Bioactive Materials 40 (2024) 182–211

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: www.keaipublishing.com/en/journals/bioactive-materials

Review article

# Tissue engineering approaches for dental pulp regeneration: The development of novel bioactive materials using pharmacological epigenetic inhibitors

Ross M. Quigley [a,b,**], Michaela Kearney [a], Oran D. Kennedy [b,c], Henry F. Duncan [a,c,*]

[a] Division of Restorative Dentistry & Periodontology, Dublin Dental University Hospital, Trinity College Dublin (TCD), University of Dublin, Lincoln Place, Dublin, Ireland
[b] Department of Anatomy and Regenerative Medicine, and Tissue Engineering Research Group, Royal College of Surgeons in Ireland (RCSI) University of Medicine and Health Sciences, Dublin, Ireland
[c] The Trinity Centre for Biomedical Engineering (TCBE) and the Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland (RCSI) and Trinity College Dublin (TCD), Dublin, Ireland

ARTICLE INFO

*Keywords:*
Epigenetics
Regenerative medicine
Regenerative endodontics
Biocompatible materials
Tissue engineering

ABSTRACT

The drive for minimally invasive endodontic treatment strategies has shifted focus from technically complex and destructive root canal treatments towards more conservative vital pulp treatment. However, novel approaches to maintaining dental pulp vitality after disease or trauma will require the development of innovative, biologically-driven regenerative medicine strategies. For example, cell-homing and cell-based therapies have recently been developed *in vitro* and trialled in preclinical models to study dental pulp regeneration. These approaches utilise natural and synthetic scaffolds that can deliver a range of bioactive pharmacological epigenetic modulators (HDACis, DNMTis, and ncRNAs), which are cost-effective and easily applied to stimulate pulp tissue regrowth. Unfortunately, many biological factors hinder the clinical development of regenerative therapies, including a lack of blood supply and poor infection control in the necrotic root canal system. Additional challenges include a need for clinically relevant models and manufacturing challenges such as scalability, cost concerns, and regulatory issues. This review will describe the current state of bioactive-biomaterial/scaffold-based engineering strategies to stimulate dentine-pulp regeneration, explicitly focusing on epigenetic modulators and therapeutic pharmacological inhibition. It will highlight the components of dental pulp regenerative approaches, describe their current limitations, and offer suggestions for the effective translation of novel epigenetic-laden bioactive materials for innovative therapeutics.

## 1. Introduction

Dental caries, while being a preventable non-communicable disease, remains a prevalent infectious condition, imposing a significant burden on healthcare systems and patients worldwide [1]. After the carious process breaches the enamel shell, it first stimulates pulpal inflammation ('pulpitis'), often with associated pain ('toothache'). If left untreated, the pulpitis will increase in severity, leading to eventual pulp death and necrosis [2]. Recent advancements in conservative endodontic treatment have led to an increased understanding of the regenerative capabilities of the dental pulp, resulting in the development of various regenerative endodontic therapies (RETs). These RETs have become a focal point for research efforts due to their potential to restore native dental tissues, in contrast to conventional reparative treatment strategies for advanced pulpitis and pulp necrosis. Traditional approaches, such as root canal treatment (RCT), involve replacing the entire pulp with an inert filling material, and vital pulp treatment (VPT), which replaces only part of the damaged pulp with a filling material [2]. RET procedures include pulp revitalisation through cell-homing and regeneration through a structured regenerative medicine technique. However, both RETs present different endpoints, with revitalisation

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author. Division of Restorative Dentistry and Periodontology, Dublin Dental University Hospital, Trinity College Dublin, Lincoln Place, Dublin, Ireland.
** Corresponding author. Division of Restorative Dentistry and Periodontology, Dublin Dental University Hospital, Trinity College Dublin, Lincoln Place, Dublin, Ireland
*E-mail address:* hduncan@tcd.ie (H.F. Duncan).

https://doi.org/10.1016/j.bioactmat.2024.06.012
Received 12 March 2024; Received in revised form 5 June 2024; Accepted 6 June 2024

| Abbreviation | Term |
|---|---|
| 5-Aza-CdR | 5-Aza-2′-Deoxycytidine |
| BMMSC | Bone Marrow Mesenchymal Stem Cells |
| BMP | Bone Morphogenetic Protein |
| BDNF | Brain-Derived Neurotrophic Factor |
| CH | Chlorhexidine |
| CM | Conditioned Media |
| DNMT | DNA Methyltransferases |
| DNMTi | DNMT Inhibitors |
| dECM | Decellularised ECM |
| DDM | Demineralised Dentine Matrix |
| DFSC | Dental Follicle Stem Cells |
| DPC | Dental Pulp Cells |
| DPSC | Dental Pulp Stem Cells |
| DMP1 | Dentin Matrix Acidic Phosphoprotein 1 |
| DSP | Dentin Sialoprotein |
| DMC | Dentine Matrix Components |
| DEX | Dexamethasone |
| EMA | European Medicines Agency |
| ECM | Extracellular Matrix |
| EV | Extracellular Vesicles |
| FGF | Fibroblast Growth Factor |
| FDA | Food and Drug Administration |
| G-CSF | Granulocyte Colony-Stimulating Factor |
| GDF | Growth Differentiation Factor |
| GF | Growth Factor |
| HDACi | HDAC Inhibitors |
| HERS | Hertwig's Epithelial Root Sheath |
| HDAC | Histone Deacetylases |
| HDMEC | Human Dermal Microvascular Endothelial Cells |
| HUVEC | Human Umbilical Vein Endothelial Cells |
| HCSC | Hydraulic Calcium Silicate Cements |
| HA | Hydroxyapatite |
| HIF-1α | Hypoxia-Inducible Factor 1 Alpha |
| LPS | Lipopolysaccharide |
| lncRNA | Long ncRNA |
| MMP | Matrix Metalloproteinase |
| MDR | Medical Device Regulation |
| MSC | Mesenchymal Stem Cells |
| mRNA | Messenger RNAs |
| miRNA | MicroRNAs |
| MS | Microsphere |
| MTA | Mineral Trioxide Aggregate |
| mDPSC | Mobilised DPSCs |
| NF | Nanofibre |
| NGF | Neural Growth Factor |
| ncRNA | Non-Coding RNA |
| OD | Odontoblastic Differentiation |
| PDL | Periodontal Ligament |
| PDLSC | Periodontal Ligament Stem Cells |
| PDGF | Platelet-Derived Growth Factor |
| PRF | Platelet-Rich Fibrin |
| PRP | Platelet-Rich Plasma |
| PLGA | Poly-L-Glycolic Acid |
| PLLA | Poly-L-Lactic Acid |
| PCL | Polycaprolactone |
| PEG | Polyethylene Glycol |
| PGA | Polyglycolic Acid |
| RISC | RNA-Induced Silencing Complex |
| RET | Regenerative Endodontic Therapies |
| RCT | Root Canal Treatment |
| SAP | Self-Assembly Peptide |
| SP | Side Population |
| SIM | Simvastatin |
| siRNA | Small Interfering RNA |
| SME | Small and Medium Enterprises |
| SC | Stem Cells |
| SHED | Stem Cells from Human-Exfoliated Deciduous Teeth |
| SCAP | Stem Cells from the Apical Papilla |
| SDF | Stromal Cell-Derived Factor |
| SI | Subcutaneous Implantation |
| SAHA | Suberoylanilide Hydroxamic Acid |
| 3D | Three-Dimensional |
| TF | Transcription Factors |
| TAZ | Transcriptional Coactivator with the PDZ-binding motif |
| TGF- β | Transforming Growth Factor β |
| TDM | Treated Dentine Matrix |
| TCP | Tri-Calcium Phosphate |
| TSA | Trichostatin A |
| TAP | Triple Antibiotic Paste |
| VPA | Valproic Acid |
| VEGF | Vascular Endothelial Growth Factor |
| VPT | Vital Pulp Treatment |

inducing angiogenesis into a previously necrotic root canal and pulp regeneration aiming to functionally re-establish the odontoblast cells, as well as angiogenic and neurogenic supply [3]. Notably, despite a host of research in this area coupled with the inherent regenerative capacity of the dental pulp, regenerative medicine approaches have yet to be successfully translated into a commercial dental product [4].

Successful RET has the potential to revolutionise the field of dentistry and endodontics, reducing the invasiveness of procedures while offering patients improved outcomes, enhancing quality of life, and increasing the focus on preserving natural dentition. Developing these new regenerative strategies hinges on the three principal components of regenerative medicine: scaffolds, cells, and signals [5]. Tailoring each of these three constituents to the confines of an often-infected root canal system presents opportunities and challenges for clinical translation. Equally, developing simple and cost-effective solutions is paramount in dentistry, limiting the feasibility of particular options, such as using growth factor (GF)-laden scaffolds. Consequently, this review provides a comprehensive analysis of advancements in the clinical translation of bioactive biomaterial-based regenerative medicine approaches, mainly focusing on the role of pharmacological inhibitors and epigenetic modulator-doped scaffolds. Furthermore, consolidating this information with key findings from culture, animal, explant, and human studies in the literature reveals promising avenues and significant hurdles in the clinical translation of these technologies.

## 2. Pulpal & apical disease: current therapies & limitations

Pulpal and periapical diseases are inflammatory dental conditions typically caused by bacterial infection [6]. The prevention and management of these diseases are at the core of clinical endodontics [7]. Current therapies for advanced pulpal disease generally involve the removal of the entire pulp in a procedure called RCT, a technically challenging, invasive, and expensive treatment that removes the reparative capacity of the dentine-pulp complex and weakens the remaining tooth substance, making it prone to fracture [8–10]. As a result, there has been a shift towards minimally invasive, biologically based techniques that maintain the vitality of the pulp or RETs that aim to re-establish vital pulp tissue [11,12].

In a healthy state, the pulp tissue is protected by an outer shell of mineralised hard tissue, specifically dentine and enamel, preserving the

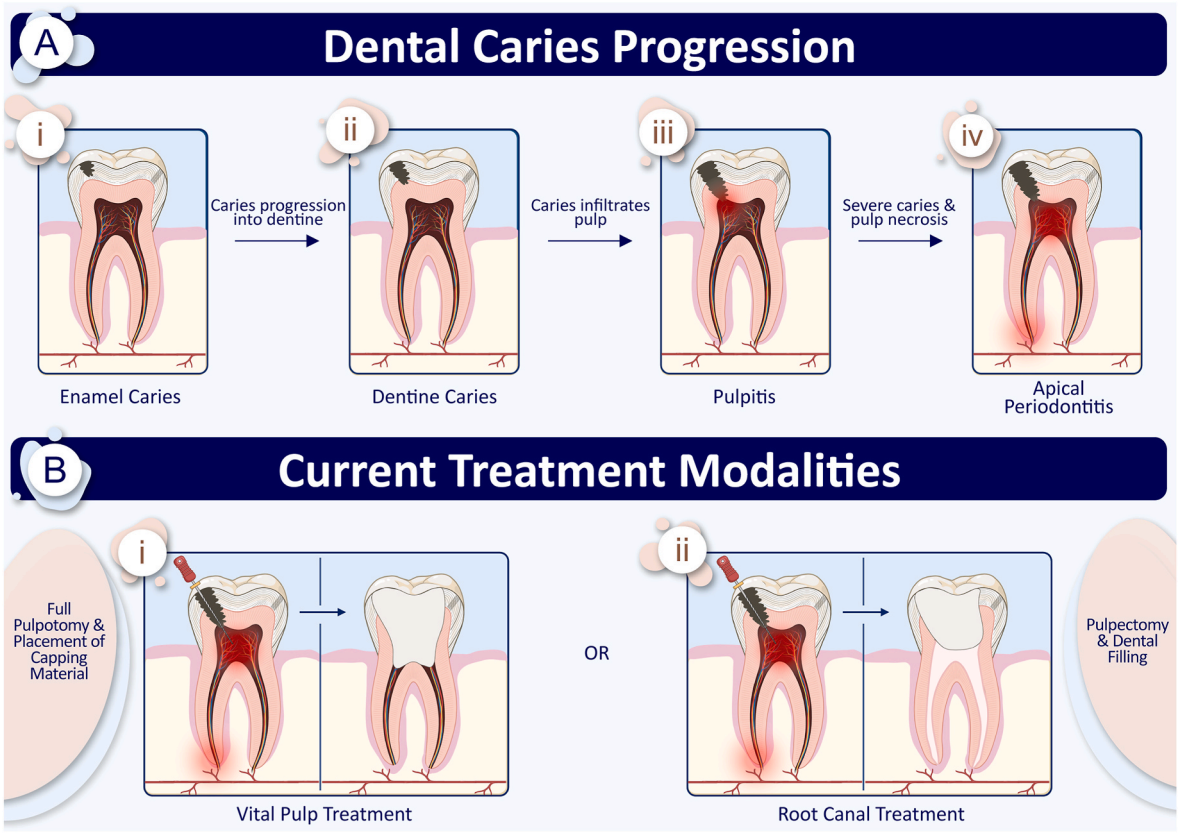


**Fig. 1. | Dental caries progression & current treatment modalities**. (A) Dental caries progression (i) Oral biofilm elicits excessive dietary carbohydrate fermentation, leading to enamel demineralisation (ii) Caries progresses into the dentine, stimulating pulpitis (iii) Caries infiltrates the dental pulp, inducing pulpitis (iv) Caries left untreated results in pulp necrosis and subsequent apical periodontitis. (B) Current treatment modalities (i) Vital pulp treatment involving a full pulpotomy & capping material induces a reparative response (ii) Root canal treatment uses a pulpectomy followed by dental filling, which is destructive and costly.

pulp's blood and nerve supply, which are critical for both tooth development and repair [13]. The close inter-relationship between the pulp and dentine has been reflected in the widespread use of the term dentine-pulp complex [14], which, although functionally helpful, has been called a biological oversimplification by some investigators [15]. The presence of a peripheral layer of terminal secretory cells called odontoblasts along the dentine-pulp interface, which represents the cells that form dentine, is central to the complex [16]. Odontoblastic processes extend into the dentinal tubules, assisting dentine formation and transmitting information to the odontoblast when threatened by caries or other irritants [14,16,17].

Caries or tooth decay occurs due to the natural accumulation of a microbial biofilm ('plaque') on teeth that produces acidic by-products that break down tooth substances [18]. The microbes in plaque are energised by recurrent exposure to fermentable dietary carbohydrates and, if not mechanically removed, lead to shifts in the bacterial microflora [19]. The persistent synthesis of acid leads to the demineralisation of dental hard tissues, which, if allowed to progress without treatment, will lead to cavitation and advancement of the lesion through dentine towards the pulp (Fig. 1) [20].

The secretion of dentine occurs by primary dentinogenesis during tooth development [17]. These primary odontoblasts remain active throughout an individual's lifetime, continuing to synthesise new dentine in a process known as secondary dentinogenesis, albeit at a much slower rate [21,22]. Additionally, existing odontoblasts or new 'odontoblast-like' cells can produce tertiary dentine in response to irritation in localised areas adjacent to the external stimuli [23]. If the stimuli are mild, moderately deep caries or a slowly advancing lesion, the primary odontoblasts survive and secrete 'reactionary' tubular dentine. If the stimuli are more aggressive (deep caries or rapidly advancing lesion), the odontoblasts may die and be replaced in a complex process called 'reparative' dentinogenesis by newly differentiated odontoblast-like cells [24].

Without intervention, the carious process will move towards the pulp, eventually entering the tertiary dentine (Fig. 2). It is not until the bacteria enter the tertiary dentine that the inflammation becomes severe, manifesting as an acute pulpitis, with areas of pulpal necrosis and eventually the development of apical periodontitis [25,26]. It should be remembered, however, that although caries remains the predominant cause of pulpal disease, severe periodontal disease and dental trauma also represent potential avenues for pulpal infection.

Although preventable, untreated caries in permanent teeth remains a prevalent health condition [1], estimated at 29 % globally, albeit showing a slight 2.6 % decrease between 1990 and 2019 [27]. Unfortunately, the burden has shifted with a 121 % and 74 % increase in low-income and low-middle-income countries, respectively, primarily driven by population growth [27]. Epidemiological studies in the United States highlight a 91 % prevalence of caries among adults, with approximately 27 % remaining untreated [28]. In younger patients, the prevalence varies between 20 % and 50 %, increasing proportionally until age 19 [29]. The socio-economic impact of caries is considerable, with an estimated global expenditure of $541 billion for dental diseases in 2015 alone [30], highlighting that current prevention approaches are inadequate and largely ineffective.

RCT is the current treatment of choice for irreversible pulpitis and pulp necrosis and, if carried out to a high technical quality, can be a very successful procedure [31]; however, the bulk of RCT is carried out in a range of primary care settings and is of mixed technical standard. This is highlighted by reports of apical disease in 10–50 % of endodontically treated teeth in cross-sectional studies of the general population in

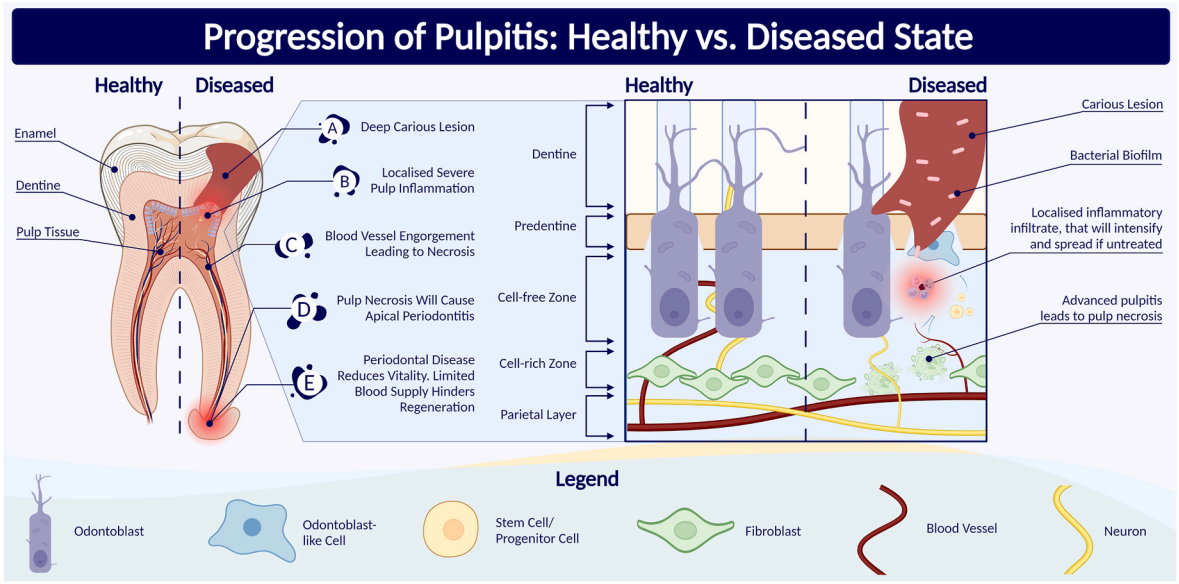


**Fig. 2. | Schematic of healthy and diseased pulp tissue.** (A) The microbial biofilm in the carious lesion will advance through dentine, stimulating pulpitis and the release of a range of inflammatory cytokines, chemokines as well as defensive molecules. (B) The pulpitic response intensifies as the lesion nears the pulp, eventually disrupting the odontoblast layer. If left untreated, the localised inflammation will spread apically and increase in severity, with the eventual death of odontoblast cells. If the carious disease is treated, the pulp can recover and repair by forming odontoblast-like cells in a process known as reparative dentinogenesis. (C) If the disease is not treated, initial blood vessel engorgement will lead to localised necrosis, which will spread apically. (D) Apical periodontitis will ensue when the canal is necrotic and the pulp space has become infected. (E) Although caries is the most common cause of pulp death, as the pulp is an end arteriole with no collateral circulation, pathogens from the periodontium can also affect vitality if the lesion extends to the tooth apex through advanced periodontal disease. Regeneration is possible if pulp cells remain in the root canal or can be 'homed' through the apex or supplemented as part of a cell-based treatment; however, this process is compromised by a lack of supplementary blood supply.

various territories [32–34].

Driven by improved biological responses, material developments, and a search for more straightforward treatments, dental practices have shifted over recent years to focus on minimally invasive and regenerative endodontic techniques [35]. These techniques are based on seminal research, showing that managing inflammatory pulp reactions is possible if the irritation is removed and the cavity conventionally restored [36,37].

VPT aims to preserve the health of all or part of the pulp by harnessing its intrinsic reparative capacity [35]. VPT is a common practice that is carried out across dental practices worldwide. Retaining part of the pulp has long demonstrated efficacy as a therapeutic modality; however, the precise quantification of its effectiveness remains unknown. A recent study conducted among endodontists in Italy and Ireland by Careddu and Duncan [38] highlights the frequency of VPTs within their respective practices. In the Italian cohort, 32 % of dentists reported completing 1–5 cases monthly, with 33 % handling 5–10 cases and 36 % managing over 10 cases. Conversely, Irish respondents exhibited a lower prevalence of VPT, with 61 % addressing 1–5 cases, 13 % handling 5–10 cases, 9 % managing over 10 cases, and 18 % abstaining from conducting any VPT procedures altogether.

Historically, calcium hydroxide [$Ca(OH)_2$] has been used as a biomaterial to contact the exposed pulp since its introduction in 1928; however, pulp capping with these materials has shown inconsistent treatment outcomes [39], potentially due to a lack of sealing ability [40–42] and cytotoxicity concerns [43]. These issues introduced concern about the efficacy of VPT as an alternative treatment to RCT. The introduction of new materials, such as hydraulic calcium silicate cements (HCSCs), including mineral trioxide aggregate (MTA) and Biodentine, has demonstrated superior histological and clinical outcomes compared to $Ca(OH)_2$ and heralded new enthusiasm for VPT in research and clinical practice [44–46].

Reflecting these changes, the European Society of Endodontology and the American Association of Endodontists have issued position statements advocating pulp retention after pulp exposure, promoting biologically based, minimally invasive approaches, and highlighting areas for further research [11,47].

While VPT effectively preserves part or all the pulp, its reparative nature often results in low-quality reparative tertiary dentine formation [48]. Furthermore, the molecular mechanisms behind the actions of direct capping materials remain incompletely understood and are generally considered unspecific [7,49]. VPT also relies on some residual pulp tissue and is not applicable when the pulp is necrotic or if there is a need to regenerate lost tissue. The lack of pulp regeneration into the pulp chamber following pulpotomy can complicate both sensibility assessments of the residual pulp and re-entry into the pulp should subsequent issues arise. The necessity for intuitive, targeted RETs highlights the next step in improving healing responses, regenerating pulp tissue, and applying direct regenerative medicine approaches.

## 3. Scaffold biomaterials & advanced fabrication techniques

Regenerative strategies using scaffolds offer promising avenues to advance treatment options beyond the traditional repair methods seen in RCT and VPT. Scaffolds in regenerative medicine are typically three-dimensional (3D) porous biomaterials which can provide structural support for regenerative processes and facilitate the delivery of cells and/or bioactive agents [50]. Recapitulation of the physiological extracellular matrix (ECM) structure of tissues through scaffolding also offers a way to control the spatial positioning of cells [51] and orchestrate cell processes such as adhesion, differentiation, proliferation, migration, and metabolism [52]. An ideal scaffold should facilitate effective gaseous exchange, nutrient delivery and waste transport [52]. A controlled biodegradation rate is also essential, as mechanical failure may occur if degradation is too rapid; in contrast, poorly designed materials can also elicit inflammatory responses [53]. Therefore, the optimal scenario is for degradation to occur at a rate that matches the production of newly developed tissue to prevent inflammation,

**Table 1**
| Natural polymeric biomaterials used within dental pulp regenerative efforts and their advantages and disadvantages.

| Biomaterial | Advantages | Disadvantages | Ref. |
|---|---|---|---|
| Collagen | · Major ECM component of the native dental pulp<br>·Cross-linking can improve strength and degradation<br>·Rough surface morphology enhances cell seeding<br>·Easy placement of cells and bioactive agents<br>·Various forms (sponges, gels, sheets)<br>·Trap for osteoconductive factors<br>·Soft & hard tissue formation<br>·Good tensile strength | ·Dependence on mineralisation for rigidity and strength<br>·Poor anti-inflammatory properties<br>·Poor stability in aqueous solution<br>·Contraction/shrinkage *in vivo*<br>·Difficult disinfection | [23,59–67] |
| Gelatin | ·Encouraging cell infiltration, adhesion, and proliferation<br>·Controlled release of bioactive agents<br>·Physicochemical tailorability<br>·Efficient cell seeding<br>·Osteoconductive | ·Effectiveness hinges on crosslinked composites reducing cost-effectiveness and reproducibility<br>·Time-consuming processing results in material and cell loss<br>·Crosslinked gelatin may lose bioactivity and not degrade<br>·Higher immunogenicity than collagen precursor<br>·Poor stability & mechanical properties | [68–77] |
| Chitosan | ·Encourages cell adhesion, proliferation, and differentiation<br>·Can be used as a thermosensitive hydrogel<br>·Promotes mineralised tissue deposition<br>·Delivery of bioactive agents<br>·Potential for cell-homing<br>·Promotes haemostasis<br>·Antimicrobial<br>·Cost-effective | ·Chemical modification can introduce toxicity<br>·Rapid and unpredictable degradation *in vivo*<br>·Difficult processing due to poor solubility<br>·Difficult to control pore size<br>·Low mechanical integrity | [78–85] |
| Fibrin | ·Encourages cell adhesion, differentiation, and angiogenesis<br>·Mimics blood clots without causing discolouration<br>·Controlled, slow release of bioactive agents<br>·Can bind and stabilise bioactive agents<br>·Promotes haemostasis<br>·Autologous (blood)<br>·Easy handling | ·Variable pore size and structure<br>·Mechanically weak<br>·Rapid degradation | [86–91] |
| Hyaluronic Acid | · Degradation products may contribute to ECM formation<br>·Contributes to the formation of native dental pulp<br>·Encourages cell adhesion and ECM secretion<br>·Promote angiogenesis and innervation<br>·Potential to provide faster healing<br>·Anti-inflammatory<br>·Bioactive | ·Required crosslinking complicates processing and increases variability<br>·Context-dependent degradation: rapid during pulpitis but slow without | [67,92–96] |
| Alginate | ·Can be modified with binding motifs to enhance adhesion<br>·Potential as efficient drug delivery platforms<br>·Supports neurotrophic factor release<br>·Rapid gelation without crosslinking<br>·Mechanically stable<br>·Cost-effective | ·Mammalian cells do not have receptors for alginate, limiting adhesion and endogenous cell migration<br>·Net negative charge inhibits protein adsorption and reduces adhesion<br>·Auto-gelation results in non-uniform bioactive agent release kinetics<br>·Alginate shrinks in acidic environments, such as in pulpitis (~6.0)<br>·Precise control of *in vivo* degradation rate is challenging<br>·Potential inflammation with less pure forms<br>·Slow degradation (months) | [73,93,97–100] |
| Silk Fibroin | ·Sites that enhance mineralisation and bind bioactive agents<br>·Supports cell attachment, proliferation, & differentiation<br>·Native mechanical strength, elasticity, & flexibility<br>·Various forms (hydrogels, implants, sponges)<br>·Minimal inflammatory reactions<br>·Oxygen and water permeability<br>·Controlled degradation rate<br>·Low immunogenicity<br>·Easy processing<br>·Cost-effective | ·Traditionally produced scaffolds are brittle and weak<br>·Poor attachment of certain cell types (neuronal)<br>·Weak osteogenic properties<br>·Biologically inert<br>·Slow degradation | [98–107] |
| PRF/PRP | ·Encourages cell adhesion, migration, proliferation, and differentiation<br>·Can be processed as injectables or liquids for easier handling<br>·PRF enhances GF entrapment and cellular migration<br>·PRF does not require anticoagulants for production<br>·Anti-microbial and anti-inflammatory properties<br>·Cytokines released enhance angiogenesis<br>·Autologous scaffold biomaterials<br>·Facilitates cell-homing<br>·Cost-effective | ·Diversity and concentration of bioactive components introduce batch-to-batch heterogeneity<br>·Scaffold is produced during treatment, increasing complexity and time<br>·PRP requires activators that can produce unfavourable rigid structures<br>·Peripheral blood lacks GFs specific for dental pulp regeneration<br>·IV blood requirement necessitates patient acceptance<br>·Uncontrollable degradation and mechanical strength<br>·No propensity for an 'off-the-shelf' product<br>·Specialised equipment and reagents needed<br>·Lack of standardised production | [108–111] |

immunogenicity, and toxicity.

The native pulp is a unique connective tissue with collagen, non-collagenous proteins, GFs and inflammatory capabilities [54]. Extensive research will be required to identify a suitable, easily applied, cost-effective biomaterial closely mimicking its composition and mechanical and molecular structure to facilitate angiogenesis and neurogenesis on the appropriate scale. Additional complexity is introduced with scaffold fabrication as the biomaterial choice dictates the fabrication methods available and the degree of control over scaffold properties [55]. Porosity, pore size, and interconnectivity are essential as these

**Table 2**
| Synthetic polymeric biomaterials used within dental pulp regenerative efforts and their advantages and disadvantages.

| Biomaterial | Advantages | Disadvantages | Ref |
|---|---|---|---|
| PCL | ·Supports cellular recruitment, differentiation, angiogenesis, and neurogenesis<br>·Mechanical properties suitable for load-bearing<br>·Potential as efficient drug delivery platforms<br>·FDA-approved for medical devices<br>·Easy and cost-effective production<br>·Biocompatible and biodegradable<br>·Printable polymer | ·Lacks support for cell adhesion<br>·Biologically inert<br>·Slow degradation<br>·Hydrophobic | [71, 114–120] |
| PLLA | ·Facilitates cell seeding, nutritional exchange, tissue formation and angiogenesis<br>·Potential as efficient drug delivery platforms<br>·FDA-approved for medical devices<br>·Controlled degradation rate<br>·Mechanical strength | ·Acidic byproducts may impair differentiation of seeded cells<br>·Degradation may liberate sequestered DMCs<br>·Rigid scaffolds are hard to maneuverer<br>·Difficult to insert into small space<br>·Lack recognition signals for cells<br>·Long resorption time<br>·Slow degradation | [91, 121–129] |
| PGA | ·Several cell types adhere and develop, including dental-derived cells<br>·Tuneable mechanical and physiochemical properties<br>·Support growth of mature tooth structures<br>·Reproducible synthesis<br>·Biodegradable | ·Acidic byproducts may hamper the regeneration of dentine–pulp complex<br>·Insoluble in most solvents introduces complex processing<br>·Initial extreme stiffness limits manoeuvrability<br>·Can induce a local inflammatory response<br>·Rapid degradation<br>·Biologically inert | [94, 130–134] |
| PEG | ·Multiple curing methods (i.e., dental curing light)<br>·Potential as efficient drug delivery platforms<br>·Potential to include bioactive motifs<br>·Tuneable mechanical properties<br>·Resistant to protein adsorption<br>·Biocompatible<br>·Water-soluble<br>·Hydrophilic<br>·Non-toxic | ·Polymerising components may be cytotoxic<br>·Poor cell attachment<br>·Biologically Inert | [91,135,136] |
| PLGA | ·Tailorable mechanical and physiochemical properties<br>·Porous architecture facilitates effective cell seeding<br>·Support growth of mature tooth structures<br>·Low toxicity and immunogenicity<br>·FDA-approved for drug delivery<br>·Controllable degradation<br>·Resistant to contraction<br>·Mechanical strength<br>·Ease of processing | ·Acidic byproducts may hamper the regeneration of dentine–pulp complex and cause inflammatory reactions<br>·Degradation pattern depends on specific monomer sequence<br>·Dissolves in a wide range of common solvents<br>· Slow degradation | [134, 137–141] |
| SAP | · Contains cleavable sites for controlled degradation<br>·Shape memory maintains integrity under stress<br>·Potential as efficient drug delivery platforms<br>·Facilitates cell survival and differentiation<br>·Formation of hydrogels with variable viscoelastic properties<br>·Mimic native dental pulp ECM<br>·Contains cell adhesive motifs<br>·Biocompatibility<br>·Biodegradability<br>·Easy synthesis<br>·Injectability | ·At low concentrations, they can be fragile and hard to inject<br>·Variable immunogenicity dependent on design<br>·Stability and consistency limitations<br>·Weak mechanical strength<br>·Complex design process<br>·Intrinsic instability<br>·Hydrophobic | [112, 142–144] |
| Bioceramics | ·Possibility for drug delivery within nanoparticles<br>·Numerous FDA-approved materials<br>·Low cytotoxicity<br>·Biocompatible<br>·Anti-microbial<br>·Bioactive | ·Well suited to direct pulp capping<br>·Possibility of tooth discolouration<br>·Mild inflammatory response<br>·Prolonged curing time<br>·Prone to fracture<br>·Osteoconductive<br>·Rigid structure<br>·High cost | [145–150] |
| Metals | ·Can improve mechanical integrity of integrated biomaterial<br>·Possibility for drug delivery within nanoparticles<br>·High uptake within cells<br>·Anti-microbial<br>·Stable *in vivo* | ·Better suited to hard tissue regeneration, such as bone<br>·Complex synthesis and handling<br>·Nanoparticles can be cytotoxic<br>·Osteoconductive<br>·Oxidative stress<br>·High cost | [151–153] |

properties facilitate effective nutrient and waste transport [56]. Interconnectivity allows for enhanced cellular signalling and migration, increased surface area for cell seeding, and enables effective angiogenesis and neurogenesis. However, excess porosity can compromise the mechanical stability of the scaffold, which is undesirable as the mechanical integrity and stiffness stimulate cell behaviour, fate, and tissue development [57].

Biomaterials and scaffolds can be categorised based on their origin as either natural or synthetic. Natural biomaterials are proteins (e.g. collagen) which possess intrinsic biocompatibility and biodegradability (Table 1). However, their degradation rate and mechanical properties are often insufficient without modification [5,58].

In contrast, synthetic scaffolds are manufactured and can be further subdivided into polymeric, bio-ceramic, or metallic materials (Table 2). Synthetic materials can be advantageous as they facilitate more significant control over mechanical properties and scaffold microarchitecture.

They are also relatively inexpensive compared with natural biomaterials [112]. However, these materials are more likely to induce inflammatory and immunogenic responses or produce toxic catabolic by-products/processes [50]. Hybrid scaffolding allows for the consolidation of multiple biomaterials to enhance the physio-mechanical properties of natural biomaterials whilst attenuating the biocompatibility concerns associated with synthetics [113].

Ultimately, recapitulation of the native dental pulp matrix is the primary goal of scaffold biomaterials within dental pulp regenerative contexts [154]. One of the most straightforward methods in approaching this is employing the native pulpal matrix. Decellularised ECM (dECM) scaffolds are derived from whole tissues, such as dental pulp, whereby the cellular components have been removed, retaining the micro- and macro-scale components, architecture, and function of ECM proteins [155]. The obvious merits of dECM-based scaffolds are their similarity to native tissues, their intrinsic bioactivity in guiding cell behaviour, and their potential to retain native vasculature [156]. Dental pulp-derived dECM contains various bioactive factors, including transforming growth factor β (TGF-β), dentin matrix acidic phosphoprotein 1 (DMP-1), dentin sialoprotein (DSP), and vascular endothelial growth factor (VEGF) [156,157]. Numerous studies have shown pulp-like tissue formation and angiogenesis, likely due to bioactive factor-induced cell homing [156–160]. Numerous studies employ allogenic dECM scaffolds, which are widely available, reseeding them with a combination of human-derived cells [156–158]. However, the major limitation of dECM-based strategies is the varying degrees of cellularity and ECM damage achieved with different processing methods, including physical, enzymatic, or chemical degradation [155,161,162]. Overall, successful dECM translation hinges on addressing complex processing challenges, difficult disinfection, and batch-to-batch variation [163].

Dentine represents a rich reservoir for various sequestered bioactive factors that may be liberated in response to certain stimuli [164]. This unique anatomical property is typically utilised within regenerative strategies by mobilisation of these dentine-matrix components (DMC) in response to some endodontic irrigants [165]. Recently, bioactive materials derived from dentine tissues have been developed, known as treated dentine matrix (TDM) or demineralised dentine matrix (DDM) [166]. TDM/DDM-based biomaterials preserve the DMCs and serve as a carrier/scaffold for their delivery in regenerative efforts [167]. Studies have consistently shown that TDM/DDM forms vascularised dentine-pulp-like tissue [168–173]. Recently, modifications to these biomaterials have enhanced their translatability; for instance, lyophilisation/cryopreservation notably lengthens their shelf-life [174,175], and incorporation within alginate hydrogels facilitate minimally invasive therapeutics that are well-aligned for soft tissue regeneration [176]. Processing of these materials is complex, involving the pulverising of dentine tissue, followed by either complete or partial EDTA-mediated demineralisation, the degree of which affects the portion of mineralised components retained [166]. While osteo-inductive and osteo-conductive biomaterials improve hard tissue regeneration, such as bone or dentine, their merits for regenerating the soft dental pulp tissue warrant consideration [137]. These materials likely result in scattered calcified tissue forming within the regenerated pulp tissue, thus failing to recapitulate the native ECM. Furthermore, the successful translation of dental tissue-derived matrices depends on developing effective and standardised sterilisation protocols, removing microbial contamination and endotoxins, and eliminating immunological risks [177].

While biomaterial choice is profoundly important in regenerative medicine strategies, the choice of fabrication method can significantly affect their suitability for different applications. Common strategies used in dental pulp regeneration studies include hydrogels, microspheres, cell sheets, and nanofibrous scaffolds (Fig. 3). Hydrogels comprise a 3D network of hydrophilic polymer chains, allowing them to absorb and retain large volumes of water and offering a flexible matrix for nutrient diffusion, waste removal, and drug delivery [95,178]. Hydrogels are advantageous as they ameliorate the severe processing issues of common scaffold materials, facilitating the incorporation and entrapment of viable cells [179]. Furthermore, the hydrated environment of hydrogels closely mimics that of native ECM, supporting cell survival, proliferation, and differentiation. This hydrophilicity also enables clinically applicable and minimally invasive strategies using liquid hydrogel injectables and implants [179]. Microspheres, injected locally, enhance nutrient diffusion, provide a 3D environment for cell interaction, and facilitate bioactive agent incorporation and controlled release [98]. Cell sheets, a scaffold-free option, offer physical support but may have limitations in mechanical properties and clinical manoeuvrability [180]; however, due to the pulp's mineralised encasement, mechanical properties are less important in the tooth than elsewhere in the body. Nanofiber scaffolds offer highly porous constructs with a large surface-to-volume ratio, though they may be mechanically compromised [181].

Various pulpal regeneration studies have employed scaffolds

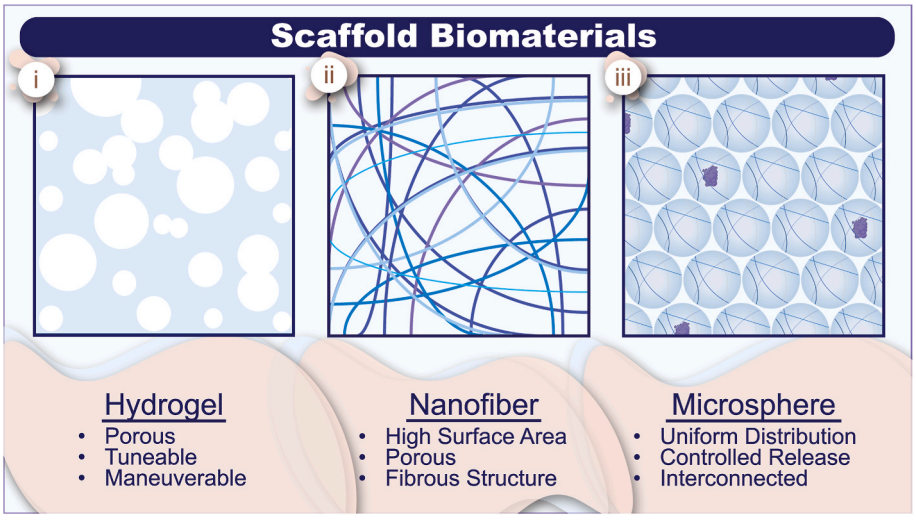


**Fig. 3. | Common biomaterial-based approaches employed in regenerative strategies.** (i) Hydrogel scaffolds are porous, mimic the extracellular matrix, facilitate effective nutrient diffusion and waste removal, and are clinically aligned due to their manoeuvrability. (ii) Nanofibrous scaffolds are highly porous and interconnected, have a large surface area to facilitate enhanced cellular adhesion during seeding, and their fibrous structure mimics that of native extracellular matrix (iii) Microsphere scaffolds allow for the controlled release of bioactive agents incorporated within.

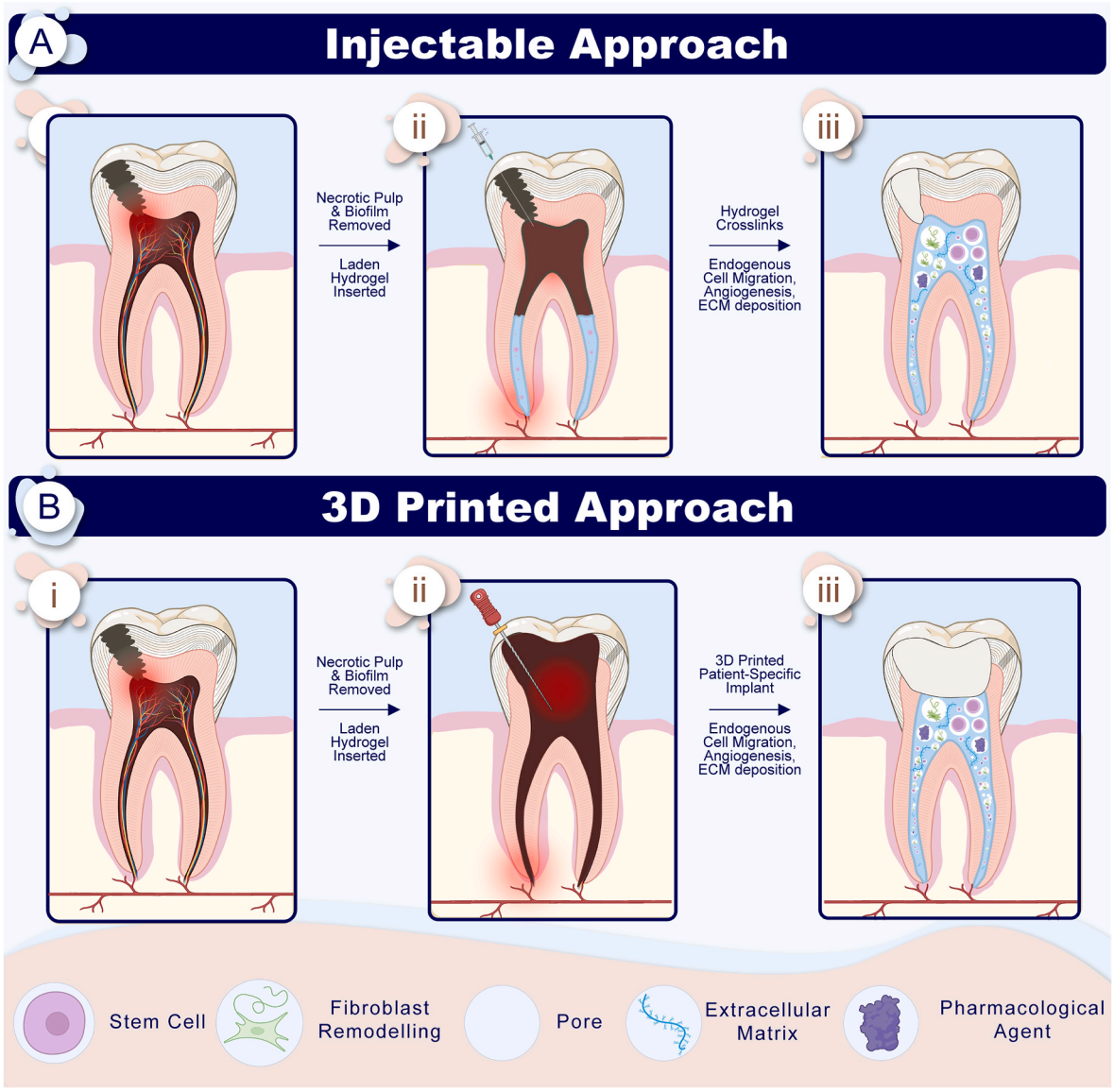


**Fig. 4. | Advanced techniques employed in novel regenerative approaches.** (A) Injectable approach (i) Caries infiltration into the dental pulp induces pulpitis (ii) The necrotic pulp and biofilm are removed, and the bioactive agent-laden hydrogel is injected into the empty root canal (ii) The hydrogel is crosslinked by an appropriate method and generates endogenous cell migration, angiogenesis, and extracellular matrix deposition. (B) 3D printed approach (i) Caries infiltration into the dental pulp induces pulpitis (ii) The necrotic pulp and biofilm are removed, followed by the formation of a large cavity to fit the patient-specific bioactive agent-laden hydrogel that induces endogenous cell migration, angiogenesis, and ECM deposition.

fabricated using traditional methods such as electrospinning or freeze-drying. However, there has been a distinct lack of clinical translation towards human use in the context of endodontic regenerative approaches. Conventional approaches cannot precisely regulate parameters such as pore size, pore geometry, pore interconnectivity, the spatial distribution of pores, and the formation of internal channels within the scaffold [182]. More advanced techniques have emerged, such as 3D printing and injectable fabrication approaches, offering certain advantages over traditional methods, which are less clinically flexible (Fig. 4).

3D printing has evolved to allow the creation of 3D shapes by printing successive biomaterial layers [183]. This offers precise control of scaffold morphology and internal microstructure for cellular growth in biomimetic tissues *in vivo* [184]. Three main techniques – inkjet, laser-assisted, and extrusion-based are employed, providing fine control, time/cost efficiency, and the unique ability for a "multiphasic" configuration [185]. Multiphasic configurations enable scaffold synthesis with regions of varying composition, which could allow for recapitulation of the cell-rich and cell-free zones of native dental pulp. Nonetheless, variables like print speed and biomaterial viscosity can be controlled to facilitate this approach's highly tuneable characteristics and enhanced scaffold resolution, ensuring that the structure accurately mimics the intricate anatomy of the dental pulp on the appropriate scale [186]. However, adapting 3D-printing technologies for biological tissues faces challenges, specifically when using inks laden with biological components. Moreover, locating and establishing suitable off-site manufacturers presents another hurdle to the clinical alignment of 3D printed

strategies. To resolve this problem, Duarte Campos et al. [187] suggested a hybrid injectable and 3D printed approach using a hand-held bioprinter for *in situ* deposition of cell-laden collagen-based bioinks.

While both 3D printing and injectable approaches have advantages in regenerative medicine, injectable approaches potentially face fewer hurdles to clinical translation. Rosa et al. [188] assessed the feasibility of injectable cell-laden hydrogels for *in vivo* experimentation in full-length human root canals. This investigation demonstrates the synthesis of vascularised soft connective tissue resembling physiological pulp, albeit with limited odontoblastic differentiation. However, further analysis of scaffold properties is necessary to optimise regenerative approaches for pulp recapitulation. Moreover, researchers have proposed using a photo-initiator for injectable scaffold crosslinking by UV light from dental curing lights, which are ubiquitous in dental practices [189].

### 3.1. Opportunities

Numerous biomaterials show great potential for dental pulp regeneration. Recently, novel advancements in producing dental tissue-derived materials present a straightforward yet effective bioactive material for dental pulp regeneration that warrants further investigation. Additionally, while the effects of mechanical stiffness on hard tissue regeneration are considerable, such as for dentine or bone tissues, it is unlikely to be as crucial for regenerating soft dental pulp. However, it would be interesting to investigate the effects of scaffolds with variable stiffness, for instance, using more rigid scaffolds in the coronal portion and softer materials within the root canal, on the pluripotency of resident pulpal stem cells. This might enhance odontoblastic differentiation, facilitating neovascularisation, endogenous cell migration, and innervation.

Furthermore, injectable and 3D-printed approaches in regenerative endodontics present significant translational opportunities. 3D bioprinting allows for the synthesis of multi-phasic scaffolds that mimic dental pulp zones and support pre-vascularisation for efficient nutrient diffusion. Injectable techniques align well with current clinical practice, offering minimally invasive and *in situ* applications of regenerative solutions. Photo-polymerisation using existing dental-curing lights, available in every dental practice, provides a clinically translatable and economical approach to crosslink scaffolds. Hybrid *in situ* approaches employing hand-held bioprinters with 3D printing technologies present a novel avenue for clinical translation.

### 3.2. Challenges

Biomaterials are the fundamental basis of any regenerative approach; however, numerous disadvantages need to be considered within dental pulp contexts, including acidic by-products, slow degradation, poor cell attachment, high cost, difficult disinfection, and insufficient manoeuvrability. Additionally, a lack of robust *in vivo* studies with clinically relevant modalities due to the current over-reliance on non-representative models. 3D printing encounters a significant limitation with patient-specific constructs requiring multiple appointments, the establishment of off-site manufacturers, large-scale changes to current clinical practice, and potential structural damage to the tooth. Standardisation and regulation of off-site manufacturers are costly and require significant investment for dentists and patients. Whilst injectable approaches are less refined, they offer a single-visit and minimally invasive chairside solution.

## 4. Cell-based therapies

Cell loading introduces an opportunity to initiate biological processes using specific cell types within the scaffold environment. Stem cells (SC) are clonogenic cells capable of self-renewal and multi-lineage differentiation [190]. The use of SCs in regenerative strategies is attractive because it offers scope for controlling cell fate and the regeneration of physiologic tissue architecture [122,191]. Cell-based therapies can induce dental pulp regeneration directly or indirectly. Direct applications involve the application and differentiation of adult SCs, whereas indirect treatments achieve regeneration by cell-homing and bioactive molecule secretion to modulate endogenous cellular processes [192].

Employing either of these methods is helpful for multiple dental pulp regenerative processes, including the vascularisation of the newly formed pulp tissue. Pulp blood vessels are end-arterioles with no lateral circulation, so unless cells are supplied within the empty pulp space, new cells can only enter through the apical foramen by cell-homing [112]. Due to these challenges, cell-homing has only shown limited success in regenerating the pulp-dentine complex in revitalisation procedures; however, the result is generally reparative bone-like tissues [193–195]. Ideally, in a true regenerative strategy, newly differentiated odontoblasts should interface with the existing dentine to regenerate the pulp-dentine complex and secrete new tertiary dentine [137]. Another crucial regenerative component is neurogenesis, as nerve fibres in the pulp tissue contribute to angiogenesis, inflammatory regulation via

**Table 3**
**| A comprehensive review of cell types and models employed in dental pulp regeneration strategies and their key findings.** Entries in **bold** indicate *in situ* approaches.

| Cell Type | Model | Procedure | Key Finding(s) | Ref. |
|---|---|---|---|---|
| $CD31^-/CD146^-$, $CD31^+/CD146^-$ | Canine | Pulpotomy | · $CD31^-/CD146^-$ SP cells induce angio- & vasculogenesis and pulp regeneration<br>· NaOCl- might have inhibited GF release | [198] |
| DPSCs | Rodent | SI HA/TCP | · DPSCs can self-renew, respond to environmental stimuli, & differentiate into adipocyte and neural cells<br>· Identified hierarchy of progenitors in DPSCs, with a SP having high proliferation & multi-potency | [190] |
| DPSCs | Rodent | SI collagen | · Regenerative triad allows for precise organisation of collagen matrix and angiogenesis<br>· DPSCs alone were insufficient for dental-pulp regeneration; triad was crucial | [63] |
| DPSCs & HUVECs | Rodent | SI GelMA | · Pulp-like tissue with organised collagen, vascular networks, & reparative dentine formation<br>· Outperformed acellular scaffolds & empty root canals in terms of attachment and organisation<br>· Human cells appeared to migrate into surrounding tissue | [76] |
| DPSCs & SCAPs | Rodent | SI tooth-slice | · Well-vascularised pulp-like tissue-filled root canal, depositing dentine without tubules | [137] |
| DPSCs, SCAPs | Rodent | SI HA root | · SCs did not induce enhanced vascularisation compared to control scaffolds | [281] |
| HUVECs & DPSCs | Rodent | SI tooth-slice | · DPSCs release pro-angiogenic factors, inhibiting HUVEC apoptosis<br>· Tissue ingrowth was limited in root segments | [143] |
| SHEDs & HDMECs | Rodent | SI tooth-slice | · Dentine-secreting odontoblast-like cells with micro-vessel formation & anastomosis<br>· No increase in microvasculature with HDMECs | [122] |
| SHEDs | Rodent | SI root | · SHEDs demonstrated potential as a single source of dental pulp regeneration<br>· Acellular inserts in root canals showed poorly organised tissue | [189] |
| SHEDs | Rodent | SI tooth-slice | · SHEDs could differentiate into functional odontoblasts and endothelial cells<br>· SHED dentine deposition was three-fold that of physiologic dentine | [207] |

**Table 4**
**| A comprehensive overview of growth factors and morphogens employed in dental regenerative studies *in vivo*.** Key findings about the *in vivo* analyses are included, whereas *in vitro* results were excluded. Components with (−) were absent in individual studies, and entries in **bold** signify a clinical trial. The findings indicated with ↑ show an increase compared to other test groups or controls.

| GF/Morphogen | Material(s) | Cell Type | Key Finding(s) | Ref. |
|---|---|---|---|---|
| BMP-2 | – | pDPCs | ↑ OD, and reparative dentine formation | [282] |
| | PLGA NF-MS | SCAPs | ↑ OD, mineralisation, and osteodentine | [185] |
| BMP-7 (+DMX) | NF-PLLA | hDPSCs | ↑ OD, pulp-like tissue, angiogenesis; high doses inhibited proliferation | [272] |
| BMP-7 | Collagen 1 | – | ↑ Reparative dentine formation and mineralisation | [66] |
| | Collagen | – | ↑ Reparative dentine and dentine bridge formation | [271] |
| | Collagen | – | A single dose is insufficient for inflammation | [270] |
| | Collagen | – | ↑ OD, reparative dentine formation, and mineralisation | [283] |
| | Chitosan/Collagen | hDPSCs | ↑ OD and proliferation | [79] |
| BMP-9 | β-TCP | hDPSCs | ↑ OD, reparative dentine formation, and mineralisation | [284] |
| DMP-1 | Collagen | hDPSCs | ↑ Matrix deposition and angiogenesis | [63] |
| | Collagen | hDPSCs | ↑ OD, matrix and calcified tissue deposition | [23] |
| FGF-2 | Gelatin | – | ↑ Root length and thickness | [285] |
| | Gelatin | – | ↑ OD and dentine-like deposits | [68] |
| | Silk fibroin | hDPSCs | ↑ OD, angiogenesis, matrix deposition, and dentine-like tissue formation | [286] |
| | Gelatin | – | ↑ Pulp-like tissue and dentine bridge formation and angiogenesis | [70] |
| G-CSF | Collagen | MDPSCs | ↑ Angio- and neurogenesis, and pulp regeneration | [287] |
| | Collagen | MDPSCs | Age-dependent decline in regeneration | [278] |
| | Collagen | MDPSCs | ↑ Pulp- and dentine-like tissue formation and angiogenesis | [288] |
| | **Collagen** | **MDPSCs** | **Safe & effective regenerative therapy** | [280] |
| | Collagen | MDPSCs | ↑ Angiogenesis and pulp-like tissue formation | [279] |
| GDF-11 | – | pDPCs | ↑ Reparative dentine formation | [289] |
| | – | pDPCs | ↑ OD, reparative and tubular dentine formation | [282] |
| NGF | PCL | – | ↑ Innervation and angiogenesis | [116] |
| SDF-1 | Collagen 1:3 | $CD105^{+}$ | ↑ Angio- and neurogenesis, pulp regeneration | [290] |
| | Collagen 1:3 | $CD31^{-}/CD146^{-}$ | ↑ Angio- and neurogenesis, pulp regeneration | [196] |
| | Collagen | BMSCs | ↑ Pulp regeneration and angiogenesis | [194] |
| | Silk fibroin | hDPSCs | ↑ Pulp regeneration, angiogenesis, and dentine deposition | [101] |
| TGF- β1 | HA | DMCCs | ↑ OD, dentine formation, and angiogenesis | [94] |
| VEGF | PLLA-MS PDO | hDPSCs | ↑ OD, pulp-like tissue formation, and angiogenesis | [126] |
| | | – | ↑ Pulp-like tissue formation and angiogenesis | [263] |

extravasation of immune cells, pulpal homeostasis, and defence mechanisms [196].

### 4.1. Cell sources

Multiple cell types within the pulp and surrounding tissues can contribute to dental pulp formation and maintenance.

DPSCs.

Dental pulp stem cells (DPSCs), which possess properties similar to mesenchymal stem cells (MSCs), have been assessed *in vitro* and *in vivo* for their proliferation and differentiation capacity. Employing hydroxyapatite/tri-calcium phosphate (HA/TCP) scaffolds in a subcutaneous implantation (SI) model, Gronthos et al. [197] demonstrated dentine-like formation with a collagen matrix perpendicular to the odontoblast-like layer. This study characterised DPSCs as clonogenic, highly proliferative postnatal SCs capable of tissue regeneration. Furthermore, these cells are also readily available within 'high-street' dental practices. However, despite promising preclinical research, *ex vivo* expansion of DPSCs through good manufacturing processes and subsequent transplantation has yet to be implemented in clinical practice [198].

SHEDs.

Another unique cell-based option in the dental field is stem cells from human exfoliated deciduous (SHED) teeth. Miura et al. [199] identified and explored SHEDs *in vitro* and *in vivo* using a HA/TCP scaffold in SI immunodeficient mice models. SHEDs demonstrated clonogenicity, high proliferation, and multi-potent differentiation into adipogenic, odontoblastic, and neurogenic cell lineages. SHEDs are readily available from any child shedding deciduous ('baby') teeth and have thereby gained attention in medicine and dentistry. Compared with DPSCs, SHEDs exhibit superior proliferation, serving as a promising allogenic SC source. They also hold the potential for autologous treatment in young patients with pulp necrosis arising from trauma [200]. Initially considered incapable of regenerating the dentine-pulp-like complex [199], though this view has been challenged, these studies employ GFs in conjunction with cellular therapies [122,188,191]. Miura et al. [199] indicated that bone formation attributed to SHEDs may result from an osteo-conductive network, now understood to result from the osteo-conductive HA/TCP scaffold rather than direct osteogenic differentiation [201].

SCAPs.

However, another option is stem cells from the apical papilla (SCAP), which Sonoyama et al. [202] identified as a multipotent population, demonstrating differentiation into odontoblast-like cells and adipocytes *in vitro*. In *ex vivo* models, SCAPs transplanted into mice with HA/TCP scaffolds formed dentine structures and connective tissue. Furthermore, SCAPs showed greater proliferative potential than DPSCs, suggesting enhanced regenerative potential. *In vivo,* studies like Wang et al. [203] and Xiao et al. [204] emphasised the importance of using SCAPs in combination with other regenerative medicine triad components for optimised odontogenic differentiation (Table 2). SCAPs sourced from third molars offer the advantage of banking for later autologous use, but the impact of freeze-thaw cycles on cell viability requires further investigation.

### 4.2. Other cell types & populations

Other oral SC populations, like periodontal ligament stem cells (PDLSC) and dental follicle stem cells (DFSC), focus on periodontal ligament (PDL) and cementum regeneration [205,206]. Some groups have employed human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HDMEC) for vasculo- and angiogenesis [122,143,207]. Using SCs tailored for these processes is beneficial for targeted neurogenesis and angiogenesis. Dental pulp

**Table 5**
**| A comprehensive overview of combinatorial and comparative analyses of growth factors and morphogens employed in dental regenerative studies *in vivo*.** Key findings about the *in vivo* analyses are included, whereas *in vitro* results were excluded. Entries with (−), (/), and (±) are absent in individual studies, combinatorial use, comparative use, and both, respectively. The findings indicated with ↑ show an increase compared to other test groups or controls.

| GFs/ Morphogens | Material(s) | Cell Type | Key Finding(s) | Ref. |
|---|---|---|---|---|
| BMP-2/-4/TGF-β1 | Collagen 1 | – | TGF-β1 inhibits regeneration | [65] |
| BMP-4, FGF-2 | Collagen/ GelMS | rDPCs | OD and angiogenesis | [291] |
| BMP-7±TGF-β1 | SA-MS | – | Combined encapsulation upregulated DSP expression. | [99] |
| FGF-2/G-CSF | Collagen | MDPSCs | No difference in regenerative potential | [292] |
| FGF-2, TGF-β1, VEGF | MDP Hydrogel | hDPSCs | ↑ OD, chemotaxis and angiogenesis | [112] |
| FGF-2/VEGF/ PDGF, NGF, BMP-7 | Collagen | – | FGF-2 demonstrated increased regenerative potential | [193] |
| FGF-4, FGF-9 | SAP | CNCLCs | ↑ OD, dentine formation, and angiogenesis | [293] |
| PDGF-BB, NGF, BDNF | Collagen 1 | BMSCs | ↑ OD, angio- and neurogenesis | [195] |
| SDF-1α/BMP-2 | VitroGel | SCAPs | ↑ OD in combinatorial use of GFs | [204] |
| SDF-1α/FGF-2/ BMP-7 | Collagen 1 | hDPCs | ↑ Angiogenesis & chemotaxis with FGF-2 | [273] |
| TGF-β1, DMX | Chitosan/ Alginate | – | ↑ Chemotaxis & angiogenesis | [294] |

cells (DPC) are a heterogeneous population of cells obtained from human primary teeth containing less than 2 % SCs [208]. Studies have also investigated diverse cell types, such as human pulpal and gingival fibroblasts, and their capacity for dental pulp regeneration [130]. However, achieving control over angiogenesis, vasculogenesis, and neurogenesis requires a multifaceted approach. This involves combining bioactive agents to interact with endogenous cells and stimulate host signalling mechanisms to elicit the desired biological response.

### 4.3. Tooth banking

A key challenge in dental regenerative strategies is the need for autologous SCs, as allogenic sources likely necessitate immunosuppressive treatment, which is prohibitive within simple dental procedures based on the risk-to-benefit ratio. Establishing commercial tooth banks could address this limitation by storing dental tissue for later use. The banking workflow involves donor eligibility assessment, tissue collection, cell isolation, quality control, cryopreservation, and post-thaw quality control assessments. Standardising European practices encompassing protocols, procedures, good manufacturing practices, and meticulous documentation is essential for the widespread adoption of these organisations [209].

Although a potentially costly option, numerous tooth banks exist globally with the ability to extract multiple SC populations from a single tissue source. This versatility offers the potential to address pathologies other than dental caries, such as myocardial infarction and diabetes, and applications in osseous and neural tissue regeneration [210]. Tooth banks also facilitate the synthesis of decellularised and native matrix-derived scaffold biomaterials for autologous use. The multifactorial use of SCs and the presence of established organisations underscores their potential efficacy in dental pulp regeneration.

### 4.4. Direct cell-based therapies

#### 4.4.1. Monodispersed cells

Many studies have employed different cell types in dental regenerative contexts (Table 3). Numerous *in vivo* studies have used SCs combined with other factors from the regenerative medicine engineering triad (Tables 4 and 5); as such, the key findings of such studies cannot be solely attributed to the presence of cells or lack thereof.

However, this analysis emphasises two significant issues in this field: a lack of *in situ* approaches in higher mammals and the repeated use of 2D models, wherein the findings poorly translate when replicated in 3D cultures. Additionally, investigators tend to tunnel vision on odontogenic differentiation, with only some studies also considering angio-/vasculo-/neurogenesis. Iohara et al. [198] addressed both limitations with an *in situ, in vivo* experiment using transplantation of side population (SP) cell-laden collagen scaffolds into canine dog teeth following partial pulpotomy, finding strong trophic potential to induce angio-/vasculogenesis and pulp regeneration.

#### 4.4.2. Cell spheroids

Numerous efforts have been made to employ cell-based dental pulp regenerative therapies *in vivo* with limited success. Scaffolds often fail to recapitulate the essential functions of native ECM; specifically, cell-scaffold interactions lack the signal integration properties that regulate intracellular crosstalk in three dimensions [211]. Additionally, incorporating monodispersed cells into a scaffold is typically achieved using enzymatic digestion of the cell-secreted ECM, severing cellular connections. In contrast, cell spheroids present a promising solution widely used in alternative regenerative strategies [212]. In this approach, cells form dense aggregates that maintain their endogenous ECM, facilitating cell-matrix and cell-cell interactions. However, several challenges persist, including the limited donor supply for autologous sources, the potential necessity for immunosuppressive therapy with allogenic sources, and extensive time and cost requirements for spheroid production [212].

Cell spheroids are attractive because they enhance cell survival *in vivo* and upregulate trophic factor secretion compared with dissociated cells [213,214]. In contrast, monodispersed MSCs, including dental progenitors, are subject to low survival rates and rapid dedifferentiation upon removing soluble osteogenic stimuli, whereby seemingly terminal differentiated cells revert to a pluripotent state [214–217]. In comparison, induced MSC spheroids exhibit improved retention of the differentiated phenotype mediated by the cell-secreted ECM, albeit short-lived [218]. This phenotypic persistence can be further enhanced by incorporating functionalised micro-/nanoparticles [219,220]. Additionally, microparticles can be used to alter the mechanical properties of the spheroid to further direct differentiation lineage [221]. In combination, this enables the development of tuneable, scaffold-free approaches with sustained-release capabilities.

While the trade-off between autologous and allogeneic cell-based therapies is a significant challenge in the field, it has been observed that, despite multiple engraftments, immune-matched allo-MSCs exhibit immune-evasive properties, thereby not mounting immunogenic reactions. Instead, dental pulp-derived MSCs demonstrate an immunomodulatory and anti-inflammatory capacity mediated by numerous chemokines [222–224]. Notably, hypoxia-inducible factor 1 alpha (HIF-1) production under hypoxic conditions, such as those created within spheroid conformations, is both angiogenic and immunomodulatory [222,225–227]. Using this property, spheroids can be pre-exposed to osteogenic induction or hypoxic conditions before transplantation to enhance regenerative outcomes. The exact mechanisms of these effects necessitate further characterisation, although a recent preclinical trial of immune mismatched DPSCs transplanted into canine incisors demonstrates no toxicity or adverse events [228].

Interestingly, the cell source challenge can be entirely circumvented using cell spheroid-conditioned media (CM). In this method, the culture

medium becomes saturated with the cell secretome containing various anti-apoptotic, immunomodulatory, angiogenic, and neurotrophic constituents [229]. When applied to primary DPCs, CM collected from Hertwig's epithelial root sheath (HERS) cells enhanced odontoblastic differentiation-associated gene expression *in vitro* and produced ordered, mineralised dentine-like tissue and odontoblast-like cells *in vivo* [227]. Likewise, CM from pulp CD31$^-$ SP cells produced pulp-like tissue with well-organised vasculature in ectopic models, demonstrating increased migration of endogenous cells, angiogenesis, and decreased apoptosis compared to CM from bone marrow or adipose SP cells, albeit less than that observed in direct CD31$^-$ pulp SP cell engraftment [198, 230]. These results were mirrored in an orthotopic model using SHED-derived CM, forming pulp-like tissue and promoting angiogenesis [231]. To expand on this, Sarra et al. [232] added DPSC-CM in a direct pulp capping method, observing distinct anti-inflammatory effects. Comparing the effects of 2D and 3D derived CM, Zhou et al. [233] delineated the enhanced *in vivo* regenerative capacity of 3D tooth germ cell CM to derive from a superior secretome profile. Collectively, this offers a framework for developing cell-free, "off-the-shelf" platforms by which endogenous cells can be exposed to the diverse range of bioactive factors spheroids produce. However, the standardisation of CM production and storage is pivotal to the success of these approaches.

Several methods to produce spheroids have been developed, including the liquid overlay method, spinner flask culturing, and the hanging drop method [234]. The latter method facilitates the formation of homogenous spheroids without the need for sieving or manual selection, attenuating the efficacy inconsistencies associated with heterogeneously sized populations [235]. Recently, embryonic dental mesenchymal and epithelial cell spheroids have been produced using this method and co-cultured on semi-solid agar with trigeminal ganglia, resulting in tooth formation with complete vascularisation associated with functional innervation [236]. Although this is not well-suited for regenerative efforts, given the ethical and scalability issues that arise alongside the use of embryonic cells, it does present an alternative model for studying the effects of bioactive agents on specific stem cell populations.

As stated before, 2D monolayer-based studies serve as the most widely adopted platform for assessing the potential of various pulpal regenerative strategies. These methods are simple, accessible, cost-effective, and well-suited to pilot investigations. Despite their frequent application, numerous concerns have been associated with these traditional methodologies concerning the inaccurate representation of *in vivo* actualities and tissue engineering solutions [237]. 3D spheroid cultures provide the unique opportunity for more illustrative models, enabling the characterisation of different cell populations and bioactive agents within intricate systems. However, this is unlikely to supplant 2D methods as gene expression analysis requires monodispersed cultures, and antibody/chemical staining may not penetrate the inner layers. Developing novel analyses for spheroid culture assessment, such as RAMAN spectroscopy, is important to advance the usefulness of *in vitro* models [238]. The widespread adoption of this culture method will provide a much-needed intermediary between monolayer-based studies and *in vivo* work. Furthermore, the xenogeneic nature and ectopic site of traditional transplantation necessitate an improved animal model to better recapitulate the native dental pulp milieu. Autologous transplantation of spheroids in clinically simulated pulp spaces of teeth in higher mammals, such as dogs or primates, presents a refined approach to observing their interactions with other bioactive agents *in vivo*.

In summary, the utility of cell spheroids within dental pulp regenerative contexts is demonstrated in (1) the development of tuneable, scaffold-free approaches that can be pre-exposed to various stimuli to enhance regenerative outcomes, (2) the production of cell-free, secretome-derived platforms, enabling "off-the-shelf" solutions, and (3) the generation of novel and robust *in vitro* and *in vivo* models to facilitate the streamlined identification of promising solutions to dental pulp regeneration. Future research should focus on furthering the *in vivo* characterisation of scaffold-free and cell-free endeavours as they present distinct gaps in the existing knowledge and herald exciting opportunities for novel and innovative approaches.

### 4.5. Indirect cell-based therapies

#### 4.5.1. Extracellular vesicles

Alongside CM, extracellular vesicles (EV) are another secretome derivative that offers insight into future cell-free platforms for dental pulp regeneration. EVs can be further subdivided into apoptotic bodies, microparticles, and exosomes. EVs contain proteins, DNAs, messenger RNAs (mRNAs), and microRNAs (miRNAs), facilitating their regenerative, angiogenic, and immunomodulatory effects [239].

#### 4.5.2. Pulp regeneration

The potential mechanisms by which EVs upregulate odontoblastic differentiation result from crosstalk between multiple intracellular signalling processes. However, this process is generally conducted by the endocytosis of EVs into target cells, releasing a host of miRNAs, mRNAs and proteins to alter gene expression and regulate cell proliferation, migration, and differentiation [240]. Recently, EVs derived from primary DPCs have been shown to outperform their DPSC counterparts *in vitro*, producing dental pulp-like connective tissue *in vivo* containing collagen, odontoblast-like cells, and vascular-like structures using ectopic models [241].

In most dental pulp regenerative studies, apoptosis is thought to suggest poor prognostic outcomes for that treatment. Consistent with previous reports, DPSC aggregates undergo apoptosis shortly after implantation yet still form dental pulp-like tissue [242]. Inhibiting apoptosis mitigated this positive effect, suggesting its role in regeneration. In fact, apoptotic bodies from DPSC aggregates, i.e. vesicles released during apoptosis, promoted *in vivo* dental pulp regeneration and stimulated angiogenesis [242]. This effect was mediated by autophagy regulation, a process by which cells remove and recycle damaged or dysfunctional components. Equally, it has also been shown that EVs from pulpitis-stimulated odontoblasts protect neighbouring odontoblasts from apoptosis during the progression of dental caries [243]. This highlights the dual role of EVs—both as modes of protection and facilitators of regeneration—and points to potential therapeutic strategies. It is worth exploring how EVs can be engineered to enhance their regenerative capabilities, for instance, if treatments could be designed to induce controlled apoptosis to kickstart regeneration.

#### 4.5.3. Angiogenesis

It is well established that rapid neovascularisation is essential for the success of regenerative efforts within the pulpal chamber, given the narrow root canals and limited apical access. To this effect, DPSC-EVs have been shown to promote the angiogenic potential of HUVECs *in vitro* [244]. Furthermore, EVs obtained from HERS cells exhibit the formation of regenerated dentine-like tissue and odontoblast-like cells *in vivo* alongside the upregulation of angiogenic and neurotrophic markers [245]. Interestingly, Wu et al. [246] investigated the angiogenic potential of exosomes from SHED aggregates *in vitro* and compared the effects to direct 3D spheroid application *in vivo* when exosome biogenesis was inhibited. Notably, dentine-pulp complex regeneration and angiogenic marker expression were highest in the co-implantation of exosomes and aggregates. Concomitantly, aggregates that were inhibited from forming exosomes showed suppressed regeneration, an effect that could be partially rescued by exosome supplementation.

### 4.6. Inflammatory modulation

Lipopolysaccharide (LPS) is the major virulence factor of gram-negative bacteria and, as such, is a chemical agent that has been classically used to simulate pulpal injury and induce inflammation [247]. Likewise, LPS-conditioned DPSC exosomes enhance Schwann cells' and

HUVECs proliferation, migration, and differentiation, supplanting the effects observed with non-conditioned counterparts [248,249]. Additionally, exosome and angiogenic markers are significantly upregulated in clinically realistic deep caries models compared to healthy pulp tissue [249]. This suggests that inflammatory conditioning of EVs might prime them for more robust interactions with endogenous cells. Furthermore, Zheng et al. [250] observe that small EVs derived from DPSCs can exert anti-inflammatory effects on macrophages, which reduces inflammation *in vivo,* presenting an opportunity for integrating EVs into direct pulp capping treatments. Nevertheless, the inhibitory effects of LPS-treated EVs on SCAP proliferation, migration, and differentiation highlight the complexity of EV-mediated interactions, which are likely context-dependent [251]. These findings highlight the potential of EV-based therapies in dental pulp regeneration and underscore the necessity of precise molecular characterisation for targeted therapeutic design.

### 4.7. Functional delivery

EVs are known to bind type I collagen and fibronectin, enabling them to be tethered to biomaterials and presenting the unique opportunity to combine primed EVs within biomaterial approaches [252]. Additionally, EVs typically suffer from rapid clearance and low accumulation in target tissues, though this limitation is likely not as significant within the dental pulp, given its isolation and restricted interaction with the rest of the body [253]. This anatomical property of teeth may enhance the retention and effectiveness of EV-based therapies in dental pulp regeneration. Regardless, due to the confined space of the pulp chamber, an injectable delivery system for EVs is desirable for translational and clinical alignment. For instance, in a direct pulp capping model, it was found that EV solution alone made it difficult to seal the exposed pulp as it could not reside *in situ* [254]. Pulp capping materials easily displaced the EV solution; in contrast, TDM-loaded EVs showed the formation of dentine mineralised layers, and as such, EVs alone are likely not suited as scaffold-free approaches in dental pulp regenerative endeavours [254].

Recently, a novel injectable method for delivering EVs has been developed, wherein a fibrin-based hydrogel is split into two constituents: EV-laden fibrinogen and a cell-loaded polymerisation solution [255]. Upon extrusion of the two components, the gel polymerises, enabling the *in-situ* delivery of EVs alongside cells. *In vitro,* a homogenous distribution of the components induced enhanced proliferation, migration, and vascular-like structure formation. Likewise, EVs derived from primary DPCs and loaded onto fibrin gels showed enhanced proliferation and migration [256]. This method holds great promise for a translatable effort for dental pulp regeneration; however, further testing in orthotopic models is required.

Overall, this reaffirms that the success of regenerative approaches employing cell-secretome-based therapies hinges on the successful development of appropriate delivery methods. Furthermore, the primary advantage of EV-based approaches is the intrinsic simplicity of production; however, this introduces a batch-to-batch variation of their bioactive constituents and limits regenerative reproducibility. Thus, generating genetically engineered EVs as effective carrier systems for specific proteins or nucleic acids, such as miRNAs, is key to achieving targeted therapeutic strategies. For instance, EV-encapsulated NF1C delivery within gelatin scaffolds *in vivo* promotes dentin and collagen formation [251]. Successful translation, therefore, depends on developing cost-effective large-scale production, quality control methods to minimise heterogeneity and effective bioactive agent loading protocols.

### 4.8. Opportunities

The literature investigating cell-based therapies for dental pulp regeneration presents promising avenues for clinical translation. Identifying diverse SC populations opens opportunities for targeted and combined regenerative approaches. These cells offer unique advantages, including enhanced proliferation, readily available sources, and potential autologous tissue sourcing. Notably, the successful translation of direct cell-based therapies requires establishing cryopreservation tooth banks. Despite inadequate infrastructure, this will enable autologous treatments and streamline SC retrieval processes across various engineering frontiers such as medicine, dentistry and veterinary fields. This approach facilitates a broader application of SCs in multiple regenerative contexts. Capitalising on the therapeutic potential of cell spheroids to develop cell/scaffold-free approaches and *in vitro* regeneration models will advance the applicability of findings and streamline the development pipeline to innovative and translatable approaches. Additionally, leveraging genetically engineered EVs as effective carrier systems for bioactive agents within scaffold-based approaches presents a unique opportunity for novel targeted therapeutics.

### 4.9. Challenges

Persistent challenges hinder clinical translation in dental pulp regenerative strategies, notably the heavy reliance on SI immunodeficient rodent models such as those introduced by Gonçalves et al. [257]. This method, aimed at preventing immunogenic responses, fails to replicate the native dental pulp microenvironment where inflammatory processes are at play, severely limiting its clinical applicability. Despite two decades of use, it lacks relevance for practical SC studies and demonstrates limited translation towards *in situ* trials in higher mammals. Adding to this approach and embracing models aligned with applied treatment strategies, such as those using LPS-simulated pulpitis, will be helpful. Meanwhile, the emerging standard practice of tooth banking addresses challenges associated with allogeneic sources. However, achieving accurate native tissue architecture remains complex, with inadequate vascularisation, limited migratory capability, and challenges in producing complete regeneration. Additional confounding factors such as standardisation of recipient eligibility, cryopreservation protocols, scaffold design, biomaterial selection, and a deeper understanding of the intricate signalling pathways also need elucidation for successful clinical translation. Moreover, despite the promise of cell spheroids and EVs in regenerative endeavours, challenges persist concerning their consistent and safe production at the necessary scale and the lack of appropriate delivery methods.

## 5. Growth factors/morphogens

Cellular-based therapies face significant clinical challenges, including introducing cells into non-vascularised tissues, residual biofilm-associated infections and potential immunogenicity. Cell-homing techniques have been developed in regenerative endodontics to circumvent the difficulties of external SC transplantation and induce the migration of endogenous SC from their periapical niche to a new site within the root canal [258]. This approach generally requires a wide apical root foramen and chemotactic factors to facilitate SCs migration from the periapical area into the empty root canal, potentially promoting regeneration. Alternatively, endogenous SCs can be stored in a tooth bank following harvesting. In the latter option, the SC population will need to be expanded *ex vivo* before replantation; however, this method is still in its infancy due to high costs, limited dental practicality and a scarcity of suitable companies willing to offer this service, as previously described [114].

A plethora of GFs, morphogens, chemokines, and cytokines are involved in an intricate interplay that regulates the cell cycle, proliferation, differentiation, and migration patterns in the dental pulp [259]; however, it should be noted that supplying an exogenous cocktail of these factors is not the only method to stimulate regenerative endodontic responses. As has previously been discussed, using certain endodontic irrigants, like EDTA, can induce the liberation of various sequestered endogenous bioactive components of the dentine, broadly

known as DMCs, which includes VEGF, matrix-metalloproteinase 9 (MMP-9), bone morphogenetic protein-7 (BMP-7) and TGF-β1 amongst others promoting regenerative responses [165]. However, other irrigants, such as sodium hypochlorite (NaOCl), have been shown to inhibit this process [260]. These bioactive agents are of particular interest within dental pulp regenerative contexts, given their central roles in achieving the primary goals of these strategies.

Angiogenesis is crucial in regenerative medicine to establish adequate blood supplies, preventing necrotic or scar tissue formation [261]. VEGF is a critical regulator of physiological angiogenesis and has shown to be a key molecular target for both inhibition in pathological angiogenesis and upregulation in regenerative strategies [262]. Within biomaterial-based approaches toward pulpal regeneration, VEGF has consistently proven indispensable for forming blood vessels through the apical foramen [112,126,196,263]. However, its beneficial effects may only occur when employed alongside cell-based therapies [126].

MMP-9 is known to cleave membrane-bound VEGF, enhancing its bioavailability and this propensity to contribute to revascularisation [264,265]. Additionally, OPN and DSP, two central components for tooth development, have been identified as substrates for MMP-9 processing [266]. MMP-9 is responsible for processing the translated protein products into their functional components [266]. In LPS-simulated pulpitis models, MMP-9 expression was recently found to be significantly upregulated alongside other members of the MMP family, alluding to their involvement in the native tooth injury response [267]. These findings suggest that modulating MMP-9 expression may present a novel avenue within regenerative solutions.

BMP-7, a member of the TGFβ superfamily, plays a significant role in dental pulp regeneration, inducing odontoblastic differentiation and mineralised tissue formation [268,269]. Previously, BMP-7 has demonstrated success as a direct pulp-capping agent that elicits substantial amounts of hard tissue formation in various animal models, such as swine and ferrets [270,271]. Within regenerative solutions, BMP-7, in combination with dexamethasone (DMX) or TGFβ-1, enhances odontoblastic differentiation and hard tissue formation [99,272]. However, despite its promise, localising the delivery of BMP-7 to target tissues whilst attenuating its short half-life and potential adverse effects *in vivo* remains challenging [79]. Furthermore, despite its effectiveness for mineralised tissue formation, BMP-7 does not enhance cell recruitment like other GFs, such as SDF1 and bFGF [273]. Therefore, although BMP-7 has been a mainstay in dental pulp regenerative efforts, facilitating its deployment in clinical applications hinges on developing biomaterials to facilitate its protection and target sustained release.

TGFβ-1 is essential for the homeostatic maintenance of the dentine-pulp complex, particularly the ECM secretion and odontoblastic differentiation of resident pulpal cells [274,275]. As mentioned previously, co-delivery of BMP-7 and TGFβ-1 enhances mineralised hard tissue formation; however, isolated TGFβ-1 delivery was insufficient to replicate this response [99]. Similarly, it has been postulated that TGFβ-1 decreases the proliferation of DPSCs and inhibits pulp-like tissue formation [65,112]. However, the *in vivo* delivery of injectable HA-based gels laden with TGFβ-1 and dental mesenchymal cells elicited pulp-like tissue formation and dentine reparation [94]. Regardless, the importance of TGFβ-1 for proliferation and differentiation within the dental pulp is generally well accepted [276].

Despite the promising opportunities of GFs, several translational hurdles impede their effective deployment. Aside from the high cost, another challenge is the necessity for supra-physiological doses of GF to elicit the desired regenerative response due to their short half-life and poor protein stability [277]. Developing appropriate delivery methods that facilitate the protection and sustained-release kinetics of GFs is the crux of achieving the translation of GF-based regenerative therapies. Additionally, such large doses inherently elevate the risk of off-target effects and adverse reactions. Moreover, considering the prevalence of dental caries in clinical settings, the volume of product necessary for treatment renders approaches solely reliant on GFs economically unfeasible.

### 5.1. Clinical studies

Analysis of the numerous *in vivo* studies employing GFs (Table 4) reveals distinct trends and challenges in regenerative endodontic research. The primary hurdle to clinical translation can be attributed to the intricate complexity and variability of biomaterials and cell types employed. These studies use a diverse array of scaffold materials, such as collagen, gelatin, and silk fibroin, which, as previously demonstrated, possess unique properties that can significantly influence GF release kinetics and cellular interactions. Further complexity is introduced by including various cell types, as cellular responses to different GFs and biomaterials may vary widely. Optimal dosage and delivery methods for GFs are essential considerations for successful clinical translation, as evidenced by the inhibitory effects observed at high doses of BMP-7 [272]. Patient demographics are crucial to therapeutic development, as highlighted by the age-dependent effects Iohara et al. [278] noted for granulocyte colony-stimulating factor (G-CSF). This intricate interplay between GFs, morphogens, scaffold materials, and cell types necessitates further elucidation and optimisation to enhance regenerative outcomes and achieve successful clinical translation. Standardised approaches, protocols, and additional research efforts will address these complexities to realise the therapeutic potential of these promising findings in clinical regenerative endodontics.

Mobilised DPSCs (MDPSCs) are an isolated subpopulation of DPSCs obtained through G–CSF–induced mobilisation. They have been characterised as being enriched for specific markers such as CD105, CXCR-4, and G-CSF receptor and demonstrated increased regenerative potential *in vivo* [279]. MDPSCs have previously demonstrated higher migratory activity and trophic effects, including anti-apoptosis and immunosuppression, than other cell types like DPSCs and induced pluripotent stem cells [279]. In the first human trial, MDPSCs were employed to evaluate the safety, efficacy, and feasibility of treating irreversible pulpitis using a regenerative medicine strategy, marking a significant stride in translating preclinical findings to clinical application [280]. Moreover, various studies engage in the combinatorial and comparative use of numerous GFs, illuminating their interactions and allowing a comprehensive understanding of their effects. Additionally, these studies allow more efficacious GFs to be identified (Table 5).

### 5.2. Opportunities

Despite the challenges of using GFs, exciting opportunities arise with their controlled release through biomaterial encapsulation. Incorporating chemotactic factors holds great promise for inducing the migration of endogenous SCs in cell-homing techniques, promoting regeneration and avoiding the challenges associated with SC transplantation. Particularly, VEGF, MMP-9, BMP-7, and TGFβ-1 comprise a well-established backbone, highlighting the feasibility of bioactive materials for dental pulp regeneration. Furthermore, combinations of GFs and subsequent comparative analyses have provided a comprehensive understanding of the interplay between different GFs, highlighting areas for new studies to analyse countless biological permutations. However, in regenerative endodontics, it has become evident that exogenous GF supplementation is not the sole method to recruit these bioactive molecules. For instance, liberating endogenous sequestered GFs using appropriate irrigants such as EDTA presents a straightforward avenue for releasing numerous relevant GFs pertinent to pulp regeneration and repair.

### 5.3. Challenges

The cost of using GFs in a dental regenerative protocol is a significant limitation. GFs are hindered by a brief half-life, poor protein stability, and rapid loss of their specialised properties at physiological

temperatures. The fragile nature of GFs in the body necessitates supra-physiological doses, leading to associated adverse effects. For instance, large quantities of VEGF can result in off-target pathological vessel formation in dormant tumours [295]. However, despite these challenges, certain recombinant GFs have received European Medicines Agency (EMA) regulatory approval, albeit for non-dental applications, such as rhG-CSF in treatment for neutropenia, offering a potential fast-track method for clinical translation.

## 6. Pharmacological agents

Successful novel translation requires a multifaceted approach; thus, exploring pharmacological interventions is crucial to developing and optimising regenerative strategies. Pharmacological inhibitors are attractive as they circumvent many drawbacks of GFs, namely high cost, short half-life and supraphysiological doses. Due to the potential risk of reinfection, the success of endodontic therapies relies significantly on antimicrobial efficacy [296,297]. While triple antibiotic paste (TAP) is commonly used for disinfection during revitalisation techniques in dental clinics due to its effectiveness against bacteria, it has been associated with limitations such as dentine demineralisation, dose-dependent cytotoxicity, DMC-release inhibition, inhibiting angiogenesis, and antibiotic overuse [260,298–300]. Recent studies have explored the drawbacks of TAP, leading to the investigation of novel antimicrobial solutions. Similarly, chlorohexidine (CH), a popular disinfectant used in dental clinics, has recently been employed as a pharmacological agent in regenerative approaches to provide antimicrobial properties; however, CH's substantivity will affect the interaction with the dentine matrix.

Simvastatin (SIM) is a first-line drug targeting hyperlipidaemia; with long-time worldwide usage, it is recognised as a low-cost, safe drug [301]. Statins, like SIM, exhibit a dose-dependent dual nature - anti-inflammatory, pro-angiogenic, and enhanced vascular endothelial cell function at low doses [302–304] but, in contrast, have an angiostatic effect at high doses [303]. *In vitro* studies on SIM have demonstrated enhanced odontogenic differentiation and upregulation of mineralisation- and angiogenic-associated factors [305–307], and as such, its use has been suggested in RETs.

### 6.1. Clinical studies

#### 6.1.1. Triple antibiotic paste (TAP)

Pagliarin et al. [308] conducted an *in vivo* study in necrotic root canal systems post-endodontic treatment, finding that propolis paste outperformed TAP regarding root canal disinfection. Propolis exhibited advantages for revascularisation, demonstrated by enhanced mineralised tissue deposition and increased tissue in treated teeth. Conversely, increased inflammation severity was observed in TAP-treated compared with untreated teeth. Similarly, Bottino et al. [309] employed an *in vivo* canine model of periapical disease, finding comparable outcomes. Heightened inflammation and osteodentine-like formation were observed. The latter was attributed to the cytotoxic effects of TAP on odontoblasts, prompting SCAPs to differentiate into osteoblast- and odontoblast-like cells. Despite these effects, apical root closure did occur, possibly due to the disinfection and SCAP activity. Subsequently, Neelamurthy et al. [310] conducted a clinical trial to evaluate the *in vivo* revascularisation potential of teeth treated with TAP. Notably, the TAP used in this study differed in composition, containing metronidazole, ciprofloxacin, and clindamycin instead of minocycline, demonstrating continued root development, apical closure, and thickening of radicular dentine in treated teeth.

#### 6.1.2. Chlorohexidine (CH)

CH is a disinfectant with a long-standing history in dental clinics. Kalyan et al. [311] recently conducted a clinical trial on CH-laden polymer scaffolds as a pulp dressing following VPT, highlighting antimicrobial properties that reduce inflammation. The proposed CH scaffold could be more economically advantageous and clinically flexible than the common pulp-capping material MTA. Finally, Nagata et al. [312] compared CH, TAP, and calcium hydroxide before revascularisation, finding similar success rates between the groups; however, discolouration was observed in TAP-treated teeth.

#### 6.1.3. Simvastatin (SIM)

Investigations on the pharmacological inhibitor SIM *in vivo* have shown accelerated mineralised tissue formation when using a HA/TCP carrier [313], although this may be partially attributed to the osteoconductive carrier used; however, it should be noted that these observations were reflected *in vitro* in the absence of HA/TCP. Miyazawa et al. [314] demonstrated effective *in vivo* odontoblastic differentiation using SIM micelles in biodegradable hydrogels, elucidating the efficacy and necessity of a prolonged-release delivery mechanism for SIM.

This sentiment is supported by an *in vivo* study of PLGA-encapsulated SIM, which demonstrated blood vessel-rich pulp-like tissue regeneration facilitated by encapsulated statins'-controlled release profile [315]. Jia et al. [72] investigated SIM as a pulp dressing agent *in vivo* in both MTA and an absorbable gelatin sponge, finding unfavourable effects with MTA, such as dentine bridge formation, which hindered the regenerative response. In contrast, with SIM-loaded sponges, *de novo* dentine deposition and native pulp-like tissue formation were observed. Soares et al. [81] studied SIM-loaded calcium hydroxide scaffolds *in vivo* for the treatment of calvarial defects, finding enhanced differentiation and mineralisation, thus chemoattracting pulp cells to its surface and reducing the over-expression of odontoblastic markers. However, SIM concentration must be optimised, as evidenced by a clinical trial by Asl Aminabadi et al. [316]. Using SIM as a capping agent, this trial found that healing with hard tissue formation and no inflammation was more frequent in SIM-treated teeth. Higher SIM concentrations were associated with cytotoxic effects on odontoblasts and odontoblast-like cells, resulting in inflammation and pulp necrosis. Additionally, Xue et al. [317] noted SIM's anti-inflammatory and pro-differentiation effects and suppression of VEGF expression, which is vital for inducing angiogenesis in regenerative approaches.

### 6.2. Other pharmacological agents

Beyond these, other pharmacological compounds that have been assessed in *vivo* for dental pulp regeneration include the glucocorticoid DMX [272,294], the bisphosphonate risedronate and non-steroidal anti-inflammatory drug lornoxicam [140], magnesium phosphate [318], calcium phosphate [138], calcium hydroxide [319], the hormone leptin [320], and the cell-permeable inhibitor dimethyloxalylglycine [117]. These studies showed enhanced odontoblastic differentiation compared with controls, and some demonstrated angiogenesis [82,117]. Notably, Gonçalves da Costa Sousa et al. [82] used ciprofloxacin and IDR-1002, a synthetic host defence peptide derivative with potent anti-inflammatory properties, alone or in combination, in a hybrid scaffold. This *in vivo* study found all nanofibrous scaffolds to demonstrate the formation of new connective tissue. Further, scaffolds using ciprofloxacin and IDR-1002 evidenced non-toxicity, reduced inflammation, and revascularisation.

### 6.3. Opportunities

Alternative medicaments capable of disinfecting the root canal before regenerative endodontic procedures, though not incorporated within the scaffold, are essential, given the residual effects of current medicaments such as NaOCl, CH and TAP. Cutting-edge alternatives provide promising avenues for surpassing the limitations of commonly used medicaments. Moreover, when employed in scaffolds, disinfectants not only allow them to demonstrate antimicrobial properties but also may allow for cost-effectiveness compared to traditional materials.

Considering more conventional pharmacological agents, numerous diverse compounds have been assessed, from DMX to magnesium phosphate, encouraging results in enhancing odontoblastic differentiation and providing many options for future research. SIM has emerged as a strong candidate possessing anti-inflammatory and pro-angiogenic effects, promising enhanced odontogenic differentiation, which is crucial for dental pulp regeneration.

### 6.4. Challenges

Despite these exciting opportunities, some challenges persist. Commonly employed medicaments face limitations such as dentine demineralisation and dose-dependent cytotoxicity. Similarly, SIM exhibits dose-dependent effects, necessitating careful optimisation before translational efforts. Moreover, further clinical validation is required for existing SIM-loaded scaffolds. Finally, each pharmacological compound, of which there are numerous, requires individual examination for unique consideration. Successful translation employing pharmacological agents hinges on adequately addressing these challenges.

## 7. Epigenetic & therapeutic modulation

Among the approaches utilised to develop next-generation regenerative solutions, epigenetic modulation has garnered significant interest. Pharmacological inhibition of DNA-associated proteins presents promising avenues for innovative and novel approaches in regenerative medicine. Epigenetics encompasses various mechanisms that govern gene expression, independent of alterations in the underlying DNA sequence [321]. These mechanisms are pivotal for cellular function, allowing cells to respond to environmental stimuli during development, repair, and other cellular processes throughout life [322–325]. Notably, epigenetic dysregulation has been implicated in a spectrum of pathologies, including neurodegenerative disease, mental illness, neurodevelopmental disorders, and various cancers [323–331]. Emerging research has begun to unveil the significant role of epigenetic influences in controlling odontoblast fate, thereby underscoring the therapeutic potential of modulating these processes to orchestrate self-renewal and differentiation [332,333].

Chromatin is the physiological manifestation of genetic information within the cell, acting as an interface between the genome and the cellular environment. Understanding the intricate mechanisms of epigenetic modulation necessitates comprehending the distinct chromatin states that stimulate or repress gene activity [334]. Chromatin consists of DNA wrapped around histone proteins, forming condensed nucleosomes which serve as a platform for epigenetic modifications [335]. Epigenetic modifications include DNA methylation, histone modification, and intricate regulatory roles of non-coding RNA (ncRNA), which are increasingly recognised in orchestrating these processes. These modifications occur on both DNA and non-DNA-associated nuclear proteins and regulate gene expression by influencing the accessibility of the DNA to transcriptional machinery [336,337].

DNA methylation, orchestrated by DNA methyltransferases (DNMTs), is correlated with transcriptional silencing and a condensed chromatin structure [338]. DNA methylation results in gene repression either by physically preventing transcription factors (TF) from associating with their target site or by attracting methyl-CpG binding proteins that subsequently recruit transcriptional repression factors, such as histone deacetylases (HDACs) [339]. Histones are subject to various modifications, including acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation [336]. Histone acetylation, mediated by histone acetyltransferases (HATs), opens the chromatin architecture, facilitating transcriptional accessibility and increased gene expression. Conversely, HDACs tighten chromatin by removing the acetyl group, causing translational repression [340]. As discussed later, inhibiting histone deacetylation is of translational benefit by promoting mineralisation and regenerative responses.

Many of the cellular effects of both histone acetylation and DNA methylation are attributed to ncRNA, which comprises miRNA, small interfering RNA (siRNA), and long ncRNA (lncRNA). Both miRNAs and siRNAs play pivotal roles in gene silencing by integrating into the RNA-induced silencing complex (RISC) and binding complementary mRNA sequences [341,342]. It is important to note that most animal miRNAs imperfectly bind to target mRNAs. In this case, the RISC complex bound to the mRNA target either represses translation or promotes mRNA deadenylation and decay [343]. However, when RISC encounters mRNAs with sites nearly perfectly complementary to the miRNA, these mRNAs are cleaved endonucleolytically and degraded in a process mediated by Argonaute 2 (AGO2) [343]. While lncRNAs are not as well characterised as miRNAs or siRNAs, emerging research underscores their multifaceted cellular functions, including their therapeutic potential in regulating miRNAs [344].

Epigenetic processes present novel therapeutic opportunities, including pharmacological HDAC inhibitors (HDACis) and DNMT inhibitors (DNMTis) or the application of ncRNA for targeted translational modulation. Broadly, there are two classes of HDACis and DNMTis: pan-inhibitors, which are broad-acting, with non-specific DNMT targets, and isoform-specific inhibitors, which target specific classes of DNMTis/HDACis [333,345].

### 7.1. HDACi (histone deacetylase inhibitors)

Various HDACi classes have been investigated for their roles in odontogenic differentiation in the dentine-pulp complex, including benzamides (MS-275 and MI-192), hydroxamic acids, such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), and short-chain fatty acids like valproic acid (VPA). Certain HDACi (e.g. MI-192) exhibit isoform selectivity profiles, targeting different sub-classes of HDACs, which partially account for the differences observed in their effects.

TSA is a potent pan-HDAC isoform inhibitor, non-selectively targeting various HDACs, which has garnered significant attention within dental pulp regenerative contexts [346]. TSA demonstrates significant anti-proliferative effects in primary DPCs when applied at 100 nM and 400 nM without affecting cell viability [347,348], reflecting previous reports using TSA at much lower concentrations [349,350]. Notably, TSA-induced caspase-3 and -9 cleavage were observed in DPSCs, a process associated with apoptosis [351]. In contrast, a positive effect on proliferation has been observed, with the most significant effect observed in 20 nM TSA-treated DPSCs [352]. However, this primary cell effect was not observed when applied to a transformed odontoblast-cell line at similar concentrations (MDPC-23) [353].

The effects of TSA on odontogenic differentiation and associated gene expression patterns are both time- and dose-dependent. In DPSCs, a concentration of 20 nM TSA notably enhanced the expression of key differentiation-associated genes like DSPP, DMP-1, and BSP while concurrently reducing OC levels, albeit only observable by day 21 [352]. Similarly, in DPSCs, 100 nM TSA triggers a significant increase in DMP-1 expression [349]. Moreover, in DPSCs, TSA at 20 nM elicits migratory, inflammatory, adhesive, and reparative responses demonstrated by upregulation of CXCR4, FGF2, MCP-1, SDF-1, FN, ICAM-1, VCAM-1, and Integrinβ1 in DPSCs, even in the absence of osteogenic induction [351]. Conversely, when applied to a MDPC-23 cell line, TSA at 1 nM increases DMP-1, OC, RUNX-2, and BMP-2 expression but does not affect DSPP levels [353]. Cell type specificity likely plays a role in TSA's effect on gene regulation. For translational relevance, the effects of TSA on primary DPCs with 25 nM TSA were found to significantly increase DSPP, OP, and BMP-2/-4 expression with no effects on DMP-1 expression [347].

Sultana et al. [353] analysed basal conditions in the absence of osteogenic induction media, which may be more reflective of *in vivo* gene expression patterns, while Duncan et al. [347] conducted experiments with primary DPCs to improve the relevance and translation of

the research to *in vivo*, albeit after osteogenic induction. This critical divergence between studies also influences mineralisation characterisation; in studies using osteogenic induction, alizarin red staining was shown to be consistently upregulated [347,349,352]. However, without osteogenic induction media, TSA at all concentrations failed to replicate this effect in culture systems [353]. Notably, Suzuki et al. [350] determined that induced TSA-treated cells fail to enhance DPSC mineralisation, though this result is significantly influenced by methodological limitations discussed later in this review. The different cellular responses of primary cells and transformed cell lines, the type and concentration of HDACi employed, and the presence or absence of osteogenic induction in studies likely account for the discrepancies observed.

Overall, these trends suggest that TSA has the potential to regulate odontogenic-associated gene expression in DPCs dose-dependently; however, its effects are influenced by factors such as concentration, cell type selectivity, and, importantly, osteogenic induction. Further research using primary pulp cells in 3D cultures and *in vivo*, without the bias introduced by osteogenic induction, is required to elucidate its usefulness in dental pulp regenerative endeavours.

Similarly, the effects of VPA on odontogenic differentiation vary depending on both dosage and duration of exposure. VPA inhibits class I and II HDACs, non-specifically targeting HDAC1 and 2 (mM) [354]. Higher VPA concentrations elicit anti-proliferative and apoptotic effects and OC suppression [349,353,355,356]. Conversely, lower concentrations (up to 1 mM) have been shown to upregulate OC and other important differentiation markers such as DMP-1, DSPP, OPN, BSP, BMP-2/4, and Nestin in both DPSCs and primary DPCs, promoting dentine and collagen matrix formation in 3D cultures (347–349, 353, 355].

Discrepancies exist between studies regarding the effects of VPA on specific gene expression patterns due to variations in experimental methods and models. For instance, BMP-2 and DMP-1 upregulation was observed in VPA-treated primary DPCs under osteogenic induction [347, 348], an effect which was not mirrored in non-induced MDPC-23 [353], underscoring the importance of representative experimental frameworks moving forward.

Knockdown experiments of HDAC1 and -2 revealed intriguing insights into the efficacy of VPA in odontogenic differentiation. Paino et al. [355] reported that HDAC1 silencing increased the expression of OPN, BSP, and OC, as did co-silencing of HDAC2; however, the isolated silencing of HDAC2 downregulated OC expression despite hyperacetylation of the OC promoter region. HDAC2-mediated silencing of OC expression is attributed to glucocorticoid receptor nuclear overexpression in both HDAC2-suppressed and VPA-treated models, subsequently acting on the OC promoter and repressing transcription [356]. Furthermore, VPA inhibits the catalytic activity of class I HDACs and induces the proteasomal degradation of HDAC2, which is strongly expressed in mature odontoblasts [357,358]. Intrinsically, while potent HDAC2 inhibitors may enhance the mineralisation activity of odontoblasts, they may have a dual nature whereby terminal differentiation is hindered, at least within DPC populations. Despite the potential for streamlined approval presented by the previous FDA and EMA approval of VPA for neurological disorders, these mechanistic nuances necessitate optimising the dosage and delivery method for leveraging its full promise for dental pulp regenerative strategies.

Entinostat (MS-275) offers a greater selectivity profile than other HDACi with specific inhibitory actions against HDAC1 (nM) followed by HDAC2/3 [346,359,360]. Inhibition of HDAC1 has previously been characterised to directly upregulate RUNX2, a potent TF responsible for the expression of odontoblast differentiation-related genes such as OC [361]. A recent comparative analysis of the effects of multiple HDACis on the odontogenic differentiation of MDPC-23 cells regarded MS-275 as the most efficient due to its isoform-selectivity and extended residence time, as it is notably slower to dissociate from HDAC1/2 than TSA and SAHA [353,360]. MS-275 exhibits increased OPN, BSP, and decreased OC expression in induced DPSCs at a concentration of 1 μM, reflecting the trend previously observed in VPA [355]. This may result from the protracted residence time of MS-275 on HDAC2 (743 m) [360]. Similarly, DMP-1, RUNX2, ALP, BCL-2 and DSPP were upregulated at all concentrations of MS-275, with notable enhancements at 5–10 nM accelerating DPSC differentiation with no apoptotic effects, though concentrations above 5 μM demonstrate anti-proliferative impacts [350, 362]. When comparing its effects on odontogenic differentiation to TSA, it was revealed that mineralised deposits are significantly increased in MS-275-treated cells; however, the monolayer of both control and TSA-treated cells had detached, altering the interpretation of results [350]. Despite the promise of MS-275, the trends observed in both this compound and VPA reinforce that significant inhibition of HDAC2 may not be well-suited to the goals of dental pulp regeneration as previously described; however, further experimentation in primary DPCs in 3D models and *in vivo* analyses are required to elucidate the effectiveness of this modality properly.

SAHA, also known as vorinostat, is another pan-HDACi like TSA, with FDA approval for treating T-cell lymphoma. Previous research has shown that benzamides, such as MS-275, and hydroxamic acids, like TSA and SAHA, exhibit cytotoxic effects under constant, high-concentration exposure in cell culture [346,348] and in primary DPCs [363]. Moreover, a negative correlation exists between HDACi treatment (VPA and SAHA) and bone density, an effect that has been attributed to derive from the susceptibility of immature osteoblasts to HDACi-mediated cytotoxicity, a property not held by terminally differentiated osteoblasts [364–366]. These findings are paradoxical when considering the pro-mineralisation effects observed in odontoblasts [347,349,351–353,355,367]. However, this contradiction is likely a result of the simultaneous excitation of pre-existing mature blasts and HDACi-mediated cytotoxicity of immature blast cells. Ultimately, though HDACi treatment increases the activity of mature osteoblasts and likely odontoblasts, the effects of constant exposure should be avoided, and controlled release systems should be developed for *in vivo* use.

While considerable efforts have been dedicated to characterising the effects of TSA, VPA, and SAHA, exploration of other HDACi remains relatively nascent. For instance, a comprehensive study of LMK-235, a potent HDAC 4 and 5 inhibitor, and its usefulness on primary DPCs has been conducted [368]. As with previous interventions, the short-term low-dose treatment appears to limit anti-proliferative effects; though, in the absence of osteogenic induction, LMK-235 failed to upregulate DSPP or OC mRNA expression but did enhance RUNX2, VEGF, and ALP activity, markers which are important for early differentiation and revascularisation. Significant improvements were observed when analysed during osteogenic induction; however, further exploration is warranted to understand its potential applications, particularly in extending the study to 3D models. Similarly, the selective HDAC2 and 3 inhibitor, MI-192, reinforced the consistent trend of potent and prolonged treatment eliciting anti-proliferative and apoptotic effects [369]. Unexpectedly, these results demonstrated the upregulation of OC, ALP, BMP-2, COL1A1 and mineralised deposits in induced MI-192-treated dental pulp stromal cells. Furthermore, HDAC3 has previously been shown to interact directly with RUNX2, causing transcriptional repression of key differentiation-associated genes such as OC, OP, and BSP [370]. These findings contradict previous studies whereby potent HDAC2 inhibition (VPA) results in OC suppression, though the direct comparison of induced MI-192 treated cells with basal conditions likely skews this finding.

The adverse effects of long-term HDACi exposure may be attenuated by pulsed, short-term exposure employing either benzamides or hydroxamates [346,363]. However, the residual effects of these classes differ, where the acetylation profile of hydroxamic-acid treated cultures returns to baseline after 18 h; in contrast, this effect takes over 96 h for benzamide-treated cultures [346]. Considering the constant, controlled exposure of HDACis to resident DPCs facilitated by doped 3D scaffolds, it is unclear whether both classes' cytotoxic and anti-mineralisation properties will persist. One potential solution is to pre-treat cells, for

instance, DPSCs, with the HDACi, ideally a benzamide such as MS-275, due to its greater isoform selectivity and extended effects on acetylation. Followed by subsequent 'soak-loading' of the pre-treated cells alongside some chemotactic cocktail into a 3D scaffold. However, though this presents a particularly applied approach, it is not clinically translatable for numerous reasons. Primarily, this 'pre-treated' cell approach requires either autologous cell harvest or allogenic sources alongside their expansion and treatment with inhibitors, a service no dental clinical is equipped to provide. Furthermore, the cost of incorporating chemotactic agents such as growth factors may not be practical within the dental market.

Notably, there is a discernible gap in our current understanding of HDACis and, more broadly, epigenetic modulators in 3D environments. This research warrants *in vivo* investigations to eliminate bias introduced by osteogenic induction and to investigate the cytotoxic/inhibitory effects of constant exposure in dental-specific contexts. Additionally, 3D analyses would enhance our understanding of HDACis ability to liberate sequestered DMCs, although less efficiently than EDTA due to the observed inability to remove the smear layer efficiently [164]. Furthermore, cells treated with HDACis have shown an upregulation of migratory activity in DPSCs and primary DPCs [348,351,363], which was not observed in MPDC-23 cells [353]; as such, analysis of HDACi-doped scaffolds *in situ* should be investigated to determine the implication of these findings on endogenous cell migration through the apical foramen.

In summary, despite the promise of HDACis effects in 2D environments, expansion to 3D and *in vivo* models is necessary to elucidate their implication on odontogenic differentiation. Incorporation of HDACis into biomaterial-based approaches will likely perpetuate cytotoxic and anti-proliferative effects; however, several persisting limitations would be addressed. Previous research has suggested the cell selectivity of HDACis, whereby varied responses were observed in non-representative cell types. Notably, recent advancements in understanding the roles of HDAC1, 2, and 3 in odontogenic differentiation highlight the necessity for targeted therapeutic interventions, retiring pan-isoform inhibitors. Addressing these limitations is essential to the successful clinical translation of the HDACi-based approaches in regenerative endodontics.

### 7.2. DMNTi (DNA methyltransferase inhibitors)

Despite being in the early stages of research, DNMTis hold promise for regenerative approaches in dentistry. 5-Aza-2′-deoxycytidine (5-Aza-CdR), a potent DNMTi, has been assessed for its effects on odontogenic differentiation [214]. It was shown that 5-Aza-CdR, even at 1 μM, exhibits significant anti-proliferative effects. However, key odontogenic differentiation-associated genes, such as DMP-1, DSPP, RUNX2, DLX5, and OSX, were markedly upregulated after short-term (24hr) 5-Aza-CdR treatment, albeit less than that of induced cells with or without treatment. However, in LPS-induced models of pulpitis, 5-Aza-CdR increased the expression of various pro-inflammatory cytokines, particularly IL-6 and IL-8, via demethylation of TRAF6, a canonical transducer of NF-kB and MAPK pathways [371]. Despite the odontogenic potential of 5-Aza-CdR, these profound adverse effects indicate that 5-Aza-CdR may exacerbate pulpitis rather than modulate it. Similar investigations using RG108, another DNMTi, have revealed significant increases in DMP-1, DSPP, and Klf4 mRNA expression using mDPC6T, a self-established pre-odontoblast cell line [372]. Notably, this study does not assess the effects of RG108 on cell viability or cell growth, nor does it elucidate the effects of RG108 in the absence of osteogenic induction.

As reiterated throughout this review, 2D monolayer-based studies, although widely adopted, present several critical limitations. 3D cell-spheroid cultures offer a novel approach to more accurately assess odontogenic potential before *in vivo* experimentation. As previously described, Raik et al. [214] compared the osteogenic potential of 2D monolayer and 3D spheroid DPSC cultures. Significantly increased RUNX2, COL1A1, and OCN mRNA expression were observed in spheroids compared to the monolayer cultures, with pronounced distinctions in the methylation of their promoter regions. Specifically, the upregulation of OCN in spheroid cultures was associated with significantly hypomethylated CpG sites. This aligns with previous findings suggesting that hypermethylation of the OCN promoter induces a condensed chromatin state, leading to reduced accessibility of transcription factors such as RUNX2 [373]. However, the differential expression of COL1A1 and RUNX2 were not correlated with altered methylation status. This may be explained by previous reports that RUNX2 methylation remains consistently low independent of osteogenic induction, whereby transcription upregulates during differentiation [374].

The role of DNA methylation in regulating odontoblastic differentiation and the extent to which modulation of these mechanisms can influence regenerative approaches remains elusive. DNMT1, DNMT3A, and DNMT3B serve different functions in DNA methylation dynamics, and TET family proteins (TET1-3) facilitate the oxidation of 5 mC, an intermediate step in DNA demethylation [375,376]. DNMT1 expression was found to be decreased in the previous study investigating RG108 for odontoblastic differentiation; however, the methylation profile of critical gene promoters was not analysed to characterise its contributions [372]. Unexpectedly, further analysis of the methylation profile of 3D spheroid DPSCs revealed DNMT3A/B upregulation in spheroid cultures. Conversely, significant downregulation of DNMT3B in monolayer cultures was observed [214]. These findings seem to contradict the typical suppressive effect of DNA methylation. However, TET1 expression was also significantly upregulated in spheroid cultures, consistent with previous reports whereby TET1 knockout reduced the expression of odontoblastic differentiation-associated genes and mineralisation-associated nodule formation [377,378]. Furthermore, recent studies have shown that family with sequence similarity 20, member C (FAM20C, formerly known as DMP-4) is a target for TET1 demethylation [378]. Odontoblastic differentiation was found to upregulate FAM20C expression, and its knockout resulted in marked decreases in DSPP and DMP-1 expression. Additionally, Raik et al. [214] found that the fold change of TET1 was, on average, 40 times that of DNMT3A/B and thus, it could be postulated that TET1 attenuated the *de novo* DNMT3A/B-mediated methylation, reinforcing that DNMTi may promote differentiation and mineralisation-associated gene expression.

While significant progress has been made in understanding the role of DNA methylation in odontoblastic differentiation, there remain critical gaps in our knowledge. Further investigations are required to delineate the specific roles of DNMTs and TETs in odontoblastic differentiation, particularly within 3D models and epigenetic modulation. Additionally, using 5-Aza-CdR in LPS-induced inflammation models has revealed intriguing insights into its potential clinical translatability. Moreover, the discrepancies observed in the DNA methylation profile between 2D and 3D models reinforce that the effects observed in monolayer-based studies are not directly translatable to *in vivo* actualities, underscoring the necessity for widespread adoption of clinically relevant models. Addressing these areas of inquiry is vital to fully elucidate the mechanisms underlying DNA methylation in odontoblastic differentiation and facilitate the development of novel regenerative approaches.

### 7.3. ncRNA-based therapies

While ncRNA-based therapies are relatively new, this niche of epigenetic modulation holds significant promise for future regenerative applications. In a recent study, Kearney et al. [379] elucidate the differential effects of 5-Aza-CdR and SAHA treatment on the miRNA expression of primary DPCs. Consistent with previous findings, SAHA elicited anti-proliferative effects, and notably, cells treated with SAHA showed decreased mineralisation at day 11. The supplementary data of this study reveals differential expression patterns of miR-377. In induced cultures treated with DEX, miR-377-5p was upregulated; conversely,

co-treatment with SAHA enhances miR-377-3p expression when compared to non-induced controls. Additionally, induced samples treated with SAHA showed increased expression of both miR-377 isoforms compared to those without SAHA.

Previous research has revealed that dexamethasone treatment increases the expression of FLH2, activating Wnt/β-catenin signalling pathways and activating the expression of RUNX2, ALP, and Col1A1 [380]. The canonical Wnt/β-catenin signalling pathway is initiated by the association of Wnt ligands with relevant cell surface receptors. This interaction inhibits the destruction complex, which usually targets β-catenin for degradation. Consequently, β-catenin is stabilised and can translocate into the nucleus, which associates with TFs to upregulate target gene expression [381]. Furthermore, luciferase assays reveal that FHL2 is a target of miR-377, which attenuates Wnt/β-catenin signalling [382]. Investigating whether the adverse effects associated with SAHA treatment can be directly attributed to the upregulation of miR-377 isoforms via knockdown experiments would be valuable for understanding the underlying mechanisms.

Additionally, miR-140-5p inhibition has been shown to upregulate Wnt1, DMP-1, and DSPP, while miR-342-5p has been found to upregulate Wnt7b, DSPP, DMP-1, OSX, OCN, ALP, and COL1A1 [383,384]. Moreover, the lncRNA H19 has previously been found to function as a "sponge" to sequester miR-140-5p activity, thereby preventing its inhibitory effect on the target genes BMP-2 and FGF [385]. Similarly, the lncRNA DANCR has been found to regulate miR-216a similarly, yet this elicits upregulation of c-Cbl, inhibiting odontoblastic differentiation [386]. Notably, previous studies have revealed that c-Cbl can induce the degradation of nuclear B-catenin and inhibit pro-angiogenic Wnt targets [387]. In a contrasting regulatory mechanism, the lncRNA SNHG1 binds miRNA-328-3p, abrogating its activation of the Wnt/B-catenin signalling and enhancing odontoblastic differentiation [388]. This finding diverges from that of previously discussed studies, whereby Wnt/B-catenin signalling is beneficial to differentiation via direct upregulation of RUNX2 expression; however, RUNX2 has been implicated in preventing the terminal differentiation of odontoblasts [389]. These findings give rise to the therapeutic potential of leveraging epigenetic modulation via targeting of miRNAs and lncRNAs implicated in Wnt/B-catenin-mediated signalling with ago/antagomiRs. Integrating these innovative approaches into bioactive materials to enhance odontoblastic differentiation holds promise for advancing regenerative endodontic efforts.

Furthermore, Gong et al. [390] conducted a similar investigation of the differential expression of miRNAs in induced primary DPCs. While no similarities are observed between this study and Kearney et al. [379], likely because of the heterogenous cell population, Gong et al. [390] find significant downregulation of miR-135b at all time points. This finding aligns well with a previous study that found miR-135b to inhibit the odontoblastic differentiation of primary DPCs by regulating Smad4/5 [391]. BMP signalling can occur via two pathways: canonical and non-canonical. In the canonical pathway, BMP ligands, such as BMP-2 and -4, associate with their receptors, leading to the formation of a complex between phosphorylated Smad1/5/8 with co-mediator Smad4 that interacts with key transcription factors such as RUNX2 to upregulate odontoblastic differentiation-associated genes such as DMP-1 and DSPP [392,393]. Collectively, these results suggest that treatment with antagomir-135b may enhance odontoblastic differentiation via R-Smad-mediated gene upregulation, although further research is necessary to investigate the feasibility of this approach.

Liu et al. [394] conducted another differential expression analysis on differentiating DPSCs, reporting a significant downregulation of miR-584-5p compared to non-induced controls. Independently, Tian et al. [395] investigated the effects of miR-584 knockdown on DPSCs and discovered that it promotes odontoblastic proliferation and migration by targeting transcriptional coactivator with the PDZ-binding motif, WWTR1 (TAZ). TAZ is a downstream coactivator that plays an important role in the Hippo signalling pathway when inactive TAZ is dephosphorylated and translocated into the nucleus, binding to transcription factors to activate specific target genes [396]. Previous research has demonstrated that TAZ overexpression significantly increases COL1A1, BSP, OC, ALP, and RUNX2 expression alongside enhancing Smad2/3/4 nuclear translocation [397]. Furthermore, TAZ has been implicated in the enhancement of the proliferative and migratory abilities of DPSCs via the TGF-β/BMP pathway [398]. Considering the breadth of these implications, it can be proposed that antagomir-584 may enhance proliferation, migration, and differentiation in pulpal tissue. However, further investigations are needed to fully understand the intricate crosstalk between the TAZ/YAP-mediated Hippo signalling and other pathways, such as ERK1/2, Wnt/β-catenin, and BCL-2 pathways.

Further understanding the role of miRNA expression and its modulation for dental pulp regeneration holds significant implications for advancing regenerative endeavours, yet considering the practical implementation of these insights is vital to their success. Angiogenic-odontogenic coupling is critical for successful pulp regeneration. Wang et al. [399] demonstrated that the angiogenic potential of primary DPCs may be enhanced via inhibition of miR-424, whereby increased tubule branching and length was observed. Moreover, recent advances in biomaterial-based delivery have yielded promising platforms, such as developing novel collagen-nano HA scaffolds [400]. These scaffolds offer a dual-loading capacity for distinct miRNAs mimics/inhibitors, presenting promising avenues for creating "off-the-shelf" solutions that facilitate angiogenic-odontogenic coupling for regenerative endodontic applications.

### 7.4. Opportunities

Epigenetic modulation presents several promising avenues for the development of next-generation doped-bioactive materials. Isoform-specific DNMTis and HDACis hold profound potential to attenuate the distinct challenges associated with pan-isoform inhibitors, synthesising effective targeted therapeutics. Moreover, the previously mentioned miRNAs (miR-377, -342-5p, -140-5p, −135b, and −584) offer exciting opportunities to incorporate miRNA mimics/inhibitors to modulate odontoblastic differentiation. Furthermore, the effects of lncRNA as miRNA sponges have profound potential for innovative regenerative strategies. Additionally, novel dual-loaded scaffolds enable angiogenic-odontogenic coupling to create 'off-the-shelf' solutions. Future research should focus on assessing the feasibility of these approaches in comparable, robust 3D models to advance the potential of regenerative endodontic solutions.

### 7.5. Challenges

Despite the promise shown by epigenetic modulation and the exciting opportunities for innovative and novel approaches, several challenges persist. Foremost, the regulatory pathways that govern odontoblastic differentiation are poorly characterised, and the intricate interplay between the various networks requires further elucidation for the development of targeted therapeutics. Furthermore, the distinct lack of comparable experimental design and robust preclinical models poses significant hurdles to translating these promising findings from bench to chairside. Additionally, as mentioned earlier, the systemic effects of epigenetic modulation from regenerative approaches are yet to be discerned, as are their toxicity and potential adverse effects. Addressing these challenges is vital to advancing regenerative solutions and facilitating their deployment within dental clinics.

## 8. Regulatory approval

Navigating regulatory approval routes to translate a new dental material requires a comprehensive understanding of the corresponding legal framework, forming crucial gateways that present opportunities

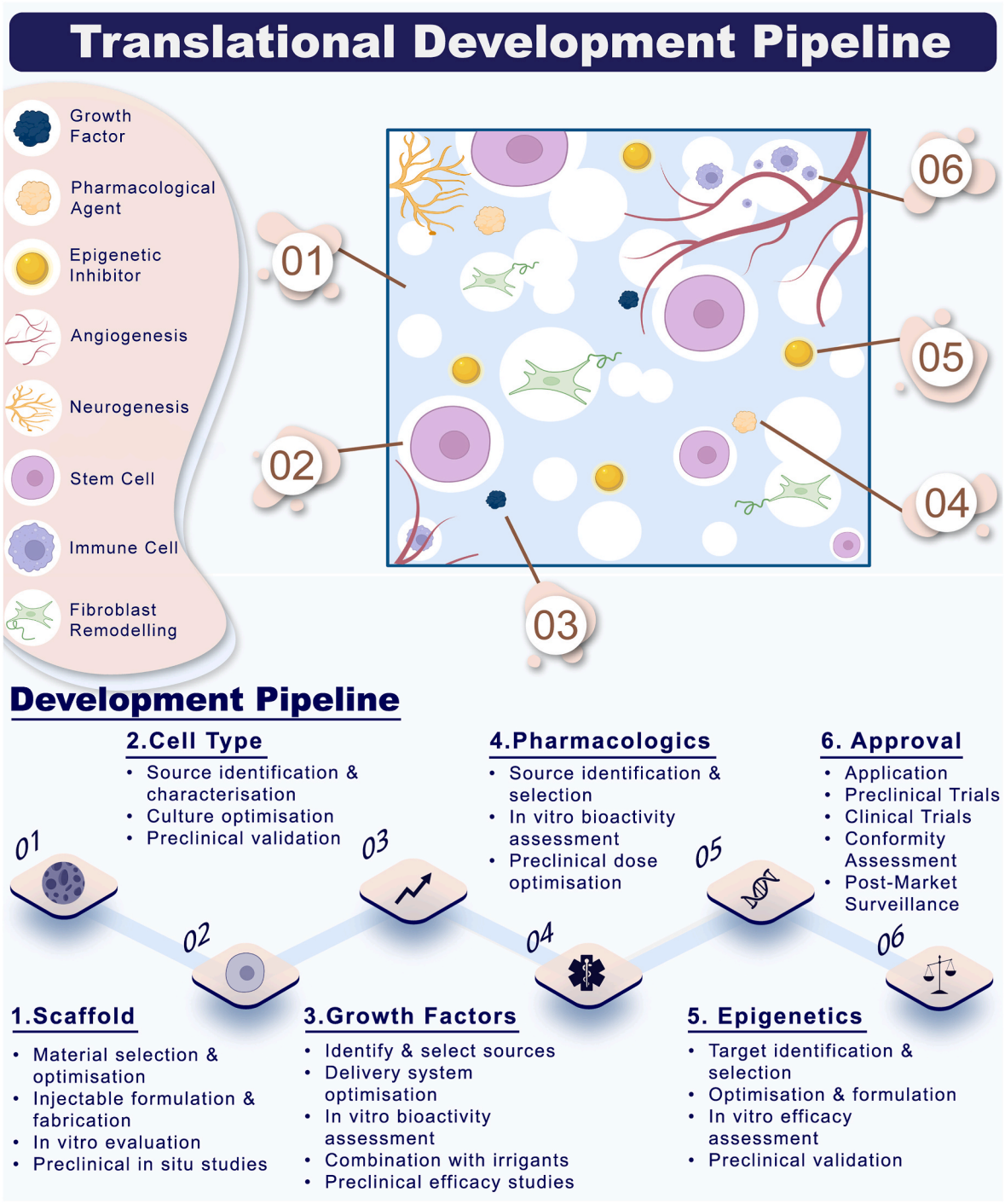


**Fig. 5.** | Translational development pipeline schematic of novel regenerative endodontic strategies.

and challenges. All dental filling materials are classed as medical devices under European and US legislation. The Medical Device Regulation (MDR) is an update of the existing European legislation, also known as EU/2017/745, which became effective in May 2021 and applies to all European Union countries. The MDR imposes strict quality control regulations on developing and using all medical devices. The transition period for meeting the requirements expires in May 2024, which denotes when all medical devices on the market must meet the standards set out in the MDR legislation. The absence of 'grandfathering' could lead to product shortages and increased financial pressure on companies, dentists and dental schools [401]. Additionally, components previously approved by the EMA or FDA, such as G-CSF [280], may no longer provide a 'fast track' to regulatory approval for regenerative applications.

The new legislation has also altered the categorisation of medical devices and, by extension, dental restorative materials, with dental materials that contact the pulp tissue, endodontic filling materials, drugs, animal-derived bone graft substitutes, and nanomaterials now classified as Class III devices, which means they are subject to the most stringent approval route. Furthermore, implantable devices that will be incorporated into the body must have an implant card; dental fillings are currently exempt from this requirement, as it would unnecessarily burden manufacturers and practitioners; however, the level of industrial responsibility has increased significantly (Article 18 (3), MDR).

Unfortunately, there are multiple grey areas with dental osteointegrated implants, abutments, and soft tissue substitutes, which are not exempt. They require a summary of safety and clinical performance to be publicly available, including a device description, previous iterations, alternatives, and an overview of the clinical evaluation (Article 32, MDR). These regulatory changes represent an immediate burden but may eventually be beneficial from a patient-centred care perspective. However, in the meantime, companies must allocate sufficient funding to ensure their products meet these standards, which may divert resources from innovative next-generation efforts and product development.

### 8.1. Opportunities

The new regulatory approval and clinical safety standards align with a patient-centred approach to healthcare, ultimately creating an ecosystem that benefits the patient and reassures practising dentists. Although fewer products will likely be available, these products will be safer. Moreover, the transition period has been extended to 2026 for Class III and 2028 for Class I devices [402]. Furthermore, the proposed introduction of a new EUDAMED database will allow a more streamlined and transparent process for verifying the regulatory status of alternative and competing products, albeit not yet fully functional.

### 8.2. Challenges

Academic groups interested in translating novel research embracing regenerative strategies may find that the industry is not enthusiastic about funding 'early-stage research' in dentistry, given that their return on investment will be lengthened. A change in the business model will be required so that manufacturing companies understand that the increased resources allocated to regulation do not diminish funds for research and development. A reduction in industrial-funded research immediately reduces any product's ability to be translated and indirectly reduces innovation in dentistry as new research areas may not move towards the clinic. These changes will have knock-on effects on small and medium enterprises (SMEs) due to limited resources, thus reducing product diversity and leading to market consolidation. Finally, notified bodies are the approved regulatory authorities that conduct the conformity assessment; however, an immediate challenge will arise as it appears unlikely that there will be enough regulators to achieve the volume of evaluations likely to evolve.

## 9. Conclusion and perspectives

The recent shift from traditional RCT toward more conservative VPT has led to the exploration of conventional regenerative medicine approaches employing scaffolds, cells, and GFs. To date, this has not resulted in successful clinical translation. This review examined recent progress in bioactive biomaterial-based strategies, focusing on pharmacological inhibitors and epigenetic modulation. The general limitations in this area are primarily attributed to the distinct lack of clinically relevant models, scalability issues, and the regulatory burden introduced by recent legislative changes (Fig. 5).

Despite these challenges, novel bioactive agents, particularly SIM and epigenetic modulators, present promising avenues for translation. Integrating HDACis, DNMTis, and miRNA-based therapies offers exciting opportunities for translation. Furthermore, this approach overcomes the high cost and dose requirements of other bioactive compounds. However, elucidation of the mechanisms and long-term consequences of epigenetic modulation in preclinical studies is essential for clinical translation.

The recent updates to the European Medical Device Regulation (MDR) align well with pulp preservation strategies, taking a patient-centred perspective. However, lengthened approval routes, resource limitations for SMEs, and a lack of sufficient infrastructure for approval pose significant challenges to translation.

In conclusion, interdisciplinary efforts are necessary to address biological issues such as inadequate blood supply, ineffective infection management within the necrotic root canal system, and logistical hurdles, including model relevance, scalability, and regulatory limitations. Addressing these issues is essential to fully leveraging the potential of bioactive biomaterial-based strategies.

## CRediT authorship contribution statement

**Ross M. Quigley:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Michaela Kearney:** Writing – review & editing, Supervision. **Oran D. Kennedy:** Writing – review & editing, Supervision. **Henry F. Duncan:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

## Ethics approval and consent to participate

Ethics approval is not applicable to this review article.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

RQ conducted the literature review, wrote the manuscript, and carried out primary editing. MK offered guidance and contributed to manuscript editing. OK contributed to planning, offered guidance, and edited the manuscript. HD contributed to planning, offered guidance, wrote specific sections of the manuscript, and edited both the manuscript and figures. This work was supported by Trinity College Dublin (Trinity Research Doctorate Award). All figures were created with BioRender.com.

## References

[1] X. Qin, H. Zi, X. Zeng, Changes in the global burden of untreated dental caries from 1990 to 2019: a Systematic analysis for the global burden of disease study, Heliyon 8 (2022) e10714, https://doi.org/10.1016/j.heliyon.2022.e10714.
[2] G. Schmalz, A.J. Smith, Pulp development, repair, and regeneration: challenges of the transition from traditional dentistry to biologically based therapies, J. Endod. 40 (2014) S2–S5, https://doi.org/10.1016/j.joen.2014.01.018.
[3] J.J. Mao, S.G. Kim, J. Zhou, L. Ye, S. Cho, T. Suzuki, S.Y. Fu, R. Yang, X. Zhou, Regenerative endodontics, Dent. Clin. 56 (2012) 639–649, https://doi.org/10.1016/j.cden.2012.05.005.
[4] M. Widbiller, H. Knüttel, N. Meschi, F. Durán, Sindreu Terol, effectiveness of endodontic tissue engineering in treatment of apical periodontitis: a systematic review, Int. Endod. J. 56 (2022), https://doi.org/10.1111/iej.13784.
[5] F.J. O'Brien, Biomaterials & scaffolds for tissue engineering, Mater. Today 14 (2011) 88–95, https://doi.org/10.1016/s1369-7021(11)70058-x.
[6] P.N.R. Nair, On the causes of persistent apical periodontitis: a review, Int. Endod. J. 39 (2006) 249–281, https://doi.org/10.1111/j.1365-2591.2006.01099.x.
[7] H.F. Duncan, Lise-Lotte Kirkevang, O.A. Peters, Ikhlas El Karim, G. Krastl, Massimo Del Fabbro, B.S. Chong, K.M. Galler, Juan José Segura-Egea, M. Kebschull, Treatment of pulpal and apical disease: the European society of Endodontology (ESE) S3-level clinical practice guideline, Int. Endod. J. 56 (2023) 238–295, https://doi.org/10.1111/iej.13974.
[8] E.S. Reeh, H.H. Messer, W.H. Douglas, Reduction in tooth stiffness as a result of endodontic and restorative procedures, J. Endod. 15 (1989) 512–516, https://doi.org/10.1016/s0099-2399(89)80191-8.
[9] J. Yang, G. Yuan, Z. Chen, Pulp regeneration: current approaches and future challenges, Front. Physiol. 7 (2016), https://doi.org/10.3389/fphys.2016.00058.
[10] F. Mannocci, B. Bhuva, M. Roig, M. Zarow, K. Bitter, European society of Endodontology position statement: the restoration of root filled teeth, Int. Endod. J. 54 (2021) 1974–1981, https://doi.org/10.1111/iej.13607.
[11] H.F. Duncan, K.M. Galler, P.L. Tomson, S. Simon, I. El-Karim, R. Kundzina, G. Krastl, T. Dammaschke, H. Fransson, M. Markvart, M. Zehnder, L. Bjørndal, European society of Endodontology position statement: management of deep caries and the exposed pulp, Int. Endod. J. 52 (2019) 923–934, https://doi.org/10.1111/iej.13080.

[12] K.M. Galler, T. Akamp, H. Knüttel, M. Widbiller, A critical analysis of clinical research methods to study regenerative endodontics, Int. Endod. J. 55 (2022), https://doi.org/10.1111/iej.13734.

[13] R.S. Lacruz, S. Habelitz, J.T. Wright, M.L. Paine, Dental enamel formation and implications for oral health and disease, Physiol. Rev. 97 (2017) 939–993, https://doi.org/10.1152/physrev.00030.2016.

[14] D.H. Pashley, Dynamics of the pulpo-dentin complex, Crit. Rev. Oral Biol. Med. 7 (1996) 104–133, https://doi.org/10.1177/10454411960070020101.

[15] M. Goldberg, J.-J. Lasfargues, Pulpo-dentinal complex revisited, J. Dent. 23 (1995) 15–20, https://doi.org/10.1016/0300-5712(95)90655-2.

[16] E. Couve, Ultrastructural changes during the life cycle of human odontoblasts, Arch. Oral Biol. 31 (1986) 643–651, https://doi.org/10.1016/0003-9969(86)90093-2.

[17] W.T. Butler, H. Ritchie, The nature and functional significance of dentin extracellular matrix proteins, Int. J. Dev. Biol. 39 (1995) 169–179. https://pubmed.ncbi.nlm.nih.gov/7626404/. (Accessed 30 May 2024).

[18] P.D. Marsh, Microbial ecology of dental plaque and its significance in health and disease, Adv. Dent. Res. 8 (1994) 263–271, https://doi.org/10.1177/08959374940080022001.

[19] B. Nyvad, W. Crielaard, A. Mira, N. Takahashi, D. Beighton, Dental caries from a molecular microbiological perspective, Caries Res. 47 (2013) 89–102, https://doi.org/10.1159/000345367.

[20] N.B. Pitts, D.T. Zero, P.D. Marsh, K. Ekstrand, J.A. Weintraub, F. Ramos-Gomez, J. Tagami, S. Twetman, G. Tsakos, A. Ismail, Dental caries, Nat. Rev. Dis. Prim. 3 (2017), https://doi.org/10.1038/nrdp.2017.30.

[21] A. Linde, M. Goldberg, Dentinogenesis, Crit. Rev. Oral Biol. Med. 4 (1993) 679–728, https://doi.org/10.1177/10454411930040050301.

[22] A. Linde, T. Lundgren, From serum to the mineral phase. The role of the odontoblast in calcium transport and mineral formation, Int. J. Dev. Biol. 39 (1995) 213–222. https://pubmed.ncbi.nlm.nih.gov/7626409/.

[23] A. Almushayt, K. Narayanan, A.E. Zaki, A. George, Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts, Gene Ther. 13 (2005) 611–620, https://doi.org/10.1038/sj.gt.3302687.

[24] A.J. Smith, Pulpal responses to caries and dental repair, Caries Res. 36 (2002) 223–232, https://doi.org/10.1159/000063930.

[25] R. Reeves, H.R. Stanley, The relationship of bacterial penetration and pulpal pathosis in carious teeth, Oral Surg. Oral Med. Oral Pathol. 22 (1966) 59–65, https://doi.org/10.1016/0030-4220(66)90143-5.

[26] P.N.R. Nair, Pathogenesis ofApicalPeriodontitis and theCauses ofEndodonticFailures, Crit. Rev. Oral Biol. Med. 15 (2004) 348–381, https://doi.org/10.1177/154411130401500604.

[27] World Health Organisation, Global Oral Health Status Report: towards Universal Health Coverage for Oral Health by 2030, 2022. Www.who.int. https://www.who.int/publications/i/item/9789240061484.

[28] B. Dye, G. Thornton-Evans, X. Li, T. Iafolla, Dental Caries and Tooth Loss in Adults in the United States, 2011-2012, NCHS Data Brief, 2015, p. 197. https://pubmed.ncbi.nlm.nih.gov/25973996/. (Accessed 30 May 2024).

[29] E. Fleming, J. Afful, Prevalence of Total and Untreated Dental Caries Among Youth: United States, 2015-2016, NCHS Data Brief, 2018, pp. 1–8. https://pubmed.ncbi.nlm.nih.gov/29717975/. (Accessed 30 May 2024).

[30] A.J. Righolt, M. Jevdjevic, W. Marcenes, S. Listl, Global-, regional-, and country-level economic impacts of dental diseases in 2015, J. Dent. Res. 97 (2018) 501–507, https://doi.org/10.1177/0022034517750572.

[31] L.E. Burns, J. Kim, Y. Wu, R. Alzwaideh, R. McGowan, A. Sigurdsson, Outcomes of primary root canal therapy: an updated systematic review of longitudinal clinical studies published between 2003 and 2020, Int. Endod. J. 55 (2022), https://doi.org/10.1111/iej.13736.

[32] W.P. Saunders, E.M. Saunders, Prevalence of periradicular periodontitis associated with crowned teeth in an adult scottish subpopulation, Br. Dent. J. 185 (1998) 137–140, https://doi.org/10.1038/sj.bdj.4809750.

[33] R.J.G. De Moor, G.M.G. Hommez, J.G. De Boever, K.I.M. Delme, G.E.I. Martens, Periapical health related to the quality of root canal treatment in a Belgian population, Int. Endod. J. 33 (2000) 113–120, https://doi.org/10.1046/j.1365-2591.2000.00295.x.

[34] G. Di Filippo, S.K. Sidhu, B.S. Chong, Apical periodontitis and the technical quality of root canal treatment in an adult sub-population in london, Br. Dent. J. 216 (2014), https://doi.org/10.1038/sj.bdj.2014.404. E22–E22.

[35] H.F. Duncan, Present status and future directions—vital pulp treatment and pulp preservation strategies, Int. Endod. J. 55 (2022), https://doi.org/10.1111/iej.13688.

[36] I.A. Mjör, L. Tronstad, The healing of experimentally induced pulpitis, Oral Surg. Oral Med. Oral Pathol. 38 (1974) 115–121, https://doi.org/10.1016/0030-4220(74)90322-3.

[37] J. Warfvinge, G. Bergenholtz, Healing capacity of human and monkey dental pulps following experimentally-induced pulpitis, Dent. Traumatol. 2 (1986) 256–262, https://doi.org/10.1111/j.1600-9657.1986.tb00158.x.

[38] R. Careddu, G. Plotino, E. Cotti, H.F. Duncan, The management of deep carious lesions and the exposed pulp amongst members of two European endodontic societies: a questionnaire-based study, Int. Endod. J. 54 (2020) 366–376, https://doi.org/10.1111/iej.13418.

[39] C. Barthel, B. Rosenkranz, A. Leuenberg, J. Roulet, Pulp capping of carious exposures: treatment outcome after 5 and 10 Years: a retrospective study, J. Endod. 26 (2000) 525–528, https://doi.org/10.1097/00004770-200009000-00010.

[40] G. Bergenholtz, C.F. Cox, W.J. Loesche, S.A. Syed, Bacterial leakage around dental restorations: its effect on the dental pulp, J. Oral Pathol. Med. 11 (1982) 439–450, https://doi.org/10.1111/j.1600-0714.1982.tb00188.x.

[41] C.F. Cox, G. Bergenholtz, D.R. Heys, S.A. Syed, M. Fitzgerald, R.J. Heys, Pulp capping of dental pulp mechanically exposed to oral microflora: a 1-2 Year observation of wound healing in the monkey, J. Oral Pathol. 14 (1985) 156–168, https://doi.org/10.1111/j.1600-0714.1985.tb00479.x.

[42] J. Mente, B. Geletneky, M. Ohle, M.J. Koch, P.G. Friedrich Ding, D. Wolff, J. Dreyhaupt, N. Martin, H.J. Staehle, T. Pfefferle, Mineral trioxide aggregate or calcium hydroxide direct pulp capping: an analysis of the clinical treatment outcome, J. Endod. 36 (2010) 806–813, https://doi.org/10.1016/j.joen.2010.02.024.

[43] K.C. da S. Modena, L.C. Casas-Apayco, M.T. Atta, C.A. de S. Costa, J. Hebling, C. R. Sipert, M.F. de L. Navarro, C.F. Santos, Cytotoxicity and biocompatibility of direct and indirect pulp capping materials, J. Appl. Oral Sci. 17 (2009) 544–554, https://doi.org/10.1590/S1678-77572009000600002.

[44] T.R.P. Ford, M. Torabinejad, H.R. Abedi, L.K. Bakland, S.P. Kariyawasam, Using mineral trioxide aggregate as a pulp-capping material, The Journal of the American Dental Association 127 (1996) 1491–1494, https://doi.org/10.14219/jada.archive.1996.0058.

[45] P.N.R. Nair, H.F. Duncan, T.R. Pitt Ford, H.U. Luder, Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial, Int. Endod. J. 0 (2007), https://doi.org/10.1111/j.1365-2591.2007.01329.x, 071026203843001-???

[46] J. Mente, S. Hufnagel, M. Leo, A. Michel, H. Gehrig, D. Panagidis, D. Saure, T. Pfefferle, Treatment outcome of mineral trioxide aggregate or calcium hydroxide direct pulp capping: long-term results, J. Endod. 40 (2014) 1746–1751, https://doi.org/10.1016/j.joen.2014.07.019.

[47] C.S. Hirschberg, G. Bogen, J.C. Galicia, R.R. Lemon, O.A. Peters, N.B. Ruparel, F. R. Tay, D.E. Witherspoon, AAE position statement on vital pulp therapy, J. Endod. 47 (2021) 1340–1344, https://doi.org/10.1016/j.joen.2021.07.015.

[48] H.F. Duncan, P.R. Cooper, A.J. Smith, Dissecting dentine–pulp injury and wound healing responses: consequences for regenerative endodontics, Int. Endod. J. 52 (2019) 261–266, https://doi.org/10.1111/iej.13064.

[49] P. Sangwan, A. Sangwan, J. Duhan, A. Rohilla, Tertiary dentinogenesis with calcium hydroxide: a review of proposed mechanisms, Int. Endod. J. 46 (2012) 3–19, https://doi.org/10.1111/j.1365-2591.2012.02101.x.

[50] S. Raghavendra, K. Gathani, Scaffolds in regenerative endodontics: a review, dental research, Journal 13 (2016) 379, https://doi.org/10.4103/1735-3327.192266.

[51] L.H. Hargreaves, Cohen's Pathways of the Pulp Expert Consult, Elsevier - Health Science, S.L., 2020.

[52] Y. Ikada, Challenges in tissue engineering, Journal of the Royal Society Interface 3 (2006) 589–601, https://doi.org/10.1098/rsif.2006.0124.

[53] G. Turnbull, J. Clarke, F. Picard, P. Riches, L. Jia, F. Han, B. Li, W. Shu, 3D bioactive composite scaffolds for bone tissue engineering, Bioact. Mater. 3 (2018) 278–314, https://doi.org/10.1016/j.bioactmat.2017.10.001.

[54] M. Goldberg, S. Lacerda-Pinheiro, N. Jegat, N. Six, D. Septier, F. Priam, M. Bonnefoix, K. Tompkins, H. Chardin, P. Denbesten, A. Veis, A. Poliard, The impact of bioactive molecules to stimulate tooth repair and regeneration as part of restorative dentistry, Dent. Clin. 50 (2006) 277–298, https://doi.org/10.1016/j.cden.2005.11.008, x.

[55] M.N. Collins, G. Ren, K. Young, S. Pina, R.L. Reis, J.M. Oliveira, Scaffold fabrication technologies and structure/function properties in bone tissue engineering, Adv. Funct. Mater. 31 (2021) 2010609, https://doi.org/10.1002/adfm.202010609.

[56] F. Causa, P.A. Netti, L. Ambrosio, A multi-functional scaffold for tissue regeneration: the need to engineer a tissue analogue, Biomaterials 28 (2007) 5093–5099, https://doi.org/10.1016/j.biomaterials.2007.07.030.

[57] S.J. Hollister, Porous scaffold design for tissue engineering, Nat. Mater. 4 (2005) 518–524, https://doi.org/10.1038/nmat1421.

[58] G.M. Cunniffe, F.J. O'Brien, Collagen scaffolds for orthopedic regenerative medicine, JOM 63 (2011) 66–73, https://doi.org/10.1007/s11837-011-0061-y.

[59] Y. Sumita, M. Honda, T. Ohara, S. Tsuchiya, H. Sagara, H. Kagami, M. Ueda, Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering, Biomaterials 27 (2006) 3238–3248, https://doi.org/10.1016/j.biomaterials.2006.01.055.

[60] W. Zhang, X. Frank Walboomers, V. Kuppevelt, W.F. Daamen, Z. Bian, J. A. Jansen, The performance of human dental pulp stem cells on different three-dimensional scaffold materials, Biomaterials 27 (2006) 5658–5668, https://doi.org/10.1016/j.biomaterials.2006.07.013.

[61] L. Niu, J. Sun, Q. Li, K. Jiao, L. Shen, D. Wu, F. Tay, J. Chen, Intrafibrillar-silicified collagen scaffolds enhance the osteogenic capacity of human dental pulp stem cells, J. Dent. 42 (2014) 839–849, https://doi.org/10.1016/j.jdent.2014.03.016.

[62] N. Mittal, S. Sharma, A comparative evaluation of natural and artificial scaffolds in regenerative endodontics: a clinical study, Saudi Endodontic Journal 6 (2016) 9, https://doi.org/10.4103/1658-5984.171995.

[63] R.S. Prescott, R. Alsanea, M.I. Fayad, B.R. Johnson, C.S. Wenckus, J. Hao, A. S. John, A. George, In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice, J. Endod. 34 (2008) 421–426, https://doi.org/10.1016/j.joen.2008.02.005.

[64] T. Qu, J. Jing, Y. Ren, C. Ma, J.Q. Feng, Q. Yu, X. Liu, Complete pulpodentin complex regeneration by modulating the stiffness of biomimetic matrix, Acta Biomater. 16 (2015) 60–70, https://doi.org/10.1016/j.actbio.2015.01.029.
[65] M. Nakashima, Induction of dentine in amputated pulp of dogs by recombinant human bone morphogenetic proteins-2 and -4 with collagen matrix, Arch. Oral Biol. 39 (1994) 1085–1089, https://doi.org/10.1016/0003-9969(94)90062-0.
[66] R. Bruce Rutherford, Larz S.W. Spångberg, M. Tucker, D.C. Rueger, M. Charette, The time-course of the induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1, Arch. Oral Biol. 39 (1994) 833–838, https://doi.org/10.1016/0003-9969(94)90014-0.
[67] Y. Inuyama, C. Kitamura, T. Nishihara, T. Morotomi, M. Nagayoshi, Y. Tabata, K. Matsuo, K.-K. Chen, M. Terashita, Effects of hyaluronic acid sponge as a scaffold on odontoblastic cell line and amputated dental pulp, J. Biomed. Mater. Res. B Appl. Biomater. 92B (2010) 120–128, https://doi.org/10.1002/jbm.b.31497.
[68] N. Kikuchi, C. Kitamura, T. Morotomi, Y. Inuyama, H. Ishimatsu, Y. Tabata, T. Nishihara, M. Terashita, Formation of dentin-like particles in dentin defects above exposed pulp by controlled release of fibroblast growth factor 2 from gelatin hydrogels, J. Endod. 33 (2007) 1198–1202, https://doi.org/10.1016/j.joen.2007.07.025.
[69] S.H. Fahmy, E.E.S. Hassanien, M.M. Nagy, K.M. El Batouty, M. Mekhemar, K. Fawzy El Sayed, E.H. Hassanein, J. Wiltfang, C. Dörfer, Investigation of the regenerative potential of necrotic mature teeth following different revascularisation protocols, Aust. Endod. J. 43 (2017) 73–82, https://doi.org/10.1111/aej.12210.
[70] H. Ishimatsu, C. Kitamura, T. Morotomi, Y. Tabata, T. Nishihara, K.-K. Chen, M. Terashita, Formation of dentinal bridge on surface of regenerated dental pulp in dentin defects by controlled release of fibroblast growth factor–2 from gelatin hydrogels, J. Endod. 35 (2009) 858–865, https://doi.org/10.1016/j.joen.2009.03.049.
[71] X. Yang, F. Yang, X.F. Walboomers, Z. Bian, M. Fan, J.A. Jansen, The performance of dental pulp stem cells on nanofibrous PCL/gelatin/nHA scaffolds, J. Biomed. Mater. Res. 93A (2009), https://doi.org/10.1002/jbm.a.32535. NA-NA.
[72] W. Jia, Y. Zhao, J. Yang, W. Wang, X. Wang, L. Ling, L. Ge, Simvastatin promotes dental pulp stem cell–induced coronal pulp regeneration in pulpotomized teeth, J. Endod. 42 (2016) 1049–1054, https://doi.org/10.1016/j.joen.2016.03.007.
[73] X. Liang, L. Xie, Q. Zhang, G. Wang, S. Zhang, M. Jiang, R. Zhang, T. Yang, X. Hu, Z. Yang, W. Tian, Gelatin methacryloyl-alginate core-shell microcapsules as efficient delivery platforms for prevascularized microtissues in endodontic regeneration, Acta Biomater. 144 (2022) 242–257, https://doi.org/10.1016/j.actbio.2022.03.045.
[74] I. Lukin, I. Erezuma, L. Maeso, J. Zarate, M.F. Desimone, T.H. Al-Tel, A. Dolatshahi-Pirouz, G. Orive, Progress in gelatin as biomaterial for tissue engineering, Pharmaceutics 14 (2022) 1177, https://doi.org/10.3390/pharmaceutics14061177.
[75] T. Yang, Q. Zhang, L. Xie, R. Zhang, R. Qian, Y. Tian, G. Chen, W. Tian, hDPSC-laden GelMA microspheres fabricated using electrostatic microdroplet method for endodontic regeneration, Mater. Sci. Eng. C 121 (2021) 111850, https://doi.org/10.1016/j.msec.2020.111850.
[76] A. Khayat, N. Monteiro, E.E. Smith, S. Pagni, W. Zhang, A. Khademhosseini, P. C. Yelick, GelMA-encapsulated hDPSCs and HUVECs for dental pulp regeneration, J. Dent. Res. 96 (2016) 192–199, https://doi.org/10.1177/0022034516682005.
[77] J. Li, Z. Wang, J. Wang, Q. Guo, Y. Fu, Z. Dai, M. Wang, Y. Bai, X. Liu, P. R. Cooper, J. Wu, W. He, Amphiregulin regulates odontogenic differentiation of dental pulp stem cells by activation of mitogen-activated protein kinase and the phosphatidylinositol 3-kinase signaling pathways, Stem Cell Res. Ther. 13 (2022), https://doi.org/10.1186/s13287-022-02971-4.
[78] H. Chen, M. Fan, Chitosan/carboxymethyl cellulose polyelectrolyte complex scaffolds for pulp cells regeneration, J. Bioact. Compat Polym. 22 (2007) 475–491, https://doi.org/10.1177/0883911507081329.
[79] X. Yang, G. Han, X. Pang, M. Fan, Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo, J. Biomed. Mater. Res. 108 (2012), https://doi.org/10.1002/jbm.a.34064 n/a-n/a.
[80] K.F. Abbas, H. Tawfik, A.A.R. Hashem, H.M.A. Ahmed, A.M. Abu-Seida, H. M. Refai, Histopathological evaluation of different regenerative protocols using chitosan-based formulations for management of immature non-vital teeth with apical periodontitis: in vivo study, Aust. Endod. J. 46 (2020) 405–414, https://doi.org/10.1111/aej.12426.
[81] D.G. Soares, E.A.F. Bordini, E.S. Bronze-Uhle, F.B. Cassiano, I.S.P. Silva, M. O. Gallinari, H.R. Matheus, J.M. Almeida, L.T.A. Cintra, J. Hebling, A. de, Chitosan-calcium-simvastatin scaffold as an inductive cell-free platform, J. Dent. Res. 100 (2021) 1118–1126, https://doi.org/10.1177/00220345211024207.
[82] G. da, G. Conceição, D. César, R. Andrade, Simoni Campos Dias, Samuel Nunes Limberger, F. Ko, Li Ting Lin, E.F. Haney, Hashem Etayash, Beverlie Baquir, M. J. Trimble, Y. Shen, Z. Su, M. Haapasalo, D. Pletzer, L. Chaves, Gláucia Schuindt Teixeira, R. Silva, Robert, antibiofilm and immunomodulatory resorbable nanofibrous filing for dental pulp regenerative procedures, Bioact. Mater. 16 (2022) 173–186, https://doi.org/10.1016/j.bioactmat.2022.01.027.
[83] M.S. Moreira, G. Sarra, G.L. Carvalho, F. Gonçalves, H.V. Caballero-Flores, A.C. F. Pedroni, C.A. Lascala, L.H. Catalani, M.M. Marques, Physical and biological properties of a chitosan hydrogel scaffold associated to photobiomodulation therapy for dental pulp regeneration: an in vitro and in vivo study, BioMed Res. Int. 2021 (2021) 1–10, https://doi.org/10.1155/2021/6684667.
[84] M. Yadav, B. Kaushik, G.K. Rao, C.M. Srivastava, D. Vaya, Advances and challenges in the use of chitosan and its derivatives in biomedical fields: a review, Carbohydrate Polymer Technologies and Applications 5 (2023) 100323, https://doi.org/10.1016/j.carpta.2023.100323.
[85] F. Croisier, C. Jérôme, Chitosan-based biomaterials for tissue engineering, Eur. Polym. J. 49 (2013) 780–792, https://doi.org/10.1016/j.eurpolymj.2012.12.009.
[86] P.P. Spicer, A.G. Mikos, Fibrin glue as a drug delivery system, J. Contr. Release 148 (2010) 49–55, https://doi.org/10.1016/j.jconrel.2010.06.025.
[87] M. Widbiller, R.B. Driesen, A. Eidt, I. Lambrichts, K.-A. Hiller, W. Buchalla, G. Schmalz, K.M. Galler, Cell homing for pulp tissue engineering with endogenous dentin matrix proteins, J. Endod. 44 (2018) 956–962.e2, https://doi.org/10.1016/j.joen.2018.02.011.
[88] K.M. Galler, A.C. Cavender, U. Koeklue, L.J. Suggs, G. Schmalz, R.N. D'Souza, Bioengineering of dental stem cells in a PEGylated fibrin gel, Regen. Med. 6 (2011) 191–200, https://doi.org/10.2217/rme.11.3.
[89] N. Ruangsawasdi, M. Zehnder, R. Patcas, C. Ghayor, F.E. Weber, Regenerative dentistry: animal model for regenerative Endodontology, Transfus. Med. Hemotherapy 43 (2016) 359–364, https://doi.org/10.1159/000447644.
[90] Nisarat Ruangsawasdi, M. Zehnder, F.E. Weber, Fibrin gel improves tissue ingrowth and cell differentiation in human immature premolars implanted in rats, J. Endod. 40 (2014) 246–250, https://doi.org/10.1016/j.joen.2013.09.022.
[91] K.M. Galler, F.P. Brandl, S. Kirchhof, M. Widbiller, A. Eidt, W. Buchalla, A. Göpferich, G. Schmalz, Suitability of different natural and synthetic biomaterials for dental pulp tissue engineering, Tissue Eng. 24 (2018) 234–244, https://doi.org/10.1089/ten.tea.2016.0555.
[92] L. Lambricht, P. De Berdt, J. Vanacker, J. Leprince, A. Diogenes, H. Goldansaz, C. Bouzin, V. Préat, C. Dupont-Gillain, A. des Rieux, The type and composition of alginate and hyaluronic-based hydrogels influence the viability of stem cells of the apical papilla, Dent. Mater. 30 (2014) e349–e361, https://doi.org/10.1016/j.dental.2014.08.369.
[93] X. Li, E. Katsanevakis, X. Liu, N. Zhang, X. Wen, Engineering neural stem cell fates with hydrogel design for central nervous system regeneration, Prog. Polym. Sci. 37 (2012) 1105–1129, https://doi.org/10.1016/j.progpolymsci.2012.02.004.
[94] L. Tan, J. Wang, S. Yin, W. Zhu, G. Zhou, Y. Cao, L. Cen, Regeneration of dentin–pulp-like tissue using an injectable tissue engineering technique, RSC Adv. 5 (2015) 59723–59737, https://doi.org/10.1039/c5ra06481c.
[95] C.R. Silva, P.S. Babo, M. Gulino, L. Costa, J.M. Oliveira, J. Silva-Correia, R.M. A. Domingues, R.L. Reis, M.E. Gomes, Injectable and tunable hyaluronic acid hydrogels releasing chemotactic and angiogenic growth factors for endodontic regeneration, Acta Biomater. 77 (2018) 155–171, https://doi.org/10.1016/j.actbio.2018.07.035.
[96] M.N. Collins, C. Birkinshaw, Hyaluronic acid based scaffolds for tissue engineering—a review, Carbohydrate Polymers 92 (2013) 1262–1279, https://doi.org/10.1016/j.carbpol.2012.10.028.
[97] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, Prog. Polym. Sci. 37 (2012) 106–126, https://doi.org/10.1016/j.progpolymsci.2011.06.003.
[98] R. Zhang, L. Xie, H. Wu, T. Yang, Q. Zhang, Y. Tian, Y. Liu, X. Han, W. Guo, M. He, S. Liu, W. Tian, Alginate/laponite hydrogel microspheres co-encapsulating dental pulp stem cells and VEGF for endodontic regeneration, Acta Biomater. 113 (2020) 305–316, https://doi.org/10.1016/j.actbio.2020.07.012.
[99] R. Oliva-Rodríguez, José Pérez-Urizar, E. Dibildox-Alvarado, María Consolación Martínez-Saldaña, Francisco Javier Avelar-González, Héctor Flores-Reyes, A. de, Alma Lilián Guerrero-Barrera, Design of a controlled release system of OP-1 and TGF-β1 based in microparticles of sodium alginate and release characterization by HPLC-UV, In Vitro Cellular & Developmental Biology – Animal 47 (2011) 681–688, https://doi.org/10.1007/s11626-011-9459-7.
[100] L. Tronstad, J.O. Andreasen, G. Hasselgren, L. Kristerson, I. Riis, pH changes in dental tissues after root canal filling with calcium hydroxide, J. Endod. 7 (1981) 17–21, https://doi.org/10.1016/s0099-2399(81)80262-2.
[101] J.-W. Yang, Y. Zhang, C. Wan, Z. Sun, S. Nie, S. Jian, L. Zhang, G. Song, Z. Chen, Autophagy in SDF-1α-mediated DPSC migration and pulp regeneration, Biomaterials 44 (2015) 11–23, https://doi.org/10.1016/j.biomaterials.2014.12.006.
[102] J. Wei, X.-Q. Sun, B.-X. Hou, Evaluation of silk fibroin-RGD-stem cell factor scaffold effect on adhesion, migration, and proliferation of stem cells of apical papilla, Stem Cell. Int. 2021 (2021) 1–10, https://doi.org/10.1155/2021/6612324.
[103] S. Jiang, Z. Yu, L. Zhang, G. Wang, X. Dai, X. Lian, Y. Yan, L. Zhang, Y. Wang, R. Li, H. Zou, Effects of different aperture-sized type I collagen/silk fibroin scaffolds on the proliferation and differentiation of human dental pulp cells, Regenerative Biomaterials 8 (2021), https://doi.org/10.1093/rb/rbab028.
[104] F. Paladini, M. Pollini, Novel approaches and biomaterials for bone tissue engineering: a focus on silk fibroin, Materials 15 (2022) 6952, https://doi.org/10.3390/ma15196952.
[105] Y. Lyu, Y. Liu, H.-Y. He, H. Wang, Application of silk-fibroin-based hydrogels in tissue engineering, Gels 9 (2023), https://doi.org/10.3390/gels9050431, 431–431.
[106] Mar Collado-González, María Pilar Pecci-Lloret, D. García-Bernal, S.D. Aznar-Cervantes, R. Elías, José María Moraleda, J.L. Cenis, Francisco javier rodríguez-lozano, biological effects of silk fibroin 3D scaffolds on stem cells from human exfoliated deciduous teeth (SHEDs), Odontology 106 (2017) 125–134, https://doi.org/10.1007/s10266-017-0310-9.
[107] W. Sun, Y. Zhang, D.A. Gregory, A. Jimenez-Franco, Mhd Anas Tomeh, Songwei Lv, J. Wang, J.W. Haycock, J.R. Lu, X. Zhao, Patterning the neuronal cells via inkjet printing of self-assembled peptides on silk scaffolds, Progress in Natural Science/Progress in Natural Science 30 (2020) 686–696, https://doi.org/10.1016/j.pnsc.2020.09.007.

[108] A.T. Ulusoy, I. Turedi, M. Cimen, Z.C. Cehreli, Evaluation of blood clot, platelet-rich Plasma, platelet-rich fibrin, and platelet pellet as scaffolds in regenerative endodontic treatment: a prospective randomized trial, J. Endod. 45 (2019) 560–566, https://doi.org/10.1016/j.joen.2019.02.002.
[109] S. Arshad, F. Tehreem, M. Rehab khan, F. Ahmed, A. Marya, M.I. Karobari, Platelet-rich fibrin used in regenerative endodontics and dentistry: current uses, limitations, and future recommendations for application, International Journal of Dentistry 2021 (2021) 1–8, https://doi.org/10.1155/2021/4514598.
[110] D.M. Elkady, Y.R. Helaly, H.O. AbuBakr Hala, A.M. Yassin, N.A. AbdElkader, A. El Dina, A. Scarano, Ahmad, an animal study on the effectiveness of platelet-rich Plasma as a direct pulp capping agent, Sci. Rep. 14 (2024), https://doi.org/10.1038/s41598-024-54162-1.
[111] Nima Farshidfar, Mohammad Amin Amiri, D. Jafarpour, Shahram Hamedani, Seyyed Vahid Niknezhad, Lobat Tayebi, The feasibility of injectable PRF (I-PRF) for bone tissue engineering and its application in oral and maxillofacial reconstruction: from bench to chairside, Biomater. Adv. 134 (2022), https://doi.org/10.1016/j.msec.2021.112557, 112557–112557.
[112] K.M. Galler, J.D. Hartgerink, A.C. Cavender, G. Schmalz, R.N. D'Souza, A customized self-assembling peptide hydrogel for dental pulp tissue engineering, Tissue Eng. 18 (2012) 176–184, https://doi.org/10.1089/ten.tea.2011.0222.
[113] M. Abbasian, B. Massoumi, R. Mohammad-Rezaei, H. Samadian, M. Jaymand, Scaffolding polymeric biomaterials: are naturally occurring biological macromolecules more appropriate for tissue engineering? Int. J. Biol. Macromol. 134 (2019) 673–694, https://doi.org/10.1016/j.ijbiomac.2019.04.197.
[114] K. Kim, C.H. Lee, B.K. Kim, J.J. Mao, Anatomically shaped tooth and periodontal regeneration by cell homing, J. Dent. Res. 89 (2010) 842–847, https://doi.org/10.1177/0022034510370803.
[115] W. Lee, J.-H. Oh, J.-C. Park, H.-I. Shin, J.-H. Baek, H.-M. Ryoo, K.M. Woo, Performance of electrospun poly(ε-caprolactone) fiber meshes used with mineral trioxide aggregates in a pulp capping procedure, Acta Biomater. 8 (2012) 2986–2995, https://doi.org/10.1016/j.actbio.2012.04.032.
[116] S. Eap, Thibault Bécavin, L. Keller, Tunay Kökten, F. Fioretti, J.-L. Weickert, E. Deveaux, N. Benkirane-Jessel, S. Kuchler-Bopp, Nanofibers implant functionalized by neural growth factor as a strategy to innervate a bioengineered tooth, Adv. Healthcare Mater. 3 (2013) 386–391, https://doi.org/10.1002/adhm.201300281.
[117] Y.-J. Yoo, J.-H. Oh, Q. Zhang, W. Lee, K.M. Woo, Dimethyloxalylglycine-embedded poly(ε-caprolactone) fiber meshes promote odontoblastic differentiation of human dental pulp-derived cells, J. Endod. 44 (2018) 98–103. e1, https://doi.org/10.1016/j.joen.2017.09.002.
[118] Z. Mousavi Nejad, A. Zamanian, M. Saeidifar, H.R. Vanaei, M. Salar Amoli, 3D bioprinting of polycaprolactone-based scaffolds for pulp-dentin regeneration: investigation of physicochemical and biological behavior, Polymers 13 (2021) 4442, https://doi.org/10.3390/polym13244442.
[119] V.K. Gopinath, S. Soumya, V.Y. Chakrapani, T.S.S. Kumar, Odontogenic differentiation of inflamed dental pulp stem cells (IDPSCs) on polycaprolactone (PCL) nanofiber blended with hydroxyapatite, Dent. Mater. J. 40 (2021) 312–321, https://doi.org/10.4012/dmj.2020-005.
[120] S. Sheela, F.M. AlGhalban, K.A. Khalil, T. Laoui, V.K. Gopinath, Synthesis and biocompatibility evaluation of PCL electrospun membranes coated with MTA/HA for potential application in dental pulp capping, Polymers 14 (2022) 4862, https://doi.org/10.3390/polym14224862.
[121] E. Capuana, F. Lopresti, M. Ceraulo, V. La Carrubba, Poly-l-Lactic acid (PLLA)-Based biomaterials for regenerative medicine: a review on processing and applications, Polymers 14 (2022) 1153, https://doi.org/10.3390/polym14061153.
[122] M.M. Cordeiro, Z. Dong, T. Kaneko, Z. Zhang, M. Miyazawa, S. Shi, A.J. Smith, J. E. Nör, Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth, J. Endod. 34 (2008) 962–969, https://doi.org/10.1016/j.joen.2008.04.009.
[123] L. Casagrande, Flávio Fernando Demarco, Z.P. Zhang, F. Borba, S.S. Shi, J.E. Nör, Dentin-derived BMP-2 and odontoblast differentiation, J. Dent. Res. 89 (2010) 603–608, https://doi.org/10.1177/0022034510364487.
[124] F.F. Demarco, L. Casagrande, Z. Zhang, Z. Dong, S.B. Tarquinio, B.D. Zeitlin, S. Shi, A.J. Smith, J.E. Nör, Effects of morphogen and scaffold porogen on the differentiation of dental pulp stem cells, J. Endod. 36 (2010) 1805–1811, https://doi.org/10.1016/j.joen.2010.08.031.
[125] J. Wang, H. Ma, X. Jin, J. Hu, X. Liu, L. Ni, P.X. Ma, The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells, Biomaterials 32 (2011) 7822–7830, https://doi.org/10.1016/j.biomaterials.2011.04.034.
[126] X. Li, C. Ma, X. Xie, H. Sun, X. Liu, Pulp regeneration in a full-length human tooth root using a hierarchical nanofibrous microsphere system, Acta Biomater. 35 (2016) 57–67, https://doi.org/10.1016/j.actbio.2016.02.040.
[127] T. Ito, T. Kaneko, Y. Sueyama, R. Kaneko, Takashi okiji, dental pulp tissue engineering of pulpotomized rat molars with bone marrow mesenchymal stem cells, Odontology 105 (2016) 392–397, https://doi.org/10.1007/s10266-016-0283-0.
[128] D.G. Soares, Z. Zhang, F. Mohamed, T.W. Eyster, C.A. de Souza Costa, P.X. Ma, Simvastatin and nanofibrous poly(l-lactic acid) scaffolds to promote the odontogenic potential of dental pulp cells in an inflammatory environment, Acta Biomater. 68 (2018) 190–203, https://doi.org/10.1016/j.actbio.2017.12.037.
[129] Z. Zhang, K.A. Warner, A. Mantesso, J.E. Nör, PDGF-BB signaling via PDGFR-β regulates the maturation of blood vessels generated upon vasculogenic differentiation of dental pulp stem cells, Front. Cell Dev. Biol. 10 (2022), https://doi.org/10.3389/fcell.2022.977725.
[130] B. Buurma, K. Gu, R.B. Rutherford, Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds, Eur. J. Oral Sci. 107 (1999) 282–289, https://doi.org/10.1046/j.0909-8836.1999.eos107408.x.
[131] K. Ceonzo, A. Gaynor, L. Shaffer, K. Kojima, C.A. Vacanti, G.L. Stahl, Polyglycolic acid-induced inflammation: role of hydrolysis and resulting complement activation, Tissue Eng. 12 (2006) 301–308, https://doi.org/10.1089/ten.2006.12.301.
[132] P.B. Maurus, C.C. Kaeding, Bioabsorbable implant material review, Operat. Tech. Sports Med. 12 (2004) 158–160, https://doi.org/10.1053/j.otsm.2004.07.015.
[133] V.K. Sugiaman, , Jeffrey, S. Naliani, N. Pranata, R. Djuanda, R.I. Saputri, Polymeric scaffolds used in dental pulp regeneration by tissue engineering approach, Polymers 15 (2023) 1082, https://doi.org/10.3390/polym15051082.
[134] M.T. Duailibi, S.E. Duailibi, C.S. Young, J.D. Bartlett, J.P. Vacanti, P.C. Yelick, Bioengineered teeth from cultured rat tooth bud cells, J. Dent. Res. 83 (2004) 523–528, https://doi.org/10.1177/154405910408300703.
[135] J. Zhu, Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering, Biomaterials 31 (2010) 4639–4656, https://doi.org/10.1016/j.biomaterials.2010.02.044.
[136] G.P. Raeber, M.P. Lutolf, J.A. Hubbell, Molecularly engineered PEG hydrogels: a novel model system for proteolytically mediated cell migration, Biophys. J. 89 (2005) 1374–1388, https://doi.org/10.1529/biophysj.104.050682.
[137] G.T.-J. Huang, T. Yamaza, L.D. Shea, F. Djouad, N.Z. Kuhn, R.S. Tuan, S. Shi, Stem/progenitor cell–mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model, Tissue Eng. 16 (2010) 605–615, https://doi.org/10.1089/ten.tea.2009.0518.
[138] L. Zheng, F. Yang, H. Shen, X. Hu, C. Mochizuki, M. Sato, S. Wang, Y. Zhang, The effect of composition of calcium phosphate composite scaffolds on the formation of tooth tissue from human dental pulp stem cells, Biomaterials 32 (2011) 7053–7059, https://doi.org/10.1016/j.biomaterials.2011.06.004.
[139] J.-M. Lü, X. Wang, C. Marin-Muller, H. Wang, P.H. Lin, Q. Yao, C. Chen, Current advances in research and clinical applications of PLGA-based nanotechnology, Expert Rev. Mol. Diagn. 9 (2009) 325–341, https://doi.org/10.1586/erm.09.15.
[140] Rehab Nabil Shamma, N.A. Elkasabgy, A.A. Mahmoud, Shaimaa Ismail Gawdat, M.M. Kataia, M.A. Abdel, Design of novel injectable in-situ forming scaffolds for non-surgical treatment of periapical lesions: in-vitro and in-vivo evaluation, Int. J. Pharm. 521 (2017) 306–317, https://doi.org/10.1016/j.ijpharm.2017.02.058.
[141] P. Gentile, V. Chiono, I. Carmagnola, P. Hatton, An overview of poly(lactic-co-glycolic) acid (PLGA)-Based biomaterials for bone tissue engineering, Int. J. Mol. Sci. 15 (2014) 3640–3659, https://doi.org/10.3390/ijms15033640.
[142] J. Chen, X. Zou, Self-assemble peptide biomaterials and their biomedical applications, Bioact. Mater. 4 (2019) 120–131, https://doi.org/10.1016/j.bioactmat.2019.01.002.
[143] W.L. Dissanayaka, K.M. Hargreaves, L. Jin, L.P. Samaranayake, C. Zhang, The interplay of dental pulp stem cells and endothelial cells in an injectable peptide hydrogel on angiogenesis and pulp regeneration in vivo, Tissue Eng. 21 (2015) 550–563, https://doi.org/10.1089/ten.tea.2014.0154.
[144] Y. Shen, M. Wang, T. Wang, M. Sun, H. Huang, X. Shi, S. Duan, Y. Wu, J. Zhu, F. Liu, Self-assembled short peptides: recent advances and strategies for potential pharmaceutical applications, Materials Today Bio 20 (2023), https://doi.org/10.1016/j.mtbio.2023.100644, 100644–100644.
[145] X. Dong, X. Xu, Bioceramics in endodontics: updates and future perspectives, Bioengineering 10 (2023) 354, https://doi.org/10.3390/bioengineering10030354.
[146] J. Zhang, L.X. Zhu, X. Cheng, Y. Lin, P. Yan, B. Peng, Promotion of dental pulp cell migration and pulp repair by a bioceramic putty involving FGFR-mediated signaling pathways, J. Dent. Res. 94 (2015) 853–862, https://doi.org/10.1177/0022034515572020.
[147] K.W. Al–Saudi, S.M. Nabih, A.M. Farghaly, E.A.-A. AboHager, Pulpal repair after direct pulp capping with new bioceramic materials: a comparative histological study, the Saudi dental, Journal 31 (2019) 469–475, https://doi.org/10.1016/j.sdentj.2019.05.003.
[148] J. Sanz, F. Rodríguez-Lozano, C. Llena, S. Sauro, L. Forner, Bioactivity of bioceramic materials used in the dentin-pulp complex therapy: a systematic review, Materials 12 (2019) 1015, https://doi.org/10.3390/ma12071015.
[149] H.-C. Lim, O.H. Nam, M.-J. Kim, A. El-Fiqi, H.-M. Yun, Y.-M. Lee, G.-Z. Jin, H.-H. Lee, H.-W. Kim, E.-C. Kim, Delivery of dexamethasone from bioactive nanofiber matrices stimulates odontogenesis of human dental pulp cells through integrin/BMP/mTOR signaling pathways, Int. J. Nanomed. 11 (2016) 2557–2567, https://doi.org/10.2147/IJN.S97846.
[150] R. Moonesi Rad, E. Pazarçeviren, E. Ece Akgün, Z. Evis, D. Keskin, S. Şahin, A. Tezcaner, In vitro performance of a nanobiocomposite scaffold containing boron-modified bioactive glass nanoparticles for dentin regeneration, J. Biomater. Appl. 33 (2018) 834–853, https://doi.org/10.1177/0885328218812487.
[151] L. Zhang, Y. Yu, C. Joubert, G. Bruder, Y. Liu, C.-C. Chang, M. Simon, S. Walker, M. Rafailovich, Differentiation of dental pulp stem cells on gutta-percha scaffolds, Polymers 8 (2016) 193, https://doi.org/10.3390/polym8050193.
[152] M. Fathi-Achacheloui, H. Knopf-Marques, C.E. Ribeiro da Silva, J. Barthès, E. Bat, A. Tezcaner, N.E. Vrana, Use of nanoparticles in tissue engineering and regenerative medicine, Front. Bioeng. Biotechnol. 7 (2019), https://doi.org/10.3389/fbioe.2019.00113.
[153] C. Niu, K. Yuan, R. Ma, L. Gao, W. Jiang, X. Hu, W. Lin, X. Zhang, Z. Huang, Gold nanoparticles promote osteogenic differentiation of human periodontal ligament stem cells via the p38 MAPK signaling pathway, Mol. Med. Rep. 16 (2017) 4879–4886, https://doi.org/10.3892/mmr.2017.7170.

[154] S.N. Kaushik, B. Kim, A.M.C. Walma, S.C. Choi, H. Wu, J.J. Mao, H.-W. Jun, K. Cheon, Biomimetic microenvironments for regenerative endodontics, Biomater. Res. 20 (2016), https://doi.org/10.1186/s40824-016-0061-7.
[155] D.A. Taylor, L.C. Sampaio, Z. Ferdous, A.S. Gobin, L.J. Taite, Decellularized matrices in regenerative medicine, Acta Biomater. 74 (2018) 74–89, https://doi.org/10.1016/j.actbio.2018.04.044.
[156] H. Alghutaimel, X. Yang, B. Drummond, H. Nazzal, M. Duggal, E. Raïf, Investigating the vascularization capacity of a decellularized dental pulp matrix seeded with human dental pulp stem cells: in vitro and preliminary in vivo evaluations, Int. Endod. J. 54 (2021), https://doi.org/10.1111/iej.13510.
[157] Q. Alqahtani, S.H. Zaky, A. Patil, E. Beniash, H. Ray, C. Sfeir, Decellularized swine dental pulp tissue for regenerative root canal therapy, J. Dent. Res. 97 (2018) 1460–1467, https://doi.org/10.1177/0022034518785124.
[158] L. Hu, Z. Gao, J. Xu, Z. Zhu, Z. Fan, C. Zhang, J. Wang, S. Wang, Decellularized swine dental pulp as a bioscaffold for pulp regeneration, BioMed Res. Int. 2017 (2017) 1–9, https://doi.org/10.1155/2017/9342714.
[159] Q. Tan, Y. Cao, X. Zheng, M. Peng, E. Huang, J. Wang, BMP4-regulated human dental pulp stromal cells promote pulp-like tissue regeneration in a decellularized dental pulp matrix scaffold, Odontology 109 (2021), https://doi.org/10.1007/s10266-021-00620-5.
[160] S. Ravindran, Y. Zhang, C.-C. Huang, A. George, Odontogenic induction of dental stem cells by extracellular matrix-inspired three-dimensional scaffold, Tissue Eng. 20 (2014) 92–102, https://doi.org/10.1089/ten.tea.2013.0192.
[161] J. Fu, J. Chen, W. Li, X. Yang, J. Yang, H. Quan, H. Huang, G. Chen, Laminin-Modified dental pulp extracellular matrix for dental pulp regeneration, Front. Bioeng. Biotechnol. 8 (2021), https://doi.org/10.3389/fbioe.2020.595096.
[162] N. Nowwarote, S. Petit, F.C. Ferre, F. Dingli, V. Laigle, D. Loew, T. Osathanon, B. P.J. Fournier, Extracellular matrix derived from dental pulp stem cells promotes mineralization, Front. Bioeng. Biotechnol. 9 (2022), https://doi.org/10.3389/fbioe.2021.740712.
[163] X. Zhang, X. Chen, H. Hong, R. Hu, J. Liu, C. Liu, Decellularized extracellular matrix scaffolds: recent trends and emerging strategies in tissue engineering, Bioact. Mater. 10 (2022) 15–31, https://doi.org/10.1016/j.bioactmat.2021.09.014.
[164] H.F. Duncan, A.J. Smith, G.J.P. Fleming, C. Reid, G. Smith, P.R. Cooper, Release of bio-active dentine extracellular matrix components by histone deacetylase inhibitors (HDACi), Int. Endod. J. 50 (2016) 24–38, https://doi.org/10.1111/iej.12588.
[165] L. Graham, P.R. Cooper, N. Cassidy, J.E. Nor, A.J. Sloan, A.J. Smith, The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components, Biomaterials 27 (2006) 2865–2873, https://doi.org/10.1016/j.biomaterials.2005.12.020.
[166] M.E. Grawish, L.M. Grawish, H.M. Grawish, M.M. Grawish, A.A. Holiel, N. Sultan, S.A. El-Negoly, Demineralized dentin matrix for dental and alveolar bone tissues regeneration: an innovative scope review, Tissue Engineering and Regenerative Medicine 19 (2022) 687–701, https://doi.org/10.1007/s13770-022-00438-4.
[167] J. Li, H. Yang, Q. Lu, D. Chen, M. Zhou, Y. Kuang, S. Ying, J. Song, Proteomics and N-glycoproteomics analysis of an extracellular matrix-based scaffold-human treated dentin matrix, Journal of Tissue Engineering and Regenerative Medicine 13 (2019), https://doi.org/10.1002/term.2866.
[168] W. Guo, Y. He, X. Zhang, W. Lu, C. Wang, H. Yu, Y. Liu, Y. Li, Y. Zhou, J. Zhou, M. Zhang, Z. Deng, Y. Jin, The use of dentin matrix scaffold and dental follicle cells for dentin regeneration, Biomaterials 30 (2009) 6708–6723, https://doi.org/10.1016/j.biomaterials.2009.08.034.
[169] R. Li, W. Guo, B. Yang, L. Guo, L. Sheng, G. Chen, Y. Li, Q. Zou, D. Xie, X. An, Y. Chen, W. Tian, Human treated dentin matrix as a natural scaffold for complete human dentin tissue regeneration, Biomaterials 32 (2011) 4525–4538, https://doi.org/10.1016/j.biomaterials.2011.03.008.
[170] S. Liu, J. Sun, S. Yuan, Y. Yang, Y. Gong, Y. Wang, R. Guo, X. Zhang, Y. Liu, H. Mi, M. Wang, M. Liu, R. Li, Treated dentin matrix induces odontogenic differentiation of dental pulp stem cells via regulation of wnt/β-catenin signaling, Bioact. Mater. 7 (2022) 85–97, https://doi.org/10.1016/j.bioactmat.2021.05.026.
[171] J. Chen, C. Cui, X. Qiao, B. Yang, M. Yu, W. Guo, W. Tian, Treated dentin matrix paste as a novel pulp capping agent for dentin regeneration, Journal of Tissue Engineering and Regenerative Medicine 11 (2017) 3428–3436, https://doi.org/10.1002/term.2256.
[172] H. Guo, B. Li, M. Wu, W. Zhao, X. He, B. Sui, Z. Dong, L. Wang, S. Shi, X. Huang, X. Liu, Z. Li, X. Guo, K. Xuan, Y. Jin, Odontogenesis-related developmental microenvironment facilitates deciduous dental pulp stem cell aggregates to revitalize an avulsed tooth, Biomaterials 279 (2021) 121223, https://doi.org/10.1016/j.biomaterials.2021.121223.
[173] H. Bakhtiar, A. Mazidi, S. Mohammadi-Asl, S. Hasannia, M.R. Ellini, M. Pezeshki-Modaress, S.N. Ostad, K. Galler, A. Azarpazhooh, A. Kishen, Potential of treated dentin matrix xenograft for dentin-pulp tissue engineering, J. Endod. 46 (2020) 57–64.e1, https://doi.org/10.1016/j.joen.2019.10.005.
[174] F. Wang, C. Xie, N. Ren, S. Bai, Y. Zhao, Human freeze-dried dentin matrix as a biologically active scaffold for tooth tissue engineering, J. Endod. 45 (2019) 1321–1331, https://doi.org/10.1016/j.joen.2019.08.006.
[175] L. Jiao, L. Xie, B. Yang, M. Yu, Z. Jiang, L. Feng, W. Guo, W. Tian, Cryopreserved dentin matrix as a scaffold material for dentin-pulp tissue regeneration, Biomaterials 35 (2014) 4929–4939, https://doi.org/10.1016/j.biomaterials.2014.03.016.
[176] A.A. Holiel, E.M. Mahmoud, W.M. Abdel-Fattah, K.Y. Kawana, Histological evaluation of the regenerative potential of a novel treated dentin matrix hydrogel in direct pulp capping, Clin. Oral Invest. 25 (2020) 2101–2112, https://doi.org/10.1007/s00784-020-03521-z.
[177] C.-C. Chang, T.-A. Lin, S.-Y. Wu, C.-P. Lin, H.-H. Chang, Regeneration of tooth with allogenous, autoclaved treated dentin matrix with dental pulpal stem cells: an in vivo study, J. Endod. 46 (2020) 1256–1264, https://doi.org/10.1016/j.joen.2020.05.016.
[178] J.L. Drury, D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications, Biomaterials 24 (2003) 4337–4351, https://doi.org/10.1016/s0142-9612(03)00340-5.
[179] S. Correa, A.K. Grosskopf, H. Lopez Hernandez, D. Chan, A.C. Yu, L.M. Stapleton, E.A. Appel, Translational applications of hydrogels, Chem. Rev. 121 (2021) 11385–11457, https://doi.org/10.1021/acs.chemrev.0c01177.
[180] S. Na, H. Zhang, F. Huang, W. Wang, Y. Ding, D. Li, Y. Jin, Regeneration of Dental pulp/dentine Complex with a three-dimensional and scaffold-free stem-cell sheet-derived Pellet, Journal of Tissue Engineering and Regenerative Medicine 10 (2013) 261–270, https://doi.org/10.1002/term.1686.
[181] R. Vasita, D.S. Katti, Nanofibers and their applications in tissue engineering, Int. J. Nanomed. 1 (2006) 15–30, https://doi.org/10.2147/nano.2006.1.1.15.
[182] S.M. Peltola, F.P.W. Melchels, D.W. Grijpma, M. Kellomäki, A review of rapid prototyping techniques for tissue engineering purposes, Ann. Med. 40 (2008) 268–280, https://doi.org/10.1080/07853890701881788.
[183] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (2014) 773–785, https://doi.org/10.1038/nbt.2958.
[184] H. Yu, X. Zhang, W. Song, T. Pan, H. Wang, T. Ning, Q. Wei, H.H.K. Xu, B. Wu, D. Ma, Effects of 3-dimensional bioprinting alginate/gelatin hydrogel scaffold extract on proliferation and differentiation of human dental pulp stem cells, J. Endod. 45 (2019) 706–715, https://doi.org/10.1016/j.joen.2019.03.004.
[185] Dobromira Shopova, A. Mihaylova, A. Yaneva, A. Mihaylova, Advancing dentistry through bioprinting: personalization of oral tissues, J. Funct. Biomater. 14 (2023), https://doi.org/10.3390/jfb14100530, 530–530.
[186] J. Han, W. Jeong, M.-K. Kim, S.-H. Nam, E.-K. Park, H.-W. Kang, Demineralized dentin matrix particle-based bio-ink for patient-specific shaped 3D dental tissue regeneration, Polymers 13 (2021) 1294, https://doi.org/10.3390/polym13081294.
[187] D.F. Duarte Campos, S. Zhang, F. Kreimendahl, M. Köpf, H. Fischer, M. Vogt, A. Blaeser, C. Apel, M. Esteves-Oliveira, Hand-held bioprinting for de novo vascular formation applicable to dental pulp regeneration, Connect. Tissue Res. 61 (2019) 205–215, https://doi.org/10.1080/03008207.2019.1640217.
[188] V. Rosa, Z. Zhang, R.H.M. Grande, J.E. Nör, Dental pulp tissue engineering in full-length human root canals, J. Dent. Res. 92 (2013) 970–975, https://doi.org/10.1177/0022034513505772.
[189] N. Monteiro, G. Thrivikraman, A. Athirasala, A. Tahayeri, C.M. França, J. L. Ferracane, L.E. Bertassoni, Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry, Dent. Mater. 34 (2018) 389–399, https://doi.org/10.1016/j.dental.2017.11.020.
[190] S. Gronthos, J. Brahim, W. Li, L.W. Fisher, N. Cherman, A. Boyde, P. DenBesten, P. G. Robey, S. Shi, Stem cell properties of human dental pulp stem cells, J. Dent. Res. 81 (2002) 531–535, https://doi.org/10.1177/154405910208100806.
[191] T.W. Lovelace, M.A. Henry, K.M. Hargreaves, A. Diogenes, Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure, J. Endod. 37 (2011) 133–138, https://doi.org/10.1016/j.joen.2010.10.009.
[192] Gordana Velikic, D.M. Maric, D.L. Maric, Gordana Supic, Miljan Puletic, O. Dulic, D. Vojvodic, Harnessing the stem cell niche in regenerative medicine: innovative avenue to combat neurodegenerative diseases, Int. J. Mol. Sci. 25 (2024), https://doi.org/10.3390/ijms25020993, 993–993.
[193] J.Y. Kim, X. Xin, E.K. Moioli, J. Chung, C.H. Lee, M. Chen, S.Y. Fu, P.D. Koch, J. J. Mao, Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing, Tissue Eng. 16 (2010) 3023–3031, https://doi.org/10.1089/ten.tea.2010.0181.
[194] L.-X. Zhang, L.-L. Shen, S.-H. Ge, L.-M. Wang, X.-J. Yu, Q.-C. Xu, P.-S. Yang, C.-Z. Yang, Systemic BMSC homing in the regeneration of pulp-like tissue and the enhancing effect of stromal cell-derived factor-1 on BMSC homing, Int. J. Clin. Exp. Pathol. 8 (2015) 10261–10271. https://pubmed.ncbi.nlm.nih.gov/26617734. (Accessed 30 May 2024).
[195] L. Li, Z. Wang, PDGF-BB, NGF and BDNF enhance pulp-like tissue regeneration via cell homing, RSC Adv. 6 (2016) 109519–109527, https://doi.org/10.1039/c6ra20290j.
[196] M. Nakashima, K. Iohara, Regeneration of dental pulp by stem cells, Adv. Dent. Res. 23 (2011) 313–319, https://doi.org/10.1177/0022034511405323.
[197] S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi, Postnatal human dental pulp stem cells (DPSCs) in vitro and invivo, Proc. Natl. Acad. Sci. USA 97 (2000) 13625–13630, https://doi.org/10.1073/pnas.240309797.
[198] K. Iohara, L. Zheng, M. Ito, R. Ishizaka, H. Nakamura, T. Into, K. Matsushita, M. Nakashima, Regeneration of dental pulp after pulpotomy by transplantation of CD31-/cd146- side population cells from a canine tooth, regenerative, Medicine 4 (2009) 377–385, https://doi.org/10.2217/rme.09.5.
[199] M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P.G. Robey, S. Shi, SHED: stem cells from human exfoliated deciduous teeth, Proc. Natl. Acad. Sci. USA 100 (2003) 5807–5812, https://doi.org/10.1073/pnas.0937635100.
[200] J.E. Nör, Buonocore memorial lecture, Operat. Dent. 31 (2006) 633–642, https://doi.org/10.2341/06-000.
[201] H. Rojbani, M. Nyan, K. Ohya, S. Kasugai, Evaluation of the osteoconductivity of α-tricalcium phosphate, β-tricalcium phosphate, and hydroxyapatite combined with or without simvastatin in rat calvarial defect, J. Biomed. Mater. Res. 98A (2011) 488–498, https://doi.org/10.1002/jbm.a.33117.
[202] W. Sonoyama, Y. Liu, D. Fang, T. Yamaza, B.-M. Seo, C. Zhang, H. Liu, S. Gronthos, C.-Y. Wang, S. Shi, S. Wang, Mesenchymal stem cell-mediated

functional tooth regeneration in swine, PLoS One 1 (2006) e79, https://doi.org/10.1371/journal.pone.0000079.
[203] W. Wang, M. Dang, Z. Zhang, J. Hu, T.W. Eyster, L. Ni, P.X. Ma, Dentin regeneration by stem cells of apical papilla on injectable nanofibrous microspheres and stimulated by controlled BMP-2 release, Acta Biomater. 36 (2016) 63–72, https://doi.org/10.1016/j.actbio.2016.03.015.
[204] M. Xiao, J. Qiu, R. Kuang, B. Zhang, W. Wang, Q. Yu, Synergistic effects of stromal cell-derived factor-1α and bone morphogenetic protein-2 treatment on odontogenic differentiation of human stem cells from apical papilla cultured in the VitroGel 3D system, Cell Tissue Res. 378 (2019) 207–220, https://doi.org/10.1007/s00441-019-03045-3.
[205] D. Menicanin, K.M. Mrozik, N. Wada, V. Marino, S. Shi, P.M. Bartold, S. Gronthos, Periodontal-ligament-derived stem cells exhibit the capacity for long-term survival, self-renewal, and regeneration of multiple tissue types in vivo, Stem Cell. Dev. 23 (2014) 1001–1011, https://doi.org/10.1089/scd.2013.0490.
[206] J. Zhang, H. Ding, X. Liu, Y. Sheng, X. Liu, C. Jiang, Dental follicle stem cells: tissue engineering and immunomodulation, Stem Cell. Dev. 28 (2019) 986–994, https://doi.org/10.1089/scd.2019.0012.
[207] Vivien Thiemy Sakai, Z.P. Zhang, R. Zhang, K.G. Neiva, M. Aparecida, S.S. Shi, C. Antonio, J.E. Nör, SHED differentiate into functional odontoblasts and endothelium, J. Dent. Res. 89 (2010) 791–796, https://doi.org/10.1177/0022034510368647.
[208] Z.Y. Kok, N.Y.A. Alaidaroos, A. Alraies, J.S. Colombo, L.C. Davies, R. J. Waddington, A.J. Sloan, R. Moseley, Dental pulp stem cell heterogeneity: finding superior quality "needles" in a dental pulpal "haystack" for regenerative medicine-based applications, Stem Cell. Int. 2022 (2022) 1–20, https://doi.org/10.1155/2022/9127074.
[209] S. Khaseb, M. Orooji, M.G. Pour, S.M. Safavi, M.J. Eghbal, M. Rezai Rad, Dental stem cell banking: techniques and protocols, Cell Biol. Int. 45 (2021) 1851–1865, https://doi.org/10.1002/cbin.11626.
[210] P. Hilkens, R.B. Driesen, E. Wolfs, Pascal Gervois, T. Vangansewinkel, J. Ratajczak, Y. Dillen, Annelies Bronckaers, Ivo Lambrichts, Cryopreservation and banking of dental stem cells, Adv. Exp. Med. Biol. 951 (2016) 199–235, https://doi.org/10.1007/978-3-319-45457-3_17.
[211] J.M. Kelm, V. Djonov, L.M. Ittner, D. Fluri, W. Born, S.P. Hoerstrup, M. Fussenegger, Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units, Tissue Eng. 12 (2006) 2151–2160, https://doi.org/10.1089/ten.2006.12.2151.
[212] K.H. Griffin, S.W. Fok, J. Kent Leach, Strategies to capitalize on cell spheroid therapeutic potential for tissue repair and disease modeling, NPJ Regenerative Medicine 7 (2022) 1–13, https://doi.org/10.1038/s41536-022-00266-z.
[213] A.I. Hoch, V. Mittal, D. Mitra, N. Vollmer, C.A. Zikry, J. Kent Leach, Cell-secreted matrices perpetuate the bone-forming phenotype of differentiated mesenchymal stem cells, Biomaterials 74 (2016) 178–187, https://doi.org/10.1016/j.biomaterials.2015.10.003.
[214] Raik Shalini, R. Thakur, V. Rattan, N. Kumar, A. Pal, S. Bhattacharyya, Temporal modulation of DNA methylation and gene expression in monolayer and 3D spheroids of dental pulp stem cells during osteogenic differentiation: a comparative study, Tissue Engineering and Regenerative Medicine 19 (2022) 1267–1282, https://doi.org/10.1007/s13770-022-00485-x.
[215] Y. Liu, X. Jiang, X. Zhang, R. Chen, T. Sun, K.L. Fok, J. Dong, L.L. Tsang, S. Yi, Y. Ruan, J. Guo, M.K. Yu, Y. Tian, Y.W. Chung, M. Yang, W. Xu, C.M. Chung, T. Li, H.C. Chan, Dedifferentiation-reprogrammed mesenchymal stem cells with improved therapeutic potential, Stem Cell. 29 (2011) 2077–2089, https://doi.org/10.1002/stem.764.
[216] Y. Rui, L. Xu, R. Chen, T. Zhang, S. Lin, Y. Hou, Y. Liu, F. Meng, Z. Liu, M. Ni, K. Sze Tsang, F. Yang, C. Wang, H. Chang Chan, X. Jiang, G. Li, Epigenetic memory gained by priming with osteogenic induction medium improves osteogenesis and other properties of mesenchymal stem cells, Sci. Rep. 5 (2015), https://doi.org/10.1038/srep11056.
[217] F. Paduano, E. Aiello, P.R. Cooper, B. Marrelli, I. Makeeva, M. Islam, G. Spagnuolo, D. Maged, D. De Vito, M. Tatullo, A dedifferentiation strategy to enhance the osteogenic potential of dental derived stem cells, Front. Cell Dev. Biol. 9 (2021), https://doi.org/10.3389/fcell.2021.668558.
[218] K.C. Murphy, A.I. Hoch, J.N. Harvestine, D. Zhou, J. Kent Leach, Mesenchymal stem cell spheroids retain osteogenic phenotype through α2β1 signaling, Stem Cells Translational Medicine 5 (2016) 1229–1237, https://doi.org/10.5966/sctm.2015-0412.
[219] P.N. Dang, N. Dwivedi, L.M. Phillips, X. Yu, S. Herberg, C. Bowerman, L. D. Solorio, W.L. Murphy, E. Alsberg, Controlled dual growth factor delivery from microparticles incorporated within human bone marrow-derived mesenchymal stem cell aggregates for enhanced bone tissue engineering via endochondral ossification, Stem Cells Translational Medicine 5 (2015) 206–217, https://doi.org/10.5966/sctm.2015-0115.
[220] J. Whitehead, A. Kothambawala, J. Kent Leach, Morphogen delivery by osteoconductive nanoparticles instructs stromal cell spheroid phenotype, Advanced Biosystems 3 (2019) 1900141, https://doi.org/10.1002/adbi.201900141.
[221] F. Abbasi, Mohammad Hossein Ghanian, Hossein Baharvand, Bahman Vahidi, Mohamadreza Baghaban Eslaminejad, Engineering mesenchymal stem cell spheroids by incorporation of mechanoregulator microparticles, Journal of the Mechanical Behavior of Biomedical Materials/Journal of Mechanical Behavior of Biomedical Materials 84 (2018) 74–87, https://doi.org/10.1016/j.jmbbm.2018.04.026.
[222] V.G. Martínez, I. Ontoria-Oviedo, Carolina Pinto Ricardo, S.E. Harding, R. Sacedón, A. Varas, A. Zapata, P. Sepúlveda, A. Vicente, Overexpression of hypoxia-inducible factor 1 alpha improves immunomodulation by dental mesenchymal stem cells, Stem Cell Res. Ther. 8 (2017), https://doi.org/10.1186/s13287-017-0659-2.
[223] Jong Won Hong, Jung Hyun Lim, C.J. Chung, Tae Jo Kang, Tae Yeon Kim, Young Seok Kim, Tae Suk Roh, Dae Hyun Lew, Immune tolerance of human dental pulp-derived mesenchymal stem cells mediated by CD4+CD25+FoxP3+ regulatory T-cells and induced by TGF-β1 and IL-10, Yonsei Medical Journal/Yonsei Medical Journal 58 (2017), https://doi.org/10.3349/ymj.2017.58.5.1031, 1031–1031.
[224] L. Luo, Y. He, X. Wang, B. Key, B.H. Lee, H. Li, Q. Ye, Potential roles of dental pulp stem cells in neural regeneration and repair, Stem Cell. Int. 2018 (2018) 1–15, https://doi.org/10.1155/2018/1731289.
[225] K. Janjić, Bledar Lilaj, A. Moritz, H. Agis, Formation of spheroids by dental pulp cells in the presence of hypoxia and hypoxia mimetic agents, Int. Endod. J. 51 (2018) e146–e156, https://doi.org/10.1111/iej.12806.
[226] H. Hsieh, T. Young, C. Yao, Y. Chen, Aggregation of human dental pulp cells into 3D spheroids enhances their migration ability after reseeding, J. Cell. Physiol. 234 (2018) 976–986, https://doi.org/10.1002/jcp.26927.
[227] Y. Duan, X. Li, S. Zhang, S. Wang, T. Wang, H. Chen, Y. Yang, S. Jia, G. Chen, W. Tian, Therapeutic potential of HERS spheroids in tooth regeneration, Theranostics 10 (2020) 7409–7421, https://doi.org/10.7150/thno.44782.
[228] K. Iohara, S. Utsunomiya, S. Kohara, M. Nakashima, Allogeneic transplantation of mobilized dental pulp stem cells with the mismatched dog leukocyte antigen type is safe and efficacious for total pulp regeneration, Stem Cell Res. Ther. 9 (2018), https://doi.org/10.1186/s13287-018-0855-8.
[229] J.K. Bar, A. Lis-Nawara, P.G. Grelewski, Dental pulp stem cell-derived secretome and its regenerative potential, Int. J. Mol. Sci. 22 (2021) 12018, https://doi.org/10.3390/ijms222112018.
[230] Y. Hayashi, M. Murakami, R. Kawamura, Ryo Ishizaka, Osamu Fukuta, M. Nakashima, CXCL14 and MCP1 are potent trophic factors associated with cell migration and angiogenesis leading to higher regenerative potential of dental pulp side population cells, Stem Cell Res. Ther. 6 (2015), https://doi.org/10.1186/s13287-015-0088-z.
[231] P. Harumi, C. Silvia, , Fernando, M. Elisabeth, D. Roberti, A. Clara, G. Carvalho, Diego Pulzatto Cury, N. Olsen, Márcia Martins Marques, Angiogenic properties of dental pulp stem cells conditioned medium on endothelial cells in vitro and in rodent orthotopic dental pulp regeneration, Heliyon 5 (2019) e01560, https://doi.org/10.1016/j.heliyon.2019.e01560.
[232] G. Sarra, M.E. de L. Machado, H.V. Caballero-Flores, M.S. Moreira, A.C. F. Pedroni, M.M. Marques, Effect of human dental pulp stem cell conditioned medium in the dentin-pulp complex regeneration: a pilot in vivo study, Tissue Cell 72 (2021) 101536, https://doi.org/10.1016/j.tice.2021.101536.
[233] T. Zhou, M. Rong, Z. Wang, H. Chu, C. Chen, J. Zhang, Z. Tian, Conditioned medium derived from 3D tooth germs: a novel cocktail for stem cell priming and early in vivo pulp regeneration, Cell Prolif. 54 (2021), https://doi.org/10.1111/cpr.13129.
[234] R. Vadivelu, H. Kamble, M. Shiddiky, N.-T. Nguyen, Microfluidic technology for the generation of cell spheroids and their applications, Micromachines 8 (2017) 94, https://doi.org/10.3390/mi8040094.
[235] S. Kuchler-Bopp, T. Bécavin, T. Kökten, J.L. Weickert, L. Keller, H. Lesot, E. Deveaux, N. Benkirane-Jessel, Three-dimensional micro-culture system for tooth tissue engineering, J. Dent. Res. 95 (2016) 657–664, https://doi.org/10.1177/0022034516634334.
[236] J.M. Kelm, N.E. Timmins, C.J. Brown, M. Fussenegger, L.K. Nielsen, Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types, Biotechnol. Bioeng. 83 (2003) 173–180, https://doi.org/10.1002/bit.10655.
[237] C. Jensen, Y. Teng, Is it time to start transitioning from 2D to 3D cell culture? Front. Mol. Biosci. 7 (2020) 33, https://doi.org/10.3389/fmolb.2020.00033.
[238] H. Kim, Y. Han, Intan Rosalina Suhito, Y. Choi, M. Kwon, H. Son, H.-R. Kim, T.-H. Kim, Raman spectroscopy-based 3D analysis of odontogenic differentiation of human dental pulp stem cell spheroids, Anal. Chem. 93 (2021) 9995–10004, https://doi.org/10.1021/acs.analchem.0c05165.
[239] I.K. Herrmann, M.J.A. Wood, G. Fuhrmann, Extracellular vesicles as a next-generation drug delivery platform, Nat. Nanotechnol. 16 (2021) 748–759, https://doi.org/10.1038/s41565-021-00931-2.
[240] X. Hu, Y. Zhong, Y. Kong, Y. Chen, J. Feng, J. Zheng, Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through tgfβ1/smads signaling pathway via transfer of microRNAs, Stem Cell Res. Ther. 10 (2019), https://doi.org/10.1186/s13287-019-1278-x.
[241] Y. Chen, Y. Ma, X. Yang, J. Chen, B. Yang, W. Tian, The application of pulp tissue derived-exosomes in pulp regeneration: a novel cell-homing approach, Int. J. Nanomed. 17 (2022) 465–476, https://doi.org/10.2147/ijn.s342685.
[242] Z. Li, M.-L. Wu, S. Liu, L. Wang, Y. Huan, Q. Ye, X. Yang, H. Guo, A. Liu, X. Huang, X. Yang, F. Ding, H.-K. Xu, J. Zhou, P. Liu, S. Liu, Y. Jin, K. Xuan, Apoptotic vesicles activate autophagy in recipient cells to induce angiogenesis and dental pulp regeneration, Mol. Ther. 30 (2022) 3193–3208, https://doi.org/10.1016/j.ymthe.2022.05.006.
[243] H.S. Wang, F.H. Yang, Y.J. Wang, F. Pei, Z. Chen, L. Zhang, Odontoblastic exosomes attenuate apoptosis in neighboring cells, J. Dent. Res. 98 (2019) 1271–1278, https://doi.org/10.1177/0022034519869580.
[244] X. Xian, Q. Gong, C. Li, B. Guo, H. Jiang, Exosomes with highly angiogenic potential for possible use in pulp regeneration, J. Endod. 44 (2018) 751–758, https://doi.org/10.1016/j.joen.2017.12.024.
[245] S. Zhang, Y. Yang, S. Jia, H. Chen, Y. Duan, X. Li, S. Wang, T. Wang, Y. Lyu, G. Chen, W. Tian, Exosome-like vesicles derived from Hertwig's epithelial root

sheath cells promote the regeneration of dentin-pulp tissue, Theranostics 10 (2020) 5914–5931, https://doi.org/10.7150/thno.43156.
[246] M. Wu, X. Liu, Z. Li, X. Huang, H. Guo, X. Guo, X. Yang, B. Li, K. Xuan, Y. Jin, SHED aggregate exosomes shuttled miR-26a promote angiogenesis in pulp regeneration via TGF-β/SMAD2/3 signalling, Cell Prolif. 54 (2021), https://doi.org/10.1111/cpr.13074.
[247] A. Brodzikowska, M. Ciechanowska, M. Kopka, A. Stachura, P.K. Włodarski, Role of lipopolysaccharide, derived from various bacterial species, in pulpitis—a systematic review, Biomolecules 12 (2022) 138, https://doi.org/10.3390/biom12010138.
[248] J. Li, Y. Ju, S. Liu, Y. Fu, S. Zhao, Exosomes derived from lipopolysaccharide-preconditioned human dental pulp stem cells regulate Schwann cell migration and differentiation, Connect. Tissue Res. 62 (2019) 277–286, https://doi.org/10.1080/03008207.2019.1694010.
[249] X. Huang, W. Qiu, Y. Pan, J. Li, Z. Chen, K. Zhang, Y. Luo, B. Wu, W. Xu, Exosomes from LPS-stimulated hDPSCs activated the angiogenic potential of HUVECs in vitro, Stem Cell. Int. 2021 (2021) 1–15, https://doi.org/10.1155/2021/6685307.
[250] J. Zheng, Y. Kong, X. Hu, Z. Li, Y. Li, Y. Zhong, X. Wei, J. Ling, MicroRNA-enriched small extracellular vesicles possess odonto-immunomodulatory properties for modulating the immune response of macrophages and promoting odontogenesis, Stem Cell Res. Ther. 11 (2020), https://doi.org/10.1186/s13287-020-02039-1.
[251] S. Yang, Q. Liu, S. Chen, F. Zhang, Y. Li, W. Fan, L. Mai, H. He, F. Huang, Extracellular vesicles delivering nuclear factor I/C for hard tissue engineering: treatment of apical periodontitis and dentin regeneration, J. Tissue Eng. 13 (2022), https://doi.org/10.1177/20417314221084095, 204173142210840-204173142210840.
[252] C.-C. Huang, R. Narayanan, S. Alapati, S. Ravindran, Exosomes as biomimetic tools for stem cell differentiation: applications in dental pulp tissue regeneration, Biomaterials 111 (2016) 103–115, https://doi.org/10.1016/j.biomaterials.2016.09.029.
[253] O.P.B. Wiklander, J.Z. Nordin, A. O'Loughlin, Y. Gustafsson, G. Corso, I. Mäger, P. Vader, Y. Lee, H. Sork, Y. Seow, N. Heldring, L. Alvarez-Erviti, C.E. Smith, K. Le Blanc, P. Macchiarini, P. Jungebluth, M.J.A. Wood, S.E. Andaloussi, Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting, J. Extracell. Vesicles 4 (2015) 26316, https://doi.org/10.3402/jev.v4.26316.
[254] B. Wen, Y. Huang, T. Qiu, F. Huo, L. Xie, L. Liao, W. Tian, W. Guo, Reparative dentin formation by dentin matrix proteins and small extracellular vesicles, J. Endod. 47 (2021) 253–262, https://doi.org/10.1016/j.joen.2020.11.017.
[255] S. Zhang, Anja Lena Thiebes, Franziska Kreimendahl, S. Ruetten, Eva Miriam Buhl, M. Wolf, S. Jockenhoevel, C. Apel, Extracellular vesicles-loaded fibrin gel supports rapid neovascularization for dental pulp regeneration, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21124226, 4226–4226.
[256] A. Ivica, Chafik Ghayor, M. Zehnder, Silvio Valdec, F.E. Weber, Pulp-derived exosomes in a fibrin-based regenerative root filling material, J. Clin. Med. 9 (2020), https://doi.org/10.3390/jcm9020491, 491–491.
[257] S. Goncalves, Z. Dong, C. Bramante, G. Holland, A. Smith, J. Nor, Tooth slice–based models for the study of human dental pulp angiogenesis, J. Endod. 33 (2007) 811–814, https://doi.org/10.1016/j.joen.2007.03.012.
[258] S. Fayazi, K. Takimoto, A. Diogenes, Comparative evaluation of chemotactic factor effect on migration and differentiation of stem cells of the apical papilla, J. Endod. 43 (2017) 1288–1293, https://doi.org/10.1016/j.joen.2017.03.012.
[259] S. Barrientos, O. Stojadinovic, M.S. Golinko, H. Brem, M. Tomic-Canic, Perspective article: growth factors and cytokines in wound healing, Wound Repair Regen. 16 (2008) 585–601, https://doi.org/10.1111/j.1524-475x.2008.00410.x.
[260] K.M. Galler, W. Buchalla, K.-A. Hiller, M. Federlin, A. Eidt, M. Schiefersteiner, G. Schmalz, Influence of root canal disinfectants on growth factor release from dentin, J. Endod. 41 (2015) 363–368, https://doi.org/10.1016/j.joen.2014.11.021.
[261] J. Zhang, X. Liu, W. Yu, Y. Zhang, C. Shi, S. Ni, Q. Liu, X. Li, Y. Sun, C. Zheng, H. Sun, Effects of human vascular endothelial growth factor on reparative dentin formation, Mol. Med. Rep. 13 (2015) 705–712, https://doi.org/10.3892/mmr.2015.4608.
[262] N. Ferrara, H.-P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nat. Med. 9 (2003) 669–676, https://doi.org/10.1038/nm0603-669.
[263] M. Yadlapati, C. Biguetti, F. Cavalla, F. Nieves, C. Bessey, P. Bohluli, G.P. Garlet, A. Letra, W.D. Fakhouri, R.M. Silva, Characterization of a vascular endothelial growth factor–loaded bioresorbable delivery system for pulp regeneration, J. Endod. 43 (2017) 77–83, https://doi.org/10.1016/j.joen.2016.09.022.
[264] G. Bergers, R. Brekken, G. McMahon, T.H. Vu, T. Itoh, K. Tamaki, K. Tanzawa, P. Thorpe, S. Itohara, Z. Werb, D. Hanahan, Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis, Nat. Cell Biol. 2 (2000) 737–744, https://doi.org/10.1038/35036374.
[265] G-One Ahn, J.M. Brown, Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells, Cancer Cell 13 (2008) 193–205, https://doi.org/10.1016/j.ccr.2007.11.032.
[266] G. Yuan, L. Chen, J. Feng, G. Yang, Q. Ni, X. Xu, C. Wan, M. Lindsey, K.J. Donly, M. MacDougall, Z. Chen, S. Chen, Dentin sialoprotein is a novel substrate of matrix metalloproteinase 9 in vitro and in vivo, Sci. Rep. 7 (2017), https://doi.org/10.1038/srep42449.
[267] H.F. Duncan, Y. Kobayashi, Y. Yamauchi, E. Shimizu, The reparative function of MMP13 in tertiary reactionary dentinogenesis after tooth injury, Int. J. Mol. Sci. 25 (2024), https://doi.org/10.3390/ijms25020875, 875–875.
[268] T. Yamashiro, M. Tummers, I. Thesleff, Expression of bone morphogenetic proteins and msx genes during root formation, J. Dent. Res. 82 (2003) 172–176, https://doi.org/10.1177/154405910308200305.
[269] B. Rutherford, M. Fitzgerald, A new biological approach to vital pulp therapy, Crit. Rev. Oral Biol. Med. 6 (1995) 218–229, https://doi.org/10.1177/10454411950060030401.
[270] R.B. Rutherford, K. Gu, Treatment of inflamed ferret dental pulps with recombinant bone morphogenetic protein-7, Eur. J. Oral Sci. 108 (2000) 202–206, https://doi.org/10.1034/j.1600-0722.2000.108003202.x.
[271] S. Jepsen, H.-K. Albers, B. Fleiner, M.M. Tucker, D.C. Rueger, Recombinant human osteogenic protein-1 induces dentin formation: an experimental study in miniature swine, J. Endod. 23 (1997) 378–382, https://doi.org/10.1016/s0099-2399(97)80187-2.
[272] J. Wang, X. Liu, X. Jin, H. Ma, J. Hu, L. Ni, P.X. Ma, The odontogenic differentiation of human dental pulp stem cells on nanofibrous poly(l-lactic acid) scaffolds in vitro and in vivo, Acta Biomater. 6 (2010) 3856–3863, https://doi.org/10.1016/j.actbio.2010.04.009.
[273] T. Suzuki, C.H. Lee, M. Chen, W. Zhao, S.Y. Fu, J.J. Qi, G. Chotkowski, S.B. Eisig, A. Wong, J.J. Mao, Induced migration of dental pulp stem cells for in vivo pulp regeneration, J. Dent. Res. 90 (2011) 1013–1018, https://doi.org/10.1177/0022034511408426.
[274] A.J. Sloan, A.J. Smith, Stimulation of the dentine–pulp complex of rat incisor teeth by transforming growth factor-β isoforms 1–3 in vitro, Arch. Oral Biol. 44 (1999) 149–156, https://doi.org/10.1016/s0003-9969(98)00106-x.
[275] W. He, J. Zhang, Z. Niu, Q. Yu, Z. Wang, R. Zhang, L. Su, L. Fu, A.J. Smith, P. R. Cooper, Regulatory interplay between NFIC and TGF-β1 in apical papilla-derived stem cells, J. Dent. Res. 93 (2014) 496–501, https://doi.org/10.1177/0022034514525200.
[276] T. Niwa, Y. Yamakoshi, H. Yamazaki, T. Karakida, R. Chiba, J.C.-C. Hu, T. Nagano, R. Yamamoto, J.P. Simmer, H.C. Margolis, K. Gomi, The dynamics of TGF-β in dental pulp, odontoblasts and dentin, Sci. Rep. 8 (2018), https://doi.org/10.1038/s41598-018-22823-7.
[277] A.C. Mitchell, P.S. Briquez, J.A. Hubbell, J.R. Cochran, Engineering growth factors for regenerative medicine applications, Acta Biomater. 30 (2016), https://doi.org/10.1016/j.actbio.2015.11.007.
[278] K. Iohara, M. Murakami, K. Nakata, M. Nakashima, Age-dependent decline in dental pulp regeneration after pulpectomy in dogs, Exp. Gerontol. 52 (2014) 39–45, https://doi.org/10.1016/j.exger.2014.01.020.
[279] M. Murakami, Hiroshi Horibe, Koichiro Iohara, Y. Hayashi, Y. Osako, Y. Takei, K. Nakata, N. Motoyama, K. Kurita, M. Nakashima, The use of granulocyte-colony stimulating factor induced mobilization for isolation of dental pulp stem cells with high regenerative potential, Biomaterials 34 (2013) 9036–9047, https://doi.org/10.1016/j.biomaterials.2013.08.011.
[280] M. Nakashima, K. Iohara, M. Murakami, H. Nakamura, Y. Sato, Y. Ariji, K. Matsushita, Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study, Stem Cell Res. Ther. 8 (2017), https://doi.org/10.1186/s13287-017-0506-5.
[281] P. Hilkens, A. Bronckaers, J. Ratajczak, P. Gervois, E. Wolfs, I. Lambrichts, The angiogenic potential of DPSCs and SCAPs in an in vivo model of dental pulp regeneration, Stem Cell. Int. 2017 (2017) 1–14, https://doi.org/10.1155/2017/2582080.
[282] K. Iohara, M. Nakashima, M. Ito, M. Ishikawa, A. Nakasima, A. Akamine, Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2, J. Dent. Res. 83 (2004) 590–595, https://doi.org/10.1177/154405910408300802.
[283] N. Six, J.-J. Lasfargues, M. Goldberg, Differential repair responses in the coronal and radicular areas of the exposed rat molar pulp induced by recombinant human bone morphogenetic protein 7 (osteogenic protein 1), Arch. Oral Biol. 47 (2002) 177–187, https://doi.org/10.1016/s0003-9969(01)00100-5.
[284] X. Li, L. Wang, Q. Su, L. Ye, X. Zhou, L. Zhang, D. Song, D. Huang, Potential roles of bone morphogenetic protein 9 in the odontogenic differentiation of dental pulp cells, J. Endod. 47 (2021) 436–443, https://doi.org/10.1016/j.joen.2020.10.018.
[285] M.M. Nagy, H.E. Tawfik, A.A.R. Hashem, A.M. Abu-Seida, Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols, J. Endod. 40 (2014) 192–198, https://doi.org/10.1016/j.joen.2013.10.027.
[286] J. Yang, Y. Zhang, Z. Sun, G. Song, Z. Chen, Dental pulp tissue engineering with bFGF-incorporated silk fibroin scaffolds, J. Biomater. Appl. 30 (2015) 221–229, https://doi.org/10.1177/0885328215577296.
[287] K. Iohara, M. Murakami, N. Takeuchi, Y. Osako, M. Ito, R. Ishizaka, S. Utunomiya, H. Nakamura, K. Matsushita, M. Nakashima, A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration, Stem Cells Translational Medicine 2 (2013) 521–533, https://doi.org/10.5966/sctm.2012-0132.
[288] K. Iohara, M. Fujita, Y. Ariji, M. Yoshikawa, H. Watanabe, A. Takashima, M. Nakashima, Assessment of pulp regeneration induced by stem cell therapy by magnetic resonance imaging, J. Endod. 42 (2016) 397–401, https://doi.org/10.1016/j.joen.2015.11.021.
[289] M. Nakashima, K. Mizunuma, T. Murakami, A. Akamine, Induction of dental pulp stem cell differentiation into odontoblasts by electroporation-mediated gene delivery of growth/differentiation factor 11 (Gdf11), Gene Ther. 9 (2002) 814–818, https://doi.org/10.1038/sj.gt.3301692.

[290] K. Iohara, K. Imabayashi, R. Ishizaka, A. Watanabe, J. Nabekura, M. Ito, K. Matsushita, H. Nakamura, M. Nakashima, Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1, Tissue Eng. 17 (2011) 1911–1920, https://doi.org/10.1089/ten.tea.2010.0615.
[291] T. Srisuwan, D.J. Tilkorn, S. Al-Benna, A. Vashi, A. Penington, H.H. Messer, K. M. Abberton, E.W. Thompson, Survival of rat functional dental pulp cells in vascularized tissue engineering chambers, Tissue Cell 44 (2012) 111–121, https://doi.org/10.1016/j.tice.2011.12.003.
[292] N. Takeuchi, Y. Hayashi, M. Murakami, F.J. Alvarez, H. Horibe, K. Iohara, K. Nakata, H. Nakamura, M. Nakashima, Similar in vitro effects and pulp regeneration in ectopic tooth transplantation by basic fibroblast growth factor and granulocyte-colony stimulating factor, Oral Dis. 21 (2015) 113–122, https://doi.org/10.1111/odi.12227.
[293] Y. Kobayashi, J. Nouet, E. Baljinnyam, Z. Siddiqui, D.H. Fine, D. Fraidenraich, V. A. Kumar, E. Shimizu, iPSC-derived cranial neural crest-like cells can replicate dental pulp tissue with the aid of angiogenic hydrogel, Bioact. Mater. 14 (2022) 290–301, https://doi.org/10.1016/j.bioactmat.2021.11.014.
[294] S. Shrestha, A. Kishen, Temporal-controlled bioactive molecules releasing core-shell nano-system for tissue engineering strategies in endodontics, Nanomed. Nanotechnol. Biol. Med. 18 (2019) 11–20, https://doi.org/10.1016/j.nano.2019.02.013.
[295] K. Lee, E.A. Silva, D.J. Mooney, Growth factor delivery-based tissue engineering: general approaches and a review of recent developments, Journal of the Royal Society Interface 8 (2010) 153–170, https://doi.org/10.1098/rsif.2010.0223.
[296] M. Trope, Clinical management of the avulsed tooth: PRESENT strategies and future directions, Dent. Traumatol. 18 (2002) 1–11, https://doi.org/10.1046/j.1600-4469.2001.00001.x.
[297] P. Verma, A. Nosrat, J.R. Kim, J.B. Price, P. Wang, E. Bair, H.H. Xu, A.F. Fouad, Effect of residual bacteria on the outcome of pulp regeneration in vivo, J. Dent. Res. 96 (2017) 100–106, https://doi.org/10.1177/0022034516671499.
[298] S. Gilbertson-Beadling, E.A. Powers, M. Stamp-Cole, P.S. Scott, T.L. Wallace, J. G. Copeland, G. Petzold, M.D. Mitchell, S.A. Ledbetter, R.A. Poorman, The tetracycline analogs minocycline and doxycycline inhibit angiogenesis in vitro by a non-metalloproteinase-dependent mechanism, J. Endod. 36 (1995) 418–424, https://doi.org/10.1007/bf00686191.
[299] S. Chuensombat, S. Khemaleelakul, S. Chattipakorn, T. Srisuwan, Cytotoxic effects and antibacterial efficacy of a 3-antibiotic combination: an in vitro study, J. Endod. 39 (2013) 813–819, https://doi.org/10.1016/j.joen.2012.11.041.
[300] G.H. Yassen, T.-M.G. Chu, G. Eckert, J.A. Platt, Effect of medicaments used in endodontic regeneration technique on the chemical structure of human immature radicular dentin: an in vitro study, J. Endod. 39 (2013) 269–273, https://doi.org/10.1016/j.joen.2012.09.020.
[301] T.R. Pedersen, L. Wilhelmsen, O. Færgeman, T.E. Strandberg, G. Thorgeirsson, L. Troedsson, J. Kristianson, K. Berg, T.J. Cook, T. Haghfelt, J. Kjekshus, T. Miettinen, A.G. Olsson, K. Pyörälä, H. Wedel, Follow-up study of patients randomized in the scandinavian simvastatin survival study (4S) of cholesterol lowering, Am. J. Cardiol. 86 (2000) 257–262, https://doi.org/10.1016/s0002-9149(00)00910-3.
[302] van Nieuw, M.A. Vermeer, Pascale Nègre-Aminou, J. Lankelma, J.J. Emeis, V. W. M, Simvastatin improves disturbed endothelial barrier function, Circulation 102 (2000) 2803–2809, https://doi.org/10.1161/01.cir.102.23.2803.
[303] M. Weis, C. Heeschen, A.J. Glassford, J.P. Cooke, Statins have biphasic effects on angiogenesis, Circulation 105 (2002) 739–745, https://doi.org/10.1161/hc0602.103393.
[304] A.S. Antonopoulos, M. Margaritis, R. Lee, K. Channon, C. Antoniades, Statins as anti-inflammatory agents in atherogenesis: molecular mechanisms and lessons from the recent clinical trials, Curr. Pharmaceut. Des. 18 (2012) 1519–1530, https://doi.org/10.2174/138161212799504803.
[305] K.-S. Min, Y.-M. Lee, S.-O. Hong, E.-C. Kim, Simvastatin promotes odontoblastic differentiation and expression of angiogenic factors via heme oxygenase-1 in primary cultured human dental pulp cells, J. Endod. 36 (2010) 447–452, https://doi.org/10.1016/j.joen.2009.11.021.
[306] L. Karanxha, S.-J. Park, W.-J. Son, J.E. Nör, K.-S. Min, Combined effects of simvastatin and enamel matrix derivative on odontoblastic differentiation of human dental pulp cells, J. Endod. 39 (2013) 76–82, https://doi.org/10.1016/j.joen.2012.10.013.
[307] D.G. Soares, G. Anovazzi, E.A.F. Bordini, U.O. Zuta, M.L.A. Silva Leite, F.G. Basso, J. Hebling, C.A. de Souza Costa, Biological analysis of simvastatin-releasing chitosan scaffold as a cell-free system for pulp-dentin regeneration, J. Endod. 44 (2018) 971–976.e1, https://doi.org/10.1016/j.joen.2018.02.014.
[308] C.M.L. Pagliarin, C. de L.D. Londero, M.C.S. Felippe, W.T. Felippe, C.C. Danesi, F. B. Barletta, Tissue characterization following revascularization of immature dog teeth using different disinfection pastes, Braz. Oral Res. 30 (2016), https://doi.org/10.1590/1807-3107bor-2016.vol30.0074.
[309] M.C. Bottino, M.T.P. Albuquerque, A. Azabi, E.A. Münchow, K.J. Spolnik, J. E. Nör, P.C. Edwards, A novel patient-specific three-dimensional drug delivery construct for regenerative endodontics, Journal of Biomedical Materials Research. Part B, Applied Biomaterials 107 (2019) 1576–1586, https://doi.org/10.1002/jbm.b.34250.
[310] P.S. Neelamurthy, R.A. Kumar, V. Balakrishnan, S.M. Venkatesan, G.S. Narayan, K. I, Revascularization in immature and mature teeth with necrotic pulp: a clinical study, J. Contemp. Dent. Pract. 19 (2018) 1393–1399. https://pubmed.ncbi.nlm.nih.gov/30602647/. (Accessed 11 February 2022).
[311] K.S.D.R. Kalyan, C. Vinay, null Arunbhupathi, K.S. Uloopi, R. Chandrasekhar, K. S. RojaRamya, Preclinical evaluation and clinical trial of chlorhexidine polymer scaffold for vital pulp therapy, J. Clin. Pediatr. Dent 43 (2019) 109–115, https://doi.org/10.17796/1053-4625-43.2.7.
[312] J.Y. Nagata, B.P. Figueiredo de Almeida Gomes, T.F. Rocha Lima, L.S. Murakami, D.E. de Faria, G.R. Campos, F.J. de Souza-Filho, A. de J. Soares, Traumatized immature teeth treated with 2 protocols of pulp revascularization, J. Endod. 40 (2014) 606–612, https://doi.org/10.1016/j.joen.2014.01.032.
[313] Y. Okamoto, W. Sonoyama, M. Ono, K. Akiyama, T. Fujisawa, M. Oshima, Y. Tsuchimoto, Y. Matsuka, T. Yasuda, S. Shi, T. Kuboki, Simvastatin induces the odontogenic differentiation of human dental pulp stem cells in vitro and in vivo, J. Endod. 35 (2009) 367–372, https://doi.org/10.1016/j.joen.2008.11.024.
[314] A. Miyazawa, T. Matsuno, K. Asano, Y. Tabata, T. Satoh, Controlled release of simvastatin from biodegradable hydrogels promotes odontoblastic differentiation, Dent. Mater. J. 34 (2015) 466–474, https://doi.org/10.4012/dmj.2014-272.
[315] X. Yuan, Z. Yuan, Y. Wang, Z. Wan, X. Wang, S. Yu, J. Han, J. Huang, C. Xiong, L. Ge, Q. Cai, Y. Zhao, Vascularized pulp regeneration via injecting simvastatin functionalized GelMA cryogel microspheres loaded with stem cells from human exfoliated deciduous teeth, Materials Today Bio 13 (2022) 100209, https://doi.org/10.1016/j.mtbio.2022.100209.
[316] N. Asl Aminabadi, E. Maljaei, L. Erfanparast, A. Ala Aghbali, H. Hamishehkar, E. Najafpour, Simvastatin versus calcium hydroxide direct pulp capping of human primary molars: a randomized clinical trial, J. Dent. Res. Dent. Clin. Dent. Prospects 7 (2013) 8–14, https://doi.org/10.5681/joddd.2013.002.
[317] D. Xue, Z. Gong, F. Zhu, Y. Qiu, X. Li, Simvastatin increases cell viability and suppresses the expression of cytokines and vascular endothelial growth factor in inflamed human dental pulp stem cells in vitro, Adv. Clin. Exp. Med.: Official Organ Wroclaw Medical University 27 (2018) 1615–1623, https://doi.org/10.17219/acem/75776.
[318] T. Qu, J. Jing, Y. Jiang, R.J. Taylor, J.Q. Feng, B. Geiger, X. Liu, Magnesium-containing nanostructured hybrid scaffolds for enhanced dentin regeneration, Tissue Eng. 20 (2014) 2422–2433, https://doi.org/10.1089/ten.tea.2013.0741.
[319] E.O. Orhan, M. Maden, B. Senguüven, Odontoblast-like cell numbers and reparative dentine thickness after direct pulp capping with platelet-rich Plasma and enamel matrix derivative: a histomorphometric evaluation, Int. Endod. J. 45 (2011) 317–325, https://doi.org/10.1111/j.1365-2591.2011.01977.x.
[320] S.-H. Choi, J.-H. Jang, J.-T. Koh, H.-S. Chang, Y.-C. Hwang, I.-N. Hwang, B.-N. Lee, W.-M. Oh, Effect of leptin on odontoblastic differentiation and angiogenesis: an in vivo study, J. Endod. 45 (2019) 1332–1341, https://doi.org/10.1016/j.joen.2019.08.003.
[321] L. Zhang, D. Wang, X. Han, F. Tang, D. Gao, Mechanism of methylation and acetylation of high GDNF transcription in glioma cells: a review, Heliyon 5 (2019) e01951, https://doi.org/10.1016/j.heliyon.2019.e01951.
[322] M. Okano, D.W. Bell, D.A. Haber, E. Li, DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development, Cell 99 (1999) 247–257, https://doi.org/10.1016/s0092-8674(00)81656-6.
[323] A. Tsumura, T. Hayakawa, Y. Kumaki, S. Takebayashi, M. Sakaue, C. Matsuoka, K. Shimotohno, F. Ishikawa, E. Li, H.R. Ueda, J. Nakayama, M. Okano, Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b, Gene Cell. 11 (2006) 805–814, https://doi.org/10.1111/j.1365-2443.2006.00984.x.
[324] J. Liao, R. Karnik, H. Gu, M.J. Ziller, K. Clement, A.M. Tsankov, V. Akopian, C. A. Gifford, J. Donaghey, C. Galonska, R. Pop, D. Reyon, S.Q. Tsai, W. Mallard, J. K. Joung, J.L. Rinn, A. Gnirke, A. Meissner, Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells, Nat. Genet. 47 (2015) 469–478, https://doi.org/10.1038/ng.3258.
[325] H. Spiers, E. Hannon, L.C. Schalkwyk, R. Smith, C.C.Y. Wong, M.C. O'Donovan, N. J. Bray, J. Mill, Methylomic trajectories across human fetal brain development, Genome Res. 25 (2015) 338–352, https://doi.org/10.1101/gr.180273.114.
[326] A.P. Feinberg, B. Vogelstein, Hypomethylation distinguishes genes of some human cancers from their normal counterparts, Nature 301 (1983) 89–92, https://doi.org/10.1038/301089a0.
[327] P.A. Jones, S.B. Baylin, The fundamental role of epigenetic events in cancer, Nat. Rev. Genet. 3 (2002) 415–428, https://doi.org/10.1038/nrg816.
[328] P.L. De Jager, G. Srivastava, K. Lunnon, J. Burgess, L.C. Schalkwyk, L. Yu, M. L. Eaton, B.T. Keenan, J. Ernst, C. McCabe, A. Tang, T. Raj, J. Replogle, W. Brodeur, S. Gabriel, H.S. Chai, C. Younkin, S.G. Younkin, F. Zou, M. Szyf, Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci, Nat. Neurosci. 17 (2014) 1156–1163, https://doi.org/10.1038/nn.3786.
[329] C.C.Y. Wong, E.L. Meaburn, A. Ronald, T.S. Price, A.R. Jeffries, L.C. Schalkwyk, R. Plomin, J. Mill, Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits, Mol. Psychiatr. 19 (2013) 495–503, https://doi.org/10.1038/mp.2013.41.
[330] E. Hannon, E. Dempster, J. Viana, J. Burrage, A.R. Smith, R. Macdonald, D. St Clair, C. Mustard, G. Breen, S. Therman, J. Kaprio, T. Toulopoulou, H.E.H. Pol, M. M. Bohlken, R.S. Kahn, I. Nenadic, C.M. Hultman, R.M. Murray, D.A. Collier, N. Bass, An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential DNA methylation, Genome Biol. 17 (2016), https://doi.org/10.1186/s13059-016-1041-x.
[331] B. Crawford, Z. Craig, G. Mansell, I. White, A. Smith, S. Spaull, J. Imm, E. Hannon, A. Wood, H. Yaghootkar, Y. Ji, N. Mullins, C.M. Lewis, J. Mill, T.M. Murphy, DNA methylation and inflammation marker profiles associated with a history of depression, Hum. Mol. Genet. 27 (2018) 2840–2850, https://doi.org/10.1093/hmg/ddy199.
[332] D. Bayarsaihan, Deciphering the epigenetic code in embryonic and dental pulp stem cells, Yale J. Biol. Med. 89 (2016) 539–563. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5168831. (Accessed 30 May 2024).

[333] Y. Liu, L. Gan, D.-X. Cui, S.-H. Yu, Y. Pan, L.-W. Zheng, M. Wan, Epigenetic regulation of dental pulp stem cells and its potential in regenerative endodontics, World J. Stem Cell. 13 (2021) 1647–1666, https://doi.org/10.4252/wjsc.v13.i11.1647.
[334] C.D. Allis, T. Jenuwein, The molecular hallmarks of epigenetic control, Nat. Rev. Genet. 17 (2016) 487–500, https://doi.org/10.1038/nrg.2016.59.
[335] K. Luger, A.W. Mäder, R.K. Richmond, D.F. Sargent, T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution, Nature 389 (1997) 251–260, https://doi.org/10.1038/38444.
[336] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705, https://doi.org/10.1016/j.cell.2007.02.005.
[337] C.P. Ponting, P.L. Oliver, W. Reik, Evolution and functions of long noncoding RNAs, Cell 136 (2009) 629–641, https://doi.org/10.1016/j.cell.2009.02.006.
[338] T.B. Miranda, P.A. Jones, DNA methylation: the nuts and bolts of repression, J. Cell. Physiol. 213 (2007) 384–390, https://doi.org/10.1002/jcp.21224.
[339] A.P. Bird, A.P. Wolffe, Methylation-induced repression— belts, braces, and chromatin, Cell 99 (1999) 451–454, https://doi.org/10.1016/s0092-8674(00)81532-9.
[340] T. Jenuwein, C.D. Allis, Translating the histone code, Science 293 (2001) 1074–1080, https://doi.org/10.1126/science.1063127.
[341] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and Function, Cell 116 (2004) 281–297, https://doi.org/10.1016/s0092-8674(04)00045-5.
[342] R.W. Carthew, E.J. Sontheimer, Origins and Mechanisms of miRNAs and siRNAs, Cell 136 (2009) 642–655, https://doi.org/10.1016/j.cell.2009.01.035.
[343] J. Krol, I. Loedige, W. Filipowicz, The widespread regulation of microRNA biogenesis, function and decay, Nat. Rev. Genet. 11 (2010) 597–610, https://doi.org/10.1038/nrg2843.
[344] L. Statello, C.-J. Guo, L.-L. Chen, M. Huarte, Gene regulation by long non-coding RNAs and its biological functions, Nat. Rev. Mol. Cell Biol. 22 (2021) 96–118, https://doi.org/10.1038/s41580-020-00315-9.
[345] Z. Zhou, S. Rajasingh, B. Barani, S. Samanta, B. Dawn, R. Wang, J. Rajasingh, Therapy of infectious diseases using epigenetic approaches, Epigenetics in Human Disease (2018) 689–715, https://doi.org/10.1016/b978-0-12-812215-0.00022-4.
[346] N. Khan, M. Jeffers, S. Kumar, C. Hackett, Ferenc Boldog, Nicholai Khramtsov, X. Qian, E. Mills, Stanny Berghs, N. Carey, P. Finn, L.C. Collins, A. Tumber, J. C. Ritchie, P.S. Jensen, H. Lichenstein, M. Sehested, Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors, Biochem. J. 409 (2008) 581–589, https://doi.org/10.1042/bj20070779.
[347] H.F. Duncan, A.J. Smith, G.J.P. Fleming, P.R. Cooper, Histone deacetylase inhibitors epigenetically promote reparative events in primary dental pulp cells, Exp. Cell Res. 319 (2013) 1534–1543, https://doi.org/10.1016/j.yexcr.2013.02.022.
[348] I. Sulistyowati, T. Sukpaita, C.N. Limjeerajarus, R.S. Ampornaramveth, Hydroxamate-based histone deacetylase inhibitors as potential mediators to induce dentine regeneration by human dental pulp cell, Frontiers in Dental Medicine 2 (2021), https://doi.org/10.3389/fdmed.2021.765462.
[349] H.F. Duncan, A.J. Smith, G.J.P. Fleming, P.R. Cooper, Histone deacetylase inhibitors induced differentiation and accelerated mineralization of pulp-derived cells, J. Endod. 38 (2012) 339–345, https://doi.org/10.1016/j.joen.2011.12.014.
[350] S. Suzuki, K. Sasaki, Rahmad Rifqi Fahreza, E. Nemoto, S. Yamada, The histone deacetylase inhibitor MS-275 enhances the matrix mineralization of dental pulp stem cells by inducing fibronectin expression, Journal of Dental Sciences (2023), https://doi.org/10.1016/j.jds.2023.11.019.
[351] Z. Luo, Z. Wang, X. He, N. Liu, B. Liu, L. Sun, J. Wang, H. Duncan, W. He, P. Cooper, Effects of histone deacetylase inhibitors on regenerative cell responses in human dental pulp cells, Int. Endod. J. 51 (2017) 767–778, https://doi.org/10.1111/iej.12779.
[352] H. Jin, J.-Y. Park, H. Choi, P.-H. Choung, HDAC inhibitor trichostatin a promotes proliferation and odontoblast differentiation of human dental pulp stem cells, Tissue Eng. 19 (2013) 613–624, https://doi.org/10.1089/ten.tea.2012.0163.
[353] S. Sultana, O. Uehara, K. Yoshida, T. Saito, Y. Abiko, The histone deacetylase inhibitor, entinostat (MS-275), induces the odontogenic differentiation of an odontoblast-like cell line in the absence of an osteoblast mineralization medium, Odontology 109 (2021) 661–671, https://doi.org/10.1007/s10266-020-00588-8.
[354] N. Gurvich, O.M. Tsygankova, J.L. Meinkoth, P.S. Klein, Histone deacetylase is a target of valproic acid-mediated cellular differentiation, Cancer Res. 64 (2004) 1079–1086, https://doi.org/10.1158/0008-5472.can-03-0799.
[355] F. Paino, M. La Noce, V. Tirino, P. Naddeo, V. Desiderio, G. Pirozzi, A. De Rosa, L. Laino, L. Altucci, G. Papaccio, Histone deacetylase inhibition with valproic acid downregulates osteocalcin gene expression in human dental pulp stem cells and osteoblasts: evidence for HDAC2 involvement, STEM CELLS 32 (2014) 279–289, https://doi.org/10.1002/stem.1544.
[356] M. La Noce, L. Mele, L. Laino, G. Iolascon, G. Pieretti, G. Papaccio, V. Desiderio, V. Tirino, F. Paino, Cytoplasmic interactions between the glucocorticoid receptor and HDAC2 regulate osteocalcin expression in VPA-treated MSCs, Cells 8 (2019) 217, https://doi.org/10.3390/cells8030217.
[357] O.H. Kramer, The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2, EMBO J. 22 (2003) 3411–3420, https://doi.org/10.1093/emboj/cdg315.
[358] F.J. Klinz, Y. Korkmaz, W. Bloch, W.H.M. Raab, K. Addicks, Histone deacetylases 2 and 9 are coexpressed and nuclear localized in human molar odontoblasts in vivo, Histochem. Cell Biol. 137 (2012) 697–702, https://doi.org/10.1007/s00418-012-0920-9.
[359] E. Hu, E. Dul, C.-M. Sung, Z. Chen, R.M. Kirkpatrick, S. Zhang, K.O. Johanson, R. Liu, Lago Amparo M, G.A. Hofmann, R. Macarron, M. de, J. Michelle Kahlenberg, A. Krawiec John, J.D. Winkler, M. Jaye, Identification of novel isoform-selective inhibitors within class I histone deacetylases, J. Pharmacol. Exp. Therapeut. 307 (2003) 720–728, https://doi.org/10.1124/jpet.103.055541.
[360] Benjamin E.L. Lauffer, R. Mintzer, R. Fong, Susmith Mukund, C. Tam, Inna Zilberleyb, B. Flicke, A. Ritscher, G. Fedorowicz, R. Vallero, D.F. Ortwine, J. L. Gunzner, Z. Modrusan, L. Neumann, C.M. Koth, P.J. Lupardus, J.S. Kaminker, C.E. Heise, P. Steiner, Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability, J. Biol. Chem. 288 (2013) 26926–26943, https://doi.org/10.1074/jbc.m113.490706.
[361] H.W. Lee, J.H. Suh, A.Y. Kim, Y.S. Lee, S.Y. Park, J.B. Kim, Histone deacetylase 1-mediated histone modification regulates osteoblast differentiation, Mol. Endocrinol. 20 (2006) 2432–2443, https://doi.org/10.1210/me.2006-0061.
[362] E.-C. Lee, Y. Kim, H.-M. Lim, G.-E. Ki, Y.-K. Seo, The histone deacetylase inhibitor (MS-275) promotes differentiation of human dental pulp stem cells into odontoblast-like cells independent of the MAPK signaling system, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21165771, 5771–5771.
[363] H.F. Duncan, A.J. Smith, G.J.P. Fleming, N.C. Partridge, E. Shimizu, G.P. Moran, P.R. Cooper, The histone-deacetylase-inhibitor suberoylanilide hydroxamic acid promotes dental pulp repair mechanisms through modulation of matrix metalloproteinase-13 activity, J. Cell. Physiol. 231 (2016) 798–816, https://doi.org/10.1002/jcp.25128.
[364] A. Boluk, The effect of valproate on bone mineral density in adult epileptic patients, Pharmacol. Res. 50 (2004) 93–97, https://doi.org/10.1016/j.phrs.2003.11.011.
[365] S.M. Senn, S. Kantor, I.J. Poulton, M.J. Morris, N.A. Sims, T.J. O'Brien, J.D. Wark, Adverse effects of valproate on bone: defining a model to investigate the pathophysiology, Epilepsia 51 (2010) 984–993, https://doi.org/10.1111/j.1528-1167.2009.02516.x.
[366] M.E. McGee-Lawrence, J.J. Westendorf, Histone deacetylases in skeletal development and bone mass maintenance, Gene 474 (2011) 1–11, https://doi.org/10.1016/j.gene.2010.12.003.
[367] A. Kwon, H.-J. Park, K. Baek, H.-L. Lee, J.-C. Park, K.M. Woo, H.-M. Ryoo, J.-H. Baek, Suberoylanilide hydroxamic acid enhances odontoblast differentiation, J. Dent. Res. 91 (2012) 506–512, https://doi.org/10.1177/0022034512443367.
[368] Z. Liu, T. Chen, Q. Han, M. Chen, J. You, F. Fang, L. Peng, B. Wu, HDAC inhibitor LMK-235 promotes the odontoblast differentiation of dental pulp cells, Mol. Med. Rep. (2017), https://doi.org/10.3892/mmr.2017.8055.
[369] K. Man, L. Lawlor, L.-H. Jiang, X.B. Yang, The selective histone deacetylase inhibitor MI192 enhances the osteogenic differentiation efficacy of human dental pulp stromal cells, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22105224, 5224–5224.
[370] T.M. Schroeder, R.A. Kahler, X. Li, J.J. Westendorf, Histone deacetylase 3 interacts with runx2 to repress the osteocalcin promoter and regulate osteoblast differentiation, J. Biol. Chem. 279 (2004) 41998–42007, https://doi.org/10.1074/jbc.M403702200.
[371] Z. Feng, M. Zhan, R. Meng, X. Wang, Q. Xu, 5-Aza-2'-deoxycytidine enhances lipopolysaccharide-induced inflammatory cytokine expression in human dental pulp cells by regulating TRAF6 methylation, Bioengineered 10 (2019) 197–206, https://doi.org/10.1080/21655979.2019.1621135.
[372] Z. Sun, S. Yu, S. Chen, H. Liu, Z. Chen, SP1 regulates KLF4 via SP1 binding motif governed by DNA methylation during odontoblastic differentiation of human dental pulp cells, J. Cell. Biochem. 120 (2019) 14688–14699, https://doi.org/10.1002/jcb.28730.
[373] A. Villagra, J. Gutiérrez, R. Paredes, J. Sierra, M. Puchi, M. Imschenetzky, A. van Wijnen, J. Lian, G. Stein, J. Stein, M. Montecino, Reduced CpG methylation is associated with transcriptional activation of the bone-specific rat osteocalcin gene in osteoblasts, J. Cell. Biochem. 85 (2002) 112–122, https://doi.org/10.1002/jcb.10113.
[374] Yoichi Ezura, I. Sekiya, H. Koga, Takeshi Muneta, M. Noda, Methylation status of CpG islands in the promoter regions of signature genes during chondrogenesis of human synovium-derived mesenchymal stem cells, Arthritis Rheum. 60 (2009) 1416–1426, https://doi.org/10.1002/art.24472.
[375] X. Zhang, Y. Zhang, C. Wang, X. Wang, TET (Ten-eleven translocation) family proteins: structure, biological functions and applications, Signal Transduct. Targeted Ther. 8 (2023), https://doi.org/10.1038/s41392-023-01537-x.
[376] L.D. Moore, T. Le, G. Fan, DNA methylation and its basic function, Neuropsychopharmacology 38 (2012) 23–38, https://doi.org/10.1038/npp.2012.112.
[377] L.-J. Rao, B.-C. Yi, Q.-M. Li, Q. Xu, TET1 knockdown inhibits the odontogenic differentiation potential of human dental pulp cells, International Journal of Oral Science/International Journal of Oral Science 8 (2016) 110–116, https://doi.org/10.1038/ijos.2016.4.
[378] Q. Li, B. Yi, Z. Feng, R. Meng, C. Tian, Q. Xu, FAM20C could Be targeted by TET1 to promote odontoblastic differentiation potential of human dental pulp cells, Cell Prolif. 51 (2017) e12426, https://doi.org/10.1111/cpr.12426.
[379] M. Kearney, P.R. Cooper, A.J. Smith, H.F. Duncan, Characterisation of miRNA expression in dental pulp cells during epigenetically-driven reparative processes, Int. J. Mol. Sci. 24 (2023) 8631, https://doi.org/10.3390/ijms24108631.
[380] Z. Hamidouche, E. Haÿ, P. Vaudin, P. Charbord, R. Schüle, P.J. Marie, O. Fromigué, FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating wnt/β-catenin signaling-dependent Runx2 expression, Faseb. J. 22 (2008) 3813–3822, https://doi.org/10.1096/fj.08-106302.
[381] J. Liu, Q. Xiao, J. Xiao, C. Niu, Y. Li, X. Zhang, Z. Zhou, G. Shu, G. Yin, Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic

opportunities, Signal Transduct. Targeted Ther. 7 (2022) 3, https://doi.org/10.1038/s41392-021-00762-6.
[382] Y. Zhu, P. Li, X. Dan, X. Kang, Y. Ma, Y. Shi, miR-377 inhibits proliferation and differentiation of bovine skeletal muscle satellite cells by targeting FHL2, Genes 13 (2022) 947, https://doi.org/10.3390/genes13060947.
[383] X. Lu, X. Chen, J. Xing, M. Lian, D. Huang, Y. Lu, G. Feng, X. Feng, miR-140-5p regulates the odontoblastic differentiation of dental pulp stem cells via the wnt1/β-catenin signaling pathway, Stem Cell Res. Ther. 10 (2019), https://doi.org/10.1186/s13287-019-1344-4.
[384] K. Zeng, W. Li, Q. Kang, Y. Li, Q. Cheng, W. Xia, miR-342-5p inhibits odonto/osteogenic differentiation of human dental pulp stem cells via targeting Wnt7b, Oral Dis. 29 (2022) 2107–2116, https://doi.org/10.1111/odi.14195.
[385] J. Zhong, X. Tu, Y. Kong, L. Guo, B. Li, W. Zhong, Y. Cheng, Y. Jiang, Q. Jiang, LncRNA H19 promotes odontoblastic differentiation of human dental pulp stem cells by regulating miR-140-5p and BMP-2/FGF9, Stem Cell Res. Ther. 11 (2020), https://doi.org/10.1186/s13287-020-01698-4.
[386] L. Chen, Z. Song, J. Wu, Q. Huang, Z. Shen, X. Wei, Z. Lin, LncRNA DANCR sponges miR-216a to inhibit odontoblast differentiation through upregulating c-cbl, Exp. Cell Res. 387 (2020) 111751, https://doi.org/10.1016/j.yexcr.2019.111751.
[387] S. Shivanna, I. Harrold, M. Shashar, R. Meyer, C. Kiang, J. Francis, Q. Zhao, H. Feng, E.R. Edelman, N. Rahimi, V.C. Chitalia, The c-cbl ubiquitin ligase regulates nuclear β-catenin and angiogenesis by its tyrosine phosphorylation mediated through the Wnt signaling pathway, J. Biol. Chem. 290 (2015) 12537–12546, https://doi.org/10.1074/jbc.M114.616623.
[388] T. Fu, Y. Liu, X. Huang, Y. Guo, J. Shen, H. Shen, LncRNA SNHG1 regulates odontogenic differentiation of human dental pulp stem cells via miR-328-3p/Wnt/β-catenin pathway, Stem Cell Res. Ther. 13 (2022), https://doi.org/10.1186/s13287-022-02979-w.
[389] S. Li, H. Kong, N. Yao, Q. Yu, P. Wang, Y. Lin, J. Wang, R. Kuang, Xiu Song Zhao, J. Xu, Q. Zhu, L. Ni, The role of runt-related transcription factor 2 (Runx2) in the late stage of odontoblast differentiation and dentin formation, Biochem. Biophys. Res. Commun. 410 (2011) 698–704, https://doi.org/10.1016/j.bbrc.2011.06.065.
[390] Q. Gong, R. Wang, H. Jiang, Z. Lin, J. Ling, Alteration of MicroRNA expression of human dental pulp cells during odontogenic differentiation, J. Endod. 38 (2012) 1348–1354, https://doi.org/10.1016/j.joen.2012.06.016.
[391] Z. Song, L.L. Chen, R.F. Wang, W. Qin, S. Huang, J. Guo, L. Zhao, Y. Tian, MicroRNA-135b inhibits odontoblast-like differentiation of human dental pulp cells by regulating Smad5 and Smad4, Int. Endod. J. 50 (2016) 685–693, https://doi.org/10.1111/iej.12678.
[392] R.N. Wang, J. Green, Z. Wang, Y. Deng, M. Qiao, M. Peabody, Q. Zhang, J. Ye, Z. Yan, S. Denduluri, O. Idowu, M. Li, C. Shen, A. Hu, R.C. Haydon, R. Kang, J. Mok, M.J. Lee, H.L. Luu, L.L. Shi, Bone morphogenetic protein (BMP) signaling in development and human diseases, Genes & Diseases 1 (2014) 87–105, https://doi.org/10.1016/j.gendis.2014.07.005.
[393] M. Liu, G. Goldman, M. MacDougall, S. Chen, BMP signaling pathway in dentin development and diseases, Cells 11 (2022) 2216, https://doi.org/10.3390/cells11142216.
[394] Z. Liu, S. Xu, J. Dao, Z. Gan, X. Zeng, Differential expression of lncRNA/miRNA/mRNA and their related functional networks during the osteogenic/odontogenic differentiation of dental pulp stem cells, J. Cell. Physiol. 235 (2020) 3350–3361, https://doi.org/10.1002/jcp.29223.
[395] S. Tian, Y. Liu, F. Dong, Y. Dou, W. Li, J. Wang, Knockdown of microRNA-584 promotes dental pulp stem cells proliferation by targeting TAZ, Cell Cycle 19 (2020) 1048–1058, https://doi.org/10.1080/15384101.2020.1744976.
[396] A. Zhou, H. Yu, J. Liu, M. Yang, Y. Jia, B. Wu, L. Xiang, Role of hippo-YAP signaling in osseointegration by regulating osteogenesis, angiogenesis, and osteoimmunology, Front. Cell Dev. Biol. 8 (2020), https://doi.org/10.3389/fcell.2020.00780.
[397] J.-Y. Yang, S.W. Cho, J.H. An, J.Y. Jung, S.W. Kim, S.Y. Kim, J.E. Kim, C.S. Shin, Osteoblast-targeted overexpression of TAZ increases bone mass in vivo, PLoS One 8 (2013) e56585, https://doi.org/10.1371/journal.pone.0056585.
[398] T. Wang, K. Li, H. Liu, E. Luo, Focusing on Hippo pathway in stem cells of oral origin, enamel formation and periodontium regeneration, Organogenesis 18 (2022), https://doi.org/10.1080/15476278.2022.2082236.
[399] W. Liu, Q. Gong, J. Ling, W. Zhang, Z. Liu, J. Quan, Role of miR-424 on angiogenic potential in human dental pulp cells, J. Endod. 40 (2014) 76–82, https://doi.org/10.1016/j.joen.2013.09.035.
[400] I.M. Castaño, R.M. Raftery, G. Chen, B. Cavanagh, B. Quinn, G.P. Duffy, C. M. Curtin, F.J. O'Brien, Dual scaffold delivery of miR-210 mimic and miR-16 inhibitor enhances angiogenesis and osteogenesis to accelerate bone healing, Acta Biomater. 172 (2023) 480–493, https://doi.org/10.1016/j.actbio.2023.09.049.
[401] D. Mohn, M. Zehnder, The medical device regulation (MDR) from a dental perspective, Frontiers in Dental Medicine 4 (2023), https://doi.org/10.3389/fdmed.2023.1155820.
[402] European Union, in: Regulation (EU) 2023/607 of the European Parliament and of the Council of 15 March 2023 Amending Regulations (Eu) 2017/745 and (Eu) 2017/746 as Regards the Transitional Provisions for Certain Medical Devices and in Vitro Diagnostic Medical Devices, 2023. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R0607.