HA/CNC/PL). Cellulose nanocrystals improved the mechanical properties of the hydrogel and enhanced its stability. In vitro experiments demonstrated that this 3D-hydrogel structure can sustainably release pro-angiogenic growth factors, promoting angiogenesis (Fig. 3B) [120]. The choice of hydrogel type depends on specific application requirements and research objectives, such as injectable hydrogels, microsphere-based hydrogels, bilayer hydrogels, photosensitive hydrogels, magnetic hydrogels, and more [121–125]. These hydrogels have the potential to serve as controlled growth factors (GFs) delivery systems and supportive matrices for tissue regeneration, particularly in vascularized soft tissues like the dentin-pulp complex.

Microspheres, with diverse functionalities such as core-shell, surface modification, hollow, microporous, and nanofibrous structures, serve crucial roles in tissue engineering (Fig. 3C) [126,127]. Innovatively, a hierarchical growth factor-loaded nanofibrous microsphere scaffolding was developed, enabling the successful regeneration of pulp-like tissues throughout the full-length root canal and the formation of blood vessels [111]. Immune cells can regulate the process of regeneration. For example, Macrophages stimulated by zinc-doped bioactive glass (BGz) micro-nanospheres show a significant decrease in M1-type pro-inflammatory markers and can significantly promote odontogenic differentiation of dental pulp cells, facilitating dentin formation [1]. While microspheres offer several advantages in tissue engineering, the non-uniformity in particle size and distribution may impact the consistency of dental pulp tissue regeneration.

The term “Tooth-on-a-Chip model” refers to a microfluidic or bioengineering system designed to mimic the physiological and mechanical characteristics of a human tooth but on a miniature scale (Fig. 3D) [128]. França et al. [129]developed the tooth-on-a-chip that mimicked the nearly physiological conditions of the pulp-dentin interface. By seeding SCAPs onto the dentin surface, the study investigated stem cell responses on the chip. Besides, this model can also serve as a platform for assessing the cytotoxicity, growth status, and metabolic effects of commonly used dental materials on the cells [130].

2.2.2.3. Requirements for scaffold materials. Tissue engineering scaffold materials are required to meet a multitude of critical criteria to effectively promote tissue regeneration and repair. These essential criteria encompass biocompatibility, ensuring that the material is compatible with human tissues, and biodegradability, allowing the degradation rate of scaffold materials matching the rate of tissue regeneration [131]. Additionally, the scaffold must possess adequate mechanical properties to provide structural support, along with an appropriate level of porosity to facilitate cell migration, vascularization, dentinogenesis, neurogeneration and tissue formation. Bioactivity is essential to encourage

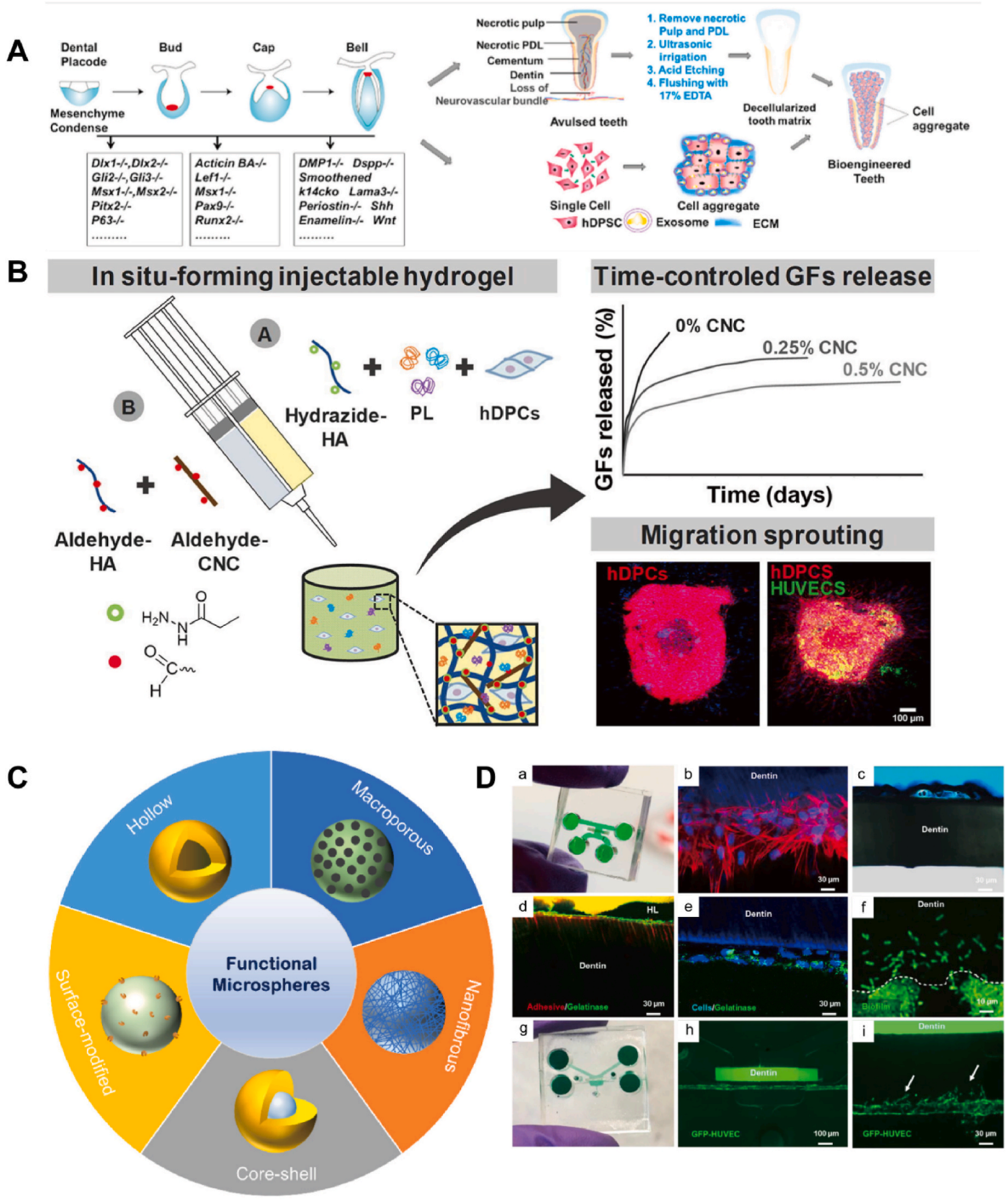

Fig. 3. Scaffolds with different structures in pulp regeneration. (A)Cell aggregates. Reprinted with permission from Ref. [119].(B)Three-dimensional injectable hydrogel. Reprinted with permission from Ref. [120]. (C)Illustration of five types of functional microspheres. Reprinted with permission from Ref. [127]. (D) Applications of the Tooth on-a-chip to test dental materials (a) Microfluidic device comprised of two parallel chambers separated by a fragment of native dentin. (b) SCAPs seeded on-chip after 7 days protrude cytoplasmic processes into the dentin tubules akin to odontoblasts. (c) Interaction of phosphoric acid and native dentin recorded in real-time. (d) Tooth-on-a-chip devices were seeded with cells, and after 48 h, fluorescein-conjugated gelatin showed gelatinolytic activity in the hybrid layer and dentin tubules. (e) The gelatinolytic activity was co-localized with cell cytoplasm suggesting a role for cells in the enzymatic degradation of the hybrid layer. (f). Streptococcus mutans seeded on-chip to evaluate real-time biofilm formation and interactions with pulp cells. (g) Tooth-on-a-chip for vasculature studies. (h) The device has a chamber on the 'pulp side' filled with collagen, seeded with mesenchymal stem cells and GFP-HUVECs. After 24 h, a pericyte-supported blood vessel is engineered with a controllable distance from the dentin. (i) Angiogenic sprouts toward the dentin on-chip. Reprinted with permission from Ref. [128].

cell adhesion, proliferation, migration and differentiation, while customizability (material types, scaffold forms, pore density and size, fiber dimensions, etc.) ensures that the scaffold can be tailored to specific applications [132].Anti-infection properties are crucial to maintain a sterile environment within the scaffold [133]. Aseptic conditions around and inside scaffolds could prevent infections, promote cell growth, maintain scaffold material functionality, and reduce the risk of inflammation, thereby ensuring the smooth progression of pulp regeneration process. Lastly, sustainability is vital to maintain the structural integrity of the scaffold throughout the dental pulp regeneration process,

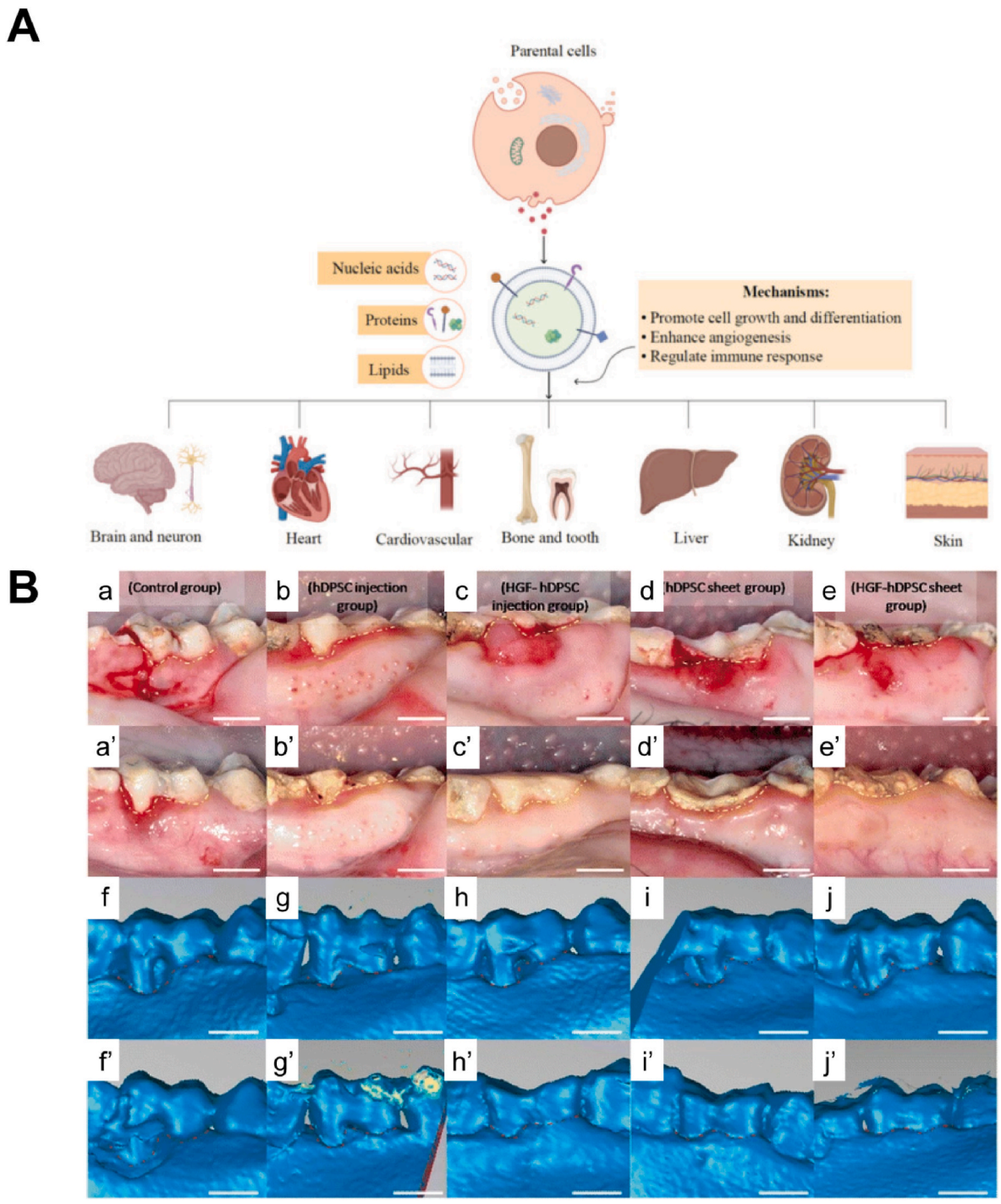

Fig. 4. EVs and gene therapy for regenerative dentistry. (A) Therapeutic mechanisms of EVs in tissue regeneration. Reprinted with permission from Ref. [148].(B) Regeneration of tissue in periodontal defects mediated by transplanted hDPSCs. Intraoral photographs were acquired a–e before transplantation or a′–e' 12 weeks after transplantation. a′ Only limited periodontal tissues were regenerated in the control group (yellow dotted line). Marked periodontal tissue formation was found in b′ the hDPSC injection group and c′ the HGF-hDPSC injection group, but they did not restore tissues to healthy levels (yellow dotted line). Periodontal tissue regeneration mediated by d′ the hDPSC sheet and e′ the HGF-hDPSC sheet achieved close to normal tissue levels (yellow dotted line). Three-dimensional CT images were acquired f–j before transplantation and f′–j' 12 weeks after transplantation. f′ Only limited bone regeneration was observed in the control group, but marked bone formation was observed g′ in the hDPSC injection group, h′ the HGF-hDPSC injection group, i′ the hDPSC sheet group, and j′ the HGF-hDPSC sheet group after cell transplantation (red dotted line). Reprinted with permission from Ref. [156].

ensuring long-term support, adaptability to varying conditions and protecting the orderly development of newly formed tissues. The selection of the appropriate scaffold material is pivotal for the success of tissue engineering projects and can vary depending on the specific application and clinical requirements.

2.2.3. *Growth factors*

Growth factors are important in endodontic regeneration, serving crucial roles in various aspects such as angiogenesis, odontoblast differentiation, and nerve growth.

Vascular Endothelial Growth Factor (VEGF) is a protein molecule that plays a crucial role in blood vessel formation and repair. When

initially discovered, VEGF was primarily associated with angiogenesis and vascular repair [134]. However, subsequent research has revealed its involvement in other biological processes, such as embryonic development, wound healing, and the development of certain diseases, particularly those related to tumors [135–137]. Injectable hydrogel microspheres carrying DPSCs and VEGF have been invented, and in vitro experiments demonstrated that the microspheres could continuously release VEGF and promote the expression of odontogenic-related genes. In a nude mouse model, it confirmed that VEGF-loaded microspheres promoted the regeneration of dentin-like tissue and the formation of new blood vessels [138]. Similarly, a study reported a degradable hybrid bio-cement, serving as a reservoir for VEGF and TGF-β1 to enable sustained growth factors release, promoting the migration of DPSCs and the formation of dentin-pulp-like structures [139]. Furthermore, a recent study revealed that VEGF/Mitogen-Activated Protein Kinase 1/Extracellular Signal-Regulated Kinase/ETS-Related Gene (VEGF/MEK1/ERK/ERG) signaling pathway played a crucial role in the anastomosis of DPSC-derived blood vessels and host blood vessels, shedding light on mechanisms for functional blood vessel generation from stem cells [140].

Bone Morphogenetic Protein 2(BMP-2) is a growth factor belonging to the bone morphogenetic protein family. It plays a crucial role in the development, regeneration, and repair processes of bones and cartilage in the human body. Divband et al. discussed a novel injectable hydrogel containing VEGF and BMP-2. This hydrogel sequentially released VEGF and BMP-2 to promote DPSCs proliferation and mineralization, mimicking the normal bone growth process [141]. By specific growth factor induction, dental pulp stem cells can differentiate into odontoblasts, promoting dentin formation and thereby achieving effective regeneration and repair of the dental pulp. A study loaded plasmid DNA with dentinogenesis-inducing factors BMP2 or DMP1 onto treated dentin scaffolds, rapidly inducing the differentiation of DPSCs into odontoblast cells, thereby reconstructing the dentin-pulp interface. The reconstructed interface exhibited similar structural and mechanical properties to natural tissue [142]. This construct shows potential as a personalized bio-filling material for the dentin-pulp interface. Moreover, researchers have discovered the molecular mechanisms of BMP2 promoting dentin formation. It confirmed that BMP2 mediated by p38 Mitogen-Activated Protein Kinase (MAPK), activated the Wingless/Integrated-β-catenin (WNT/β-catenin) signaling pathway, thereby promoting the differentiation of DPSCs and reparative dentin formation [143].

Nerve Growth Factor (NGF) is a crucial neurotrophic factor that plays a key role in the development, maintenance, and repair of the nervous system. It promotes neuronal growth, synaptic connectivity, and survival by activating intracellular signaling pathways. In dentistry, age-related changes may be governed by the NGF signaling mechanism, which plays crucial roles in odontoblast differentiation, dentine matrix synthesis and neuronal attraction [144,145]. NGF can promote the neural differentiation of DPSCs and is a potential growth factor for achieving functional dental pulp regeneration. For insistence, Zhang et al. successfully induced neural differentiation of DPSCs through the use of NGF and bFGF. After induction, DPSCs expressed higher levels of neural-related genes and protein expression. It has been confirmed that this process may be facilitated through the Extracellular Signal-Regulated Kinase (ERK) and Protein Kinase B (AKT) signaling pathways [146].

2.2.4. Extracellular vesicles(EVs) and gene therapy

2.2.4.1. Extracellular vesicles (EVs). Extracellular vesicles (EVs) are tiny lipid-covered vesicles released by cells. They carry nucleic acids, proteins, lipids, and other molecules, facilitating cell-to-cell communication and supporting normal cellular functions(Fig. 4A) [147]. Based on their biogenesis, EVs can be categorized into three main types: apoptotic bodies, microvesicles, and exosomes [148]. For example, exosomes derived from DPSCs cultured in odontogenic differentiation media were used to investigate the induction of odontogenic differentiation of DPSCs. It was confirmed that DPSCs could endocytose exosomes, leading to the activation of the P38 MAPK pathway and upregulation of odontogenic marker genes. In vivo experiments also demonstrated the regeneration of dental pulp-like tissue [149]. Similarly, Schwann cell-derived EVs promoted DPSCs proliferation, migration, osteogenesis while facilitated vessel and neurite formation, primarily through the stromal cell-derived factor 1/C-X-C Chemokine Receptor Type 4 (SDF-1/CXCR4) axis [150]. In another study, researchers investigated that apoptotic vesicles from SHEDs (SHED-apoVs) could be endocytosed by endothelial cells. And mitochondrial Tu translation elongation factor (TUFM) derived from SHED-apoVs could activate the Transcription Factor EB (TFEB)-autophagy pathway, promoting the proliferation, migration, and differentiation of endothelial cells (ECs). This resulted in an upregulation of genes associated with angiogenesis, thus facilitating vascularization of the dental pulp [151]. Due to the numerous advantages of EVs, recent years various engineering techniques, including preconditioning, drug loading, surface modification, artificial EVs have also been used to maximize the therapeutic potential of EVs to achieve functional tissue regeneration [152]. Although DPSC-derived extracellular vesicles (DPSC-EVs) have demonstrated acceptable safety and efficacy in both preclinical and clinical studies, reports of their successful application in humans are scarce yet. Several factors, including donor characteristics, vesicle preparation, and recipient health status, influence the success rate of DPSC-EVs in regenerating dental pulp. Additional research and clinical trials are necessary to optimize treatment strategies and confirm the regenerative capacity and efficacy of DPSC-EVs for dental pulp regeneration [153].

2.2.4.2. Gene therapy. Gene therapy can improve the growth, repair, and regenerative capacity of damaged tissues by regulating gene expression. Gene therapy begins by identifying the target gene and selecting an appropriate viral vector such as adenovirus or non-viral vector such as plasmid to deliver the target gene. Subsequently, the target gene is inserted into the cells using appropriate techniques. For instance, numerous genes like vascular endothelial growth factors (VEGF), bone morphogenetic protein2 (BMP-2), fibroblast growth factor (FGF) have been directly transfected into tissues [154,155]. In dentistry, Cao et al. transferred the hepatocyte growth factor gene into DPSCs using an adenovirus. Later, hepatocyte growth factor (HGF)-modified DPSCs was used in a swine model to regenerated periodontal tissue. The results showed that HGF-DPSCs significantly decreased DPSCs apoptosis and improved periodontal bone and blood vessel regeneration(Fig. 4B) [156]. Similarly, a study developed a gene therapy using Growth/differentiation factor 11 (Gdf11) electrotransfected pulp cells. It revealed that the electrotransfection promoted the differentiation of cells into dentin, and in vivo experiments demonstrated the formation of reparative dentin [157]. Furthermore, Zhang et al. designed PDGF-BB gene--modified DPSCs and showed its ability in promoting cell proliferation, angiogenesis, and odontogenic differentiation. PDGF-BB secretion by these modified cells aided in stem cell recruitment and improved the regeneration of dentin-pulp complexes in vivo [158]. In summary, gene therapy has the potential to enhance the potential of stem cells to differentiate into the desired cell types, thereby promoting the repair and regeneration of damaged tissues. However, extensive research and clinical trials are still needed to ensure its safety and effectiveness. Furthermore, regulatory, ethical, and societal issues must be appropriately addressed to ensure the sustainable development of gene therapy in the field of regenerative medicine.

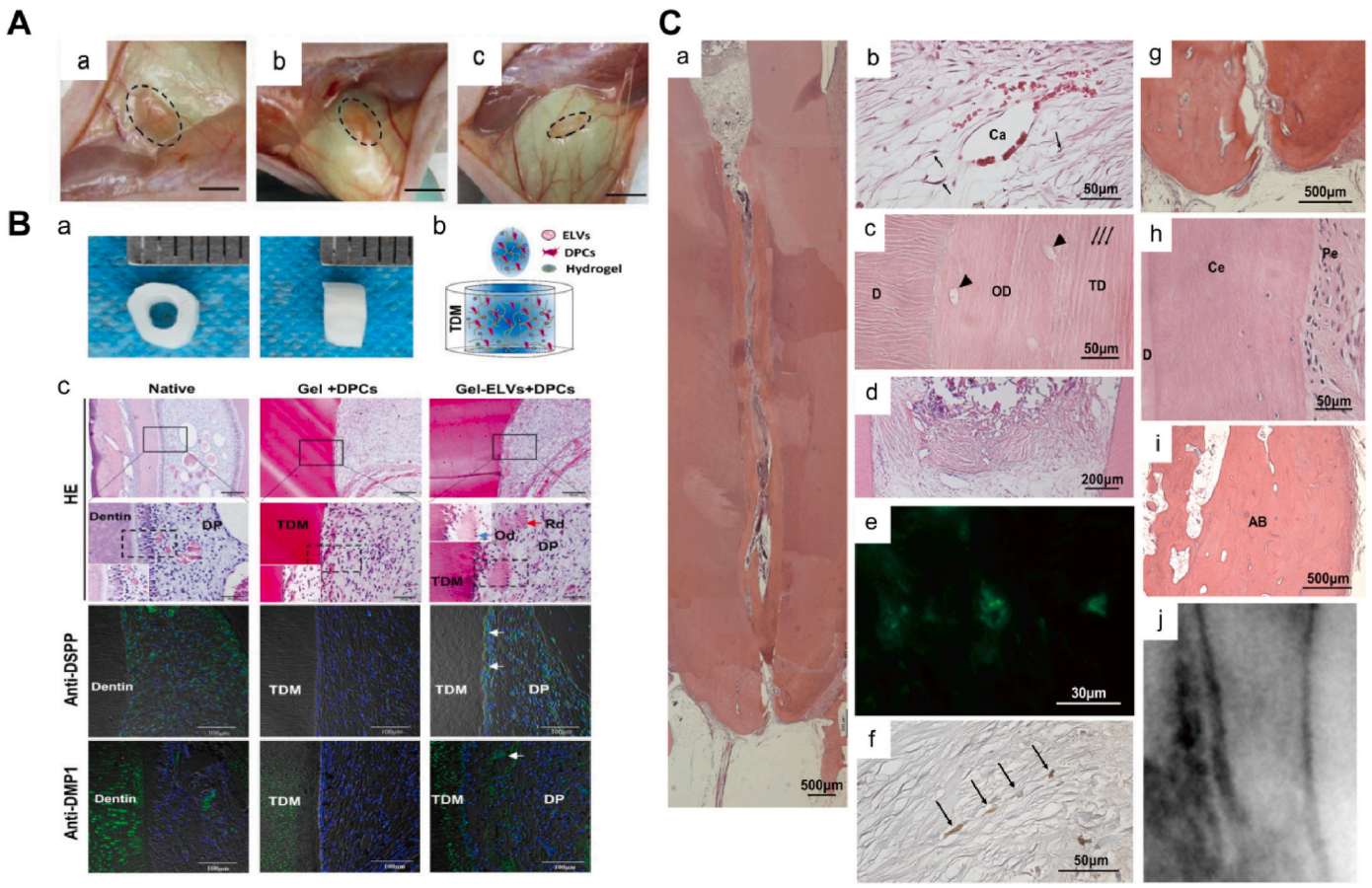

Fig. 5. Different kinds of research models for regenerative endodontics. (A) Ectopic regeneration model. Gross views of neo tissue in (a) NF-SMS group (b)NF-MS group and (c) S-MS group. Reprinted with permission from Ref. [160]. (B) Semiorthotopic regeneration model. (a) The implanted tooth root slice, with an internal diameter of 2mm and a height of 3mm. (b) Schematic of the preparation of in vivo transplants. (c) HE staining showing odontoblast-like cells and regenerated dentin-like tissue (red arrows) at the interface between the dentin and pulp-like tissue. Immunofluorescence analysis showing the upregulated expression of odontogenic differentiation markers in the Gel-ELVs + DPCs group, with positive staining (white arrows) represented by green stains. Reprinted with permission from Ref. [162]. (C) Orthotopic regeneration model. (a) A large amount of dentin-like mineralized tissue is formed along the dentinal wall. (b) Regenerated pulp tissue. Spindle-shaped pulp-like cells (arrows) and a capillary (Ca). (c) Higher magnification of osteodentin-like mineralized tissue (OD) and tubular dentin-like mineralized tissue (TD) formation. Osteodentinocytes in the lacunae (arrow heads). Dentinal tubules (arrows). Original dentin (D). (d) Osteodentin-like matrix formation on the regenerated pulp in the crown part. (e) BS1-lectin staining for angiogenesis. (f) PGP9.5 staining for neurite extension (arrows). (g) Periapical tissue. (h) Periodontal ligament (Pe) in the periapical region. Cementum (Ce). Original dentin (D). (i) Alveolar bone (AB). (j) X-ray analysis in the periapical region. Reprinted with permission from Ref[168].

2.3. In vivo models for regenerative endodontics

2.3.1. Ectopic regeneration model

Ectopic transplantation in tissue engineering refers to the process of transplanting artificially cultured tissues or cells to a location different from where they physiologically exist. To investigate the regenerative potential of DPSCs, they were isolated in vitro and co-transplanted with hydroxyapatite/tricalcium phosphate (HA/TCP) powder subcutaneously into immunodeficient mice. Following in vivo transplantation, the generation of dentin-pulp complexes was observed, indicating the potential application of DPSCs for dentin repair [21]. Likewise, Lee et al. constructed an ectopic model that transplanted subcutaneously a compound of DPSCs mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) with or without preameloblast-conditioned medium (PA-CM) or rhBMP-2 into immunocompromised mice. The results indicated that the addition of PA-CM to DPSCs resulting more extensive dentin/pulp-like structure formation, closely resembling the normal dentin-pulp complex [159]. Moreover, co-injection of DPSCs and microspheres under the skin of nude mice to establish an ectopic regeneration model confirmed the generation of a greater amount of dentin-like tissue (Fig. 5A) [160].

2.3.2. Semiorthotopic regeneration model

Semiorthotopic model is an experimental animal model that bridges the gap between orthotopic and ectopic models, allowing researchers to transplant cells or tissues to a location that is relatively close to the original but not entirely identical, in order to better study the effects of tissue regeneration and transplantation. Tooth slice is a valid model for studying semiorthotopic regeneration [161]. For example, Zhang et al. (Fig. 5B) [162]employed a method in which they filled root slice with a collagen gel containing DPSCs and exosome-like vesicles derived from Hertwig's epithelial root sheath (HERS) cell line to achieve pulp regeneration by subcutaneously implanting in mice. The results revealed that these constructs exhibited the formation of new structures resembling normal pulp-dentin tissue, offering potential avenues for tooth regeneration and therapy. Similarly, tooth fragment is also used as a semiorthotopic model in regeneration medicine. A vivo study established a semi-orthotopic model to investigate the impact of culture medium derived from 3D tooth germs (3D TGO-CM) on the regenerative potential of DPSCs. Subcutaneous implantation of tooth fragments filled with DPSCs and 3D TGO-CM was performed in nude mice. The results showed that 3D TGO-CM led to impressive early in vivo pulp regeneration, featuring well-organized pulp structures, attachment of odontogenic cells, and increased vascular formation [163]. Because of retaining

Table 3
Summary of in vivo models for pulp regeneration.

Scaffold	Agents loaded	Animals	Function	References
TDMs	SC-EVs; SDF-1	Semiorthotopic mice mode	Extracellular vesicles released by Schwann cells could recruit endogenous stem cells and promote dental pulp tissue regeneration.	[150]
TDMs	DPT-exos; DPC-exos	Semiorthotopic mice mode	DPT-exos better recruited SCAPs to regenerate dental pulp-like connective tissue	[55]
Tooth fragment/ SF scaffold	SDF-1α	Semiorthotopic mice mode	SDF-1α could trigger autophagy, promoting DPSCs migration and highly vascularized pulp and dentin-like tissue regeneration.	[172]
Three types of fibrin	eDMP	Semiorthotopic mice mode	Fibrin scaffolds with dentin matrix proteins increased the potential of stem cell chemotaxis and supported pulplike tissue formation and odontoblast-like cell differentiation.	[173]
nBG-coated dentin		Semiorthotopic mice mode	Nanobioactive glass (nBG) attracted stem cells toward dentin, enhanced cell adhesion, revascularization, and facilitated odontogenic differentiation, contributing to the regeneration of biomimetic pulp-dentin tissue structure.	[174]
Human-derived ACM		Orthotopic dog model	The human-derived composite amnion-chorion membrane cell homing model exhibited increased generation of fibrous tissue and dentin-like cells, along with reduced infection, suggesting promise for improving regenerative procedures in mature teeth.	[175]
Collagen gel	VEGF; bFGF; NGF; BMP7; PDGF	Semiorthotopic mice model	Pulp-like tissue was successful regenerated using a cell homing approach, involving the delivery of growth factors like bFGF, VEGF, or PDGF, along with NGF and BMP7.	[176]

certain anatomical and microenvironmental features, the semi-orthotopic model is relatively closer to the natural physiological environment compared to the ectopic model. However, it may still introduce some unnatural factors, such as blood flow and organ movement, which could potentially influence research outcomes.

2.3.3. Orthotopic regeneration model

The orthotopic model involves the direct implantation of scaffolds, growth factors, cells, and other components into the prepared root canals of animal teeth. It provides a better simulation of the physiological environment, allowing researchers to study tissue regeneration under conditions closely resembling the natural human processes. Due to the limited space within root canals, working with small animal models can be complex and challenging. Therefore, large animal models such as pigs, dogs, ferrets and sheep are often used in dental pulp regeneration research as orthotopic models [164].

Swine teeth share structural and functional similarities with human teeth, making pig a valuable in vivo model for oral research and specific studies in dental pulp tissue engineering. Recent research indicated that, in a miniature swine model, successful orthotopic pulp regeneration was achieved by implanting hydrogel carrying autologous stem cells into the root canal space. This resulted in the development of vascularized pulp-like tissue, accompanied by dentin-like structure [165]. However, Mangione et al. [166]also implanted porcine dental pulp cells with self-assembling nanopeptide hydrogel into minipigs' root canal but found that it primarily resulted in osteodentin bridges rather than pulp

Table 4
Representative clinical applications of pulp regeneration therapy.

Scaffolds	Stem Cells	Types of teeth in clinical trials	Outcomes	References
1.Periapical bleeding 2.Platelet-rich fibrin (PRF) 3. Collagen 4.Hydroxyapatite		Mature necrotic teeth	The readings of pulp sensibility did not exhibit significant differences across all intervals; however, in the cold test, the PRF group displayed the highest positive response rate, indicating the formation of new pulp-like tissues.	[177]
	SHED aggregates	Pulp necrosis after traumatic dental injuries	The SHEDs implantation group achieved regeneration of three-dimensional dental pulp tissue at 12 months, with increased root length, reduced width of the apical foramen, and regenerated pulp tissue containing sensory nerves.	[34]
Plasma-derived biomaterial	Human umbilical cord mesenchymal stem cells	Pulp necrosis and apical periodontitis	During the 12-month follow-up period, the umbilical cord mesenchymal stem cells implantation group exhibited significant vascular regeneration, and sensation tests indicated a notable increase in the proportion of positive dental pulp responses.	[178]
Leukocyte platelet - rich fibrin (L-PRF)	Autologous dental pulp stem cells	Symptomatic irreversible pulpitis	After 6 months and 3 years postoperatively, the tooth exhibited normal clinical responses, and periapical X-rays and CBCT imaging showed continued normal periapical area. However, vitality testing revealed decreased blood perfusion units, with delayed response to cold sensation but responsive to electric pulp testing.	[179]
1. Platelet - rich plasma 2. Platelet - rich fibrin 3.Platelet pellet 4. Blot clot		Immature necrotic incisors	During the follow-up period, all teeth exhibited similar and high levels of success, including periapical healing, radiographic root development, and positive responses to sensitivity tests. Sensitivity tests revealed that 86 % of the teeth had similar initial response times and responded positively to stimuli.	[15]
	Mobilized dental pulp stem cells	Irreversible pulpitis	At 4 weeks, the electric pulp test (EPT) exhibited a strong positive response. After 24 weeks, magnetic resonance imaging (MRI) showed signal intensity of the regenerated tissue in the root canal similar to that of normal dental pulp. Cone beam computed tomography (CBCT) revealed functional dentin formation in three out of the five patients.	[28]
	Autologous DPSCs with granulocyte colony-stimulating factor	Mature multirooted molars with pulpitis	At 4 weeks, the pulp vitality test showed a positive response; After 24 weeks, the signal intensity of the regenerated pulp tissue in the affected teeth on MRI was similar to that of the normal pulp in adjacent teeth. After 48 weeks, radiographs and CBCT showed no periapical radiolucency.	[180]

regeneration, indicating the need for further research to create a more favorable environment for pulp regeneration.

Canines are often considered as ideal models for dental research due to their similar growth patterns and pathophysiology to humans, as well as their ease of manipulation in research settings [167]. In a study involving beagle dogs, dental pulp extracellular matrix (DP-ECM) was implanted into debrided root canals for 8 weeks, resulting in the formation of dental pulp-like tissues with neo-vascularization and mineralized structures [77]. Interestingly, Iohara et al. explored the impact of trypsin pretreatment on pulpectomized teeth in aged dogs and its effects on pulp regeneration. Trypsin pretreatment increased the amount of regenerated pulp, enhanced the differentiation of cells into odontoblast-like cells, and influenced the expression of relevant genes (Fig. 5C) [168].

Ferrets serve as a promising animal model for regenerative endodontics due to their similarities to humans in craniofacial research. Their cuspid teeth size and root canal anatomy are suitable for various endodontic procedures, making them valuable for research in this field [169]. For insistence, blood clot and SynOss Putty were compared in immature ferret teeth to investigated endodontic treatment outcomes. The results showed that blood clot treatment created hard tissue formation in the root canal, while SynOss Putty was less effective, with minimal tissue regeneration and increased inflammation [170].

Due to their physiological and anatomical similarities to humans, sheep teeth can be used to test and evaluate potential treatment methods, including pulp regeneration, root canal therapy, periodontal disease treatment, and more. For example, a study aimed to assess the effectiveness of a pulp regeneration protocol on immature infected sheep teeth. After treatment, significant improvements were observed in root length, root wall thickness and apical canal diameter, with no notable differences compared to control teeth, indicating positive outcomes for endodontic regeneration and tooth revitalization in these sheep teeth [171].

Variations of these in vivo models and their semiorthotopic and orthotopic applications are summarized in Table 3.

2.4. Clinical applications or trials of pulp regeneration

With the advances in *in vitro* and in vivo researches on the pulp regeneration therapy, some clinical applications have been conducted and demonstrated positive results within different review periods (Table 4). Although animal model studies have provided valuable insights and evidences of treatment results of current pulp regeneration strategies, there are still many important issues to be clarified. For instance, the lack of standardized treatment protocols and procedural guidelines makes it difficult to compare different study outcomes, potentially affecting the stability of clinical treatment efficacy. Some treatment methods may pose potential safety risks, such as using allogeneic stem cells or genetic risks of gene-based strategy. The absence of long-term follow-up data on the efficacy of pulp regeneration therapy necessitates further observation and validation regarding treatment durability and potential side effects. These challenges hinder the clinical applications of pulp regeneration therapy. What's more, large size multicenter randomized clinical studies are required before the different pulp regeneration therapies could be properly used in clinical practices.

3. Challenges and future prospects

Pulp regeneration aims to eliminate symptoms, restore the pulp-dentin complex structure and promote dental pulp neurovascular regeneration for functional pulp regeneration. Cell homing-based dental pulp regeneration often leads to complications such as root canal calcification and tooth discoloration. Balancing the degree of root canal disinfection with residual cell viability is a critical issue to address. There is a need to design ideal root canal filling materials to eliminate residual microorganisms and promote stem cell homing. To successfully develop cell-based therapeutic approaches, it is essential to achieve controlled in vitro stem cell proliferation to generate a sufficient number of cells to meet therapeutic demands. Additionally, gene therapy can be employed to introduce target genes into stem cells to facilitate their directed differentiation, potentially generating pluripotent stem cells. Consideration must also be given to the immune response after stem cell implantation and monitoring the cell cycle to ensure genetic stability. To avoid immune reactions associated with cell transplantation, cell-free therapy based on EVs can circumvent ethical concerns and reduce the risk of tumor formation, immunogenicity, and infections associated with direct cell therapy. However, the current low yield and poor purity of EVs necessitate solutions for obtaining and purifying EVs to achieve standardized and large-scale production. Additionally, studies about EV storage conditions are needed to maximize the utilization of EV properties.

Scaffold materials play a crucial role in tissue engineering, and their design should take into account their biological, structural, physical, and chemical properties to meet the requirements of tissue regeneration. Thus far, a range of different scaffold types have been designed, from natural materials to synthetic materials, from single materials to composite materials, all meeting the design criteria for pulp-dentine complex tissue engineering. However, more research is needed to develop ideal scaffolds that fully meet the requirements for pulp-dentine complex tissue engineering in a clinical setting. Scaffolds, cells and bioactive factors constitute the regenerative microenvironment, which can mimic tissue microenvironments, affect the state of stem cells and regulate differentiation. Therefore, new perspectives on synthetic materials, such as those with antimicrobial effects, stimuli responsiveness (thermal, electric, magnetic field, etc.), and the loading of bioactive factors, show promising prospects for achieving pulp regeneration. Furthermore, standardization and homogenization of materials need to be further studied. A variety of technologies, such as electrospinning and 3D bioprinting, have been used to prepare structures with certain porosity that is suitable for cell migration, proliferation, and differentiation. However, the choice of the most suitable materials and technologies may hinder clinical translation, as different tissues and cells require different characteristics of biomaterials. So more clinical trials are necessary and future research breakthroughs in biomaterials are hoped to achieve the closer resemblance to normal tissues.

Achieving functional dental pulp regeneration, which involves the generation of dental pulp tissue with a normal dentin structure and neurovascular composition, is one of the most challenging aspects in the field of regenerative endodontics. For example, regenerative dentin may exhibit distinct microstructural features (such as tissue density, degree of mineralization, etc.) and chemical composition, which can influence its sensory function and vascularization level [28,33,39]. Selecting the appropriate stem cells and cell sources is a pivotal challenge. While various types of stem cells have been studied, determining the most suitable cell types for dental pulp regeneration still requires further research. Additionally, obtaining a sufficient quantity of cells can also be challenging. Therefore, it is essential to explore alternative cell sources and establish stem cell banks to enhance the feasibility of this process. The regulation of stem cell differentiation into various directions through growth factors and signaling molecules necessitates in-depth research. Balancing the intricate interactions among signaling pathways and understanding the mechanisms of neural and vascular regeneration are critical. Scaffolds design is equally important, as these scaffolds should mimic the natural structure of dental pulp tissue, be compatible with root canal morphology, facilitate the attachment of stem cells and growth factors, and support vascular, dentin and neural regeneration. Furthermore, the properties of scaffolds used for neural regeneration, dentin regeneration and vascular regeneration may differ, and consideration should include scaffold hardness, the relationship between degradation rate and tissue regeneration rate, and the characteristics of degradation products. More importantly, in the development and application of tissue-engineered dental pulp or scaffolds, space

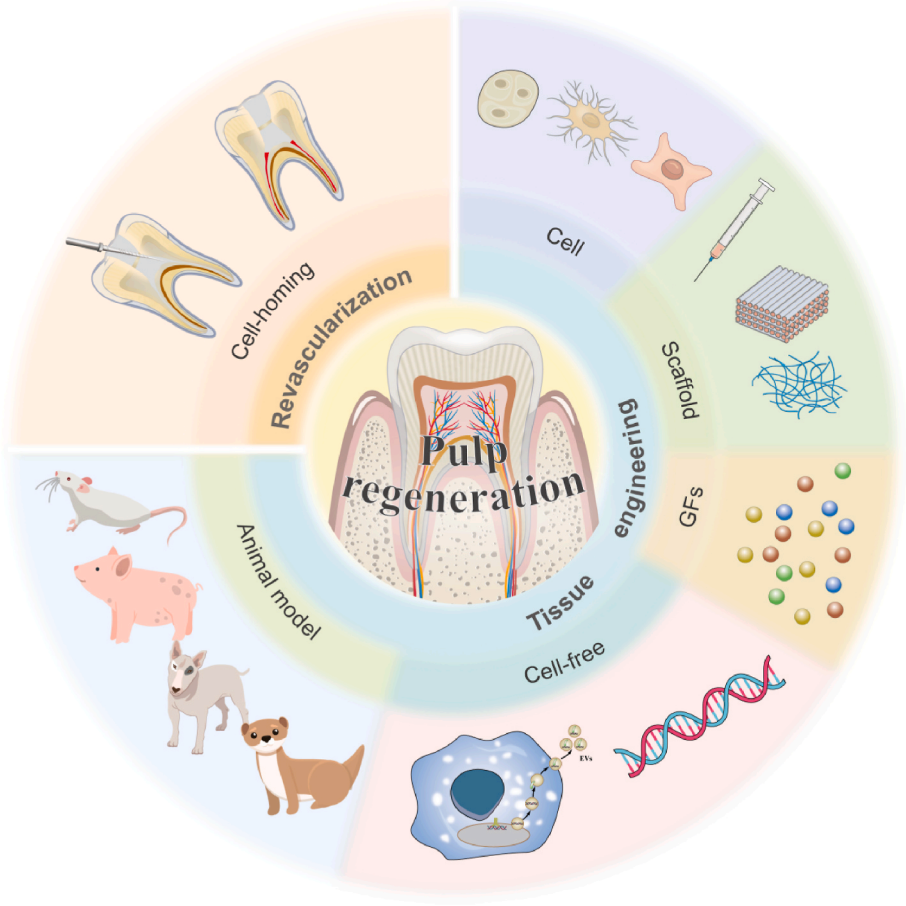

Fig. 6. Strategies for regenerative endodontics.

issues, correct placement, and clinical handling are crucial, as these factors directly impact the effectiveness and success rate of treatment. For instance, during dental pulp regeneration or scaffold placement, considerations must be given to the anatomical structure of the tooth, size of the pulp chamber, condition of surrounding tissues, and accuracy of clinical procedures. Dental pulp regeneration often involves a lengthy process. While numerous laboratory studies have been conducted, translating functional dental pulp regeneration methods from animal models to clinical practice remains a challenge. Clinical trials need to address ethical and regulatory issues while also focusing on strategies to reduce regeneration time and ensure the safety and efficacy of new methods.

In conclusion, selecting the ideal regeneration approaches, appropriate cells, growth factors, and scaffolds to meet the requirements for functional dental pulp regeneration remains a challenge. Despite extensive research efforts, tissue engineering for functional pulp regeneration requires deeper investigations into the molecular mechanisms behind the pulp regeneration and interactions among the cells, scaffold and bioactive molecules (Fig. 6).

Ethics approval and consent to participate

N/A.

CRediT authorship contribution statement

Xin-Lu Li: Writing – original draft, Investigation. **Fan Wei:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Bing Fan:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

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