Bioactive Materials 27 (2023) 574–593

Contents lists available at ScienceDirect

Bioactive Materials

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

# Epigenetic therapeutics in dental pulp treatment: Hopes, challenges and concerns for the development of next-generation biomaterials

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ARTICLE INFO

*Keywords:*
Epigenetics
Vital pulp treatment
Histone deacetylase inhibitors
Regenerative endodontics
Pulp capping

ABSTRACT

This opinion-led review paper highlights the need for novel translational research in vital-pulp-treatment (VPT), but also discusses the challenges in translating evidence to clinics. Traditional dentistry is expensive, invasive and relies on an outmoded mechanical understanding of dental disease, rather than employing a biological perspective that harnesses cell activity and the regenerative-capacity. Recent research has focussed on developing minimally-invasive biologically-based 'fillings' that preserve the dental pulp; research that is shifting the paradigm from expensive high-technology dentistry, with high failure rates, to smart restorations targeted at biological processes. Current VPTs promote repair by recruiting odontoblast-like cells in a material-dependent process. Therefore, exciting opportunities exist for development of next-generation biomaterials targeted at regenerative processes in the dentin-pulp complex. This article analyses recent research using pharmacological-inhibitors to therapeutically-target histone-deacetylase (HDAC) enzymes in dental-pulp-cells (DPCs) that stimulate pro-regenerative effects with limited loss of viability. Consequently, HDAC-inhibitors have the potential to enhance biomaterial-driven tissue responses at low concentration by influencing the cellular processes with minimal side-effects, providing an opportunity to develop a topically-placed, inexpensive bio-inductive pulp-capping material. Despite positive results, clinical translation of these innovations requires enterprise to counteract regulatory obstacles, dental-industry priorities and to develop strong academic/industry partnerships. The aim of this opinion-led review paper is to discuss the potential role of therapeutically-targeting epigenetic modifications as part of a topical VPT strategy in the treatment of the damaged dental pulp, while considering the next steps, material considerations, challenges and future for the clinical development of epigenetic therapeutics or other 'smart' restorations in VPT.

## 1. Introduction

This opinion-led review paper uses epigenetic-therapeutic dental materials as an example, to illustrate the need to develop new pulp capping materials for conservative management of the exposed pulp in vital pulp treatment (VPT), while also illustrating the obstacles to clinical translation in this area. These pulp capping materials are applied to the dental pulp, which is a dynamic connective tissue encased in health by mineralized dentin and enamel. The secretory cells of the pulp, the odontoblasts, form a peripheral layer in contact with dentin and are interconnected in what is known as the dentin-pulp complex [1]. The pulp and specifically the odontoblasts have both a formative role in tooth development (primary dentinogenesis) and throughout life (secondary dentinogenesis), as well as a protective role by acting as a biosensor and secreting new tertiary dentin after challenge by caries, trauma and microbial leakage [2,3]. The pulp is generally threatened by microbes (*e.g.,* dental caries) and if this infection is not managed it can overwhelm the pulp, leading to a progressive pulpitis, odontoblast death and eventually pulp necrosis [4,5]. However, if the inflammation can be controlled by removing the injurious threat and placing a sealing restoration, the pulp can recover, and dentin can be repaired and regenerated [6]. The prevention and control of pulpitis and apical periodontitis forms the basis of operative dentistry; however, the difficulties of predictably treating deep caries has been highlighted in a recent international position statement [7]. The European Society of Endodontology statement [7] concluded that 'the control of pulpitis and

Peer review under responsibility of KeAi Communications Co., Ltd.
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https://doi.org/10.1016/j.bioactmat.2023.04.013
Received 14 March 2023; Received in revised form 11 April 2023; Accepted 11 April 2023

conservative management of the exposed pulp should be a future focus of research activity', in order to reduce the provision of root canal treatment (RCT) and promote biomimetic solutions [7,8]. Although RCT preserves the tooth, complete removal of the irreversibly damaged pulp tissue is a destructive, technically-difficult process [7,9], which removes any inherent attributes of the pulp including perception, immunity/repair and leads to greater risk of fracture and tooth loss [10, 11]. As a result, there is an urgent need to develop our understanding of the 'key' mediators orchestrating pulpal repair processes and tertiary dentin formation in order to develop more predictable 'smarter' VPTs and immunotherapies, with the ultimate goal of creating new biologically-based dental strategies aimed at creating novel regenerative techniques that therapeutically target tertiary dentin repair and regeneration [12–14].

Currently, after pulp exposure, the material of choice is a hydraulic calcium silicate cement (HCSC) applied directly to the pulpal wound, which has been shown since its introduction in the early 1990s, in experimental pulp capping studies to promote the formation of thicker, more homogenous reparative tertiary dentin formation than traditional calcium hydroxide materials [6,15]. Furthermore, these histological results have translated into better clinical outcomes for management of the cariously-exposed pulp [16–18]. Notably, however, the reparative dentin bridge formed after exposure and primary odontoblast death rarely exhibits tubular structure and may be considered reparative rather than regenerative in nature [2,19,20]. Mechanistically, HCSC's reparative action has been attributed to a range of sources including, stimulating the release of bioactive dentin matrix components [21], high pH, increasing migration, differentiation and biomineralization of dental pulp cells (DPCs) [22,23]; however, the exact mechanism of action is unknown and not targeted to a specific pathway or biological process.

Recently, in an attempt to improve the induction and differentiation of odontoblast-like cells, several attempts to stimulate regenerative dentinogenesis using pharmacological inhibitors or activators, often already European Union (EU) or Food and Drug Administration (FDA)-approved as drugs for another indication (*e.g.,* cancer, Alzheimer's' disease) have been reported [24]. Proposed therapeutic strategies have included, targeting Wnt signaling with GSK-3 inhibitors such as Tideglusib [25], using the anti-cholesterol drug Simvastatin [26] or targeting acetylation of DNA-associated proteins by our group with histone deacetylase inhibitors (HDACi) [27–29]. These drugs have shown considerable promise in improving mineralization and regenerative processes in comparison to existing treatments *in vitro* and *in vivo*; however, translating these novel next-generation treatments to the dental clinic is challenging with obstacles, related to regulation, funding and the development of industrial partnerships needing to be addressed.

As a result, the aim of this opinion-led review paper is two-fold. Firstly, the progress in terms of epigenetic therapeutics to improve DPC mineralization processes will be highlighted, prior to secondly the obstacles, barriers and problems with the incorporation of medicinal products and the development of next-generation smart dental materials being discussed with additional reference to opinion.

## 2. Why do we need next-generation bioactive pulp capping materials?

### 2.1. To improve pulpitis management

Preservation of healthy dental pulp tissue and the subsequent prevention of apical disease, form the biological basis of operative dental care and the core of preventative endodontics. As described previously, in health, the dental pulp is naturally protected by a mineralized outer shell of enamel and dentin, while as a connective tissue it also possesses a series of defense strategies including inflammation and mineral secretion designed to protect itself against injury [2]. The pulp is principally irritated by bacteria in the form of dental caries or microleakage around restorations, but can also be challenged trauma, dental materials and as a result of restorative procedures, all of which provoke inflammatory responses in the pulp, the extent of which reflects the severity of the challenge [5,9,30]. Microbial infection by 'leakage' around restorations provides a considerable challenge particularly when the restoration is deep, as bacterial products diffusing through the dentinal tubules induce inflammation even when the restoration has not yet reached the pulp [31], with histological studies showing that this process intensifies when the cavity encroaches to within 0.5 mm of the pulp [4,32]. The secretory odontoblast initially reacts immunologically to the microbes by initial pathogen recognition, but other pulp cells including fibroblasts, dental pulp stem cells (DPSCs) and immune cells also contribute to the defensive response; thereafter, a complex series of antibacterial, immune and inflammatory responses is orchestrated [33, 34]. If the bacterial stimulation is not managed (*e.g.,* by placement of a new 'sealing' biologically-based material), the microbial biofilm will advance and the associated bacteria will invade the tissue; this aggressive bacterial challenge invariably leads to irreversible pulpits, pulp necrosis and subsequent apical periodontitis [35]. Over time the bacterial flora in the diseased pulp and the subsequent necrotic root canal system changes from comprising principally facultative anaerobic bacteria to more gram-negative, obligate anaerobic bacteria [36,37]. Pulp necrosis will necessitate remedial dental treatment, such as tooth extraction or RCT; however, removal of the bacteria and placement of a bioactive dental material can promote pulp repair and maintain vitality [5]. The choice of dental material has been shown for several years to be an important factor that determines the quality of the dental pulp defensive response [6,38,39]; however, it is accepted that no existing material is ideal [2]. Although a role for anti-inflammatory [40] or immunotherapeutic materials [41] in the treatment of pulpitis have been proposed, currently no pulp capping materials specifically target inflammatory processes except for medicaments such as Ledermix (Henry Schein, New York, USA) which contain steroids and have been used only historically for this purpose [42].

***Opinion*** The need for improved dental restorative solutions for the management of deep caries and the exposed pulp has been highlighted by global position statements [7], as dental caries in permanent teeth remains the most prevalent disease in the world today and affects patients in all countries and social classes [43]. Although the benefits of inflammatory suppression have been advocated, no currently available commercial dental or pulp capping material has specific immunomodulatory or targeted anti-inflammatory actions.

### 2.2. To promote pulpal repair processes

Pulp tissue, however, has an innate ability to heal if the injurious challenge is removed and the tooth suitably restored [5]. In deep cavities, the group of strategies aimed at maintaining the vitality of the pulp are called VPT [7]. Interestingly, although limiting pulpal inflammation is critical during VPT, it appears that a controlled level of pulpitis is helpful, at least in the initial stages, to drive reparative processes [44, 45]. If possible, minimally invasive, biologically-based VPTs (*e.g.,* pulp capping and pulpotomy) that preserve at least part of the pulp are preferable to conventional RCT, which is costly, technically complex [46]; destructive of tooth tissue [47] and often poorly carried out in dental practice [48,49].

In a response to challenge and injury the dentin-pulp complex not only reacts immunologically, but also forms tertiary dentin that provides a local physical reparative barrier adjacent to the injurious challenge and contributes to restoration of tissue integrity [2]. The term tertiary dentin actually describes a heterogeneous range of secretory responses, from a regular, tubular structure in continuity with primary and orthodentin, to the secretion of a dysplastic, defective and atubular matrix [6,50,51]. The cell responsible for the secretory responses also differs with reactionary tertiary dentin formed by an upregulation of activity of the existing primary odontoblast [52], while reparative

tertiary dentin is formed by a newly differentiated odontoblast-like cell [53]. This subdivision highlights not only differing reactions in response to mild and more severe external stimuli, but also the effect of severe stimuli on odontoblast survival and subsequent pulpal response (Fig. 1). In contrast to reactionary dentinogenesis, reparative dentin formation is a more complex process that will normally occur in response to stronger stimuli such as extremely deep caries [9] and will subsequently involve the recruitment of progenitor cells, which differentiate into odontoblast-like cells to form reparative dentin [53]. The reparative dentin in comparison with reactionary dentin displays a broad range of responses, which can vary in quality from tubular dentin replacement to an incomplete amorphous hard tissue bridge [2,6,51,54]. For descriptive and educational purposes, reactionary and reparative dentinogenesis are often described as separate processes, however, when the carious lesion is close to the pulp both processes are almost certain to occur simultaneously [55].

From a biological perspective, the cellular response is not only promoted by release of growth factors (GFs) and pro-mineralization cues from DPCs [56], but also orchestrated and regulated by bioactive molecules, including GFs, which are 'fossilized' in the dentin matrix during crown formation [57–59]. These bioactive dentin matrix components

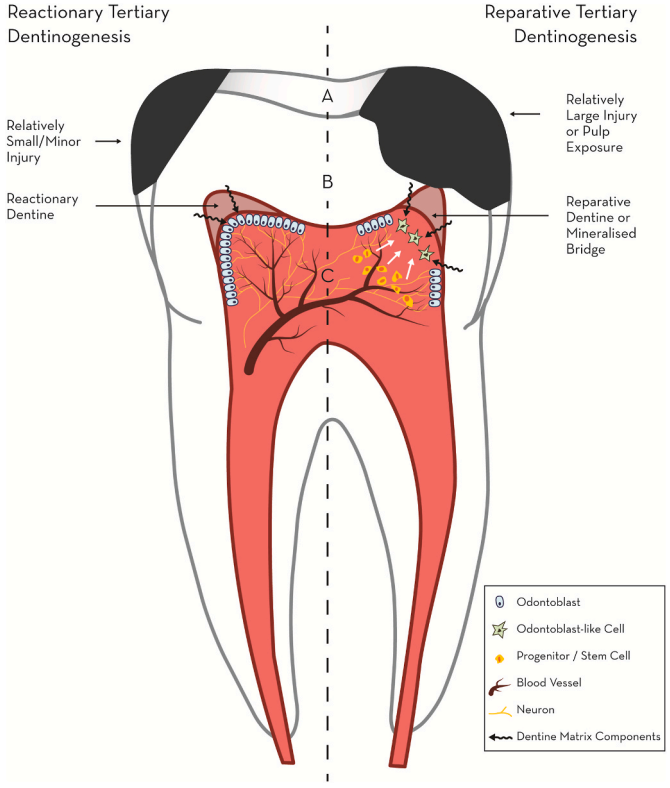


**Fig. 1. Schematic of the processes of tertiary dentin formation.** Reactionary and reparative dentinogenesis processes differ in the source of the secreting cell. Reactionary dentin is formed by the existing primary odontoblast with a mild stimulus (e.g. early stage of carious disease) activating the upregulation of existing odontoblast activity. During reactionary dentinogenesis the odontoblasts recognize the bacterial products and released DMCs diffusing through the dentin tubules, which increases cellular activity. Reparative dentin formation involves a more complex sequence of events in which a severe stimulus (e.g. increasing carious involvement of dentin) causes death of the primary odontoblasts, which are subsequently replaced following differentiation of progenitor or stem cells into odontoblast-like cells under the regulation of bioactive molecules (including DMCs). Although the nature of the cellular response is likely to be dependent upon the pulp environment, the mineralized tissue deposited at the pupal wound site will likely display a spectrum of dysplasia. **A** Enamel, **B** Dentin, **C** Pulp.

(DMCs) can be released by caries, irrigants and dental materials to stimulate the reparative process [60–63]. Indeed, it has been shown that calcium hydroxide [60], the HCSC mineral trioxide aggregate (MTA) [61] and epigenetic modifying drugs such as HDACi [63] can all modify the release profile of DMCs and alter the cellular reparative response.

***Opinion*** Continuing pulpal health and the secretion of a high-quality mineralized barrier define a successful response to a pulp capping procedure. The hard tissue response and the degree of regeneration of tubular dentin has been linked to several factors including the type of pulp capping material used. The influence of the material in modifying DMC release and the predictability, quality of the pulpal defensive response is important as it can enhance the regenerative responses in the exposed pulp, by improving not only tissue handling but also developing the biological properties of the material itself. Traditionally, however, dental filling material development has focused on physical attributes (*e.g.,* wear and strength) and esthetic issues rather than biological properties, despite the pulp capping material coming into often direct contact with the cells of the dental pulp and the opportunity to directly target cellular processes.

### *2.3. To potentially regenerate dentin after pulp exposure and biomaterial placement*

After pulp exposure and placement of a biomaterial in a pulp capping procedure, although hard tissue is formed it is generally considered to lack the tubular structure that defines dentin [64]. This questions whether the new secretory cell is producing dentin or simply dystrophic mineral has led to some investigators casting doubt on the veracity of the stem cell-led odontoblast-like cell theory with alternative suggestions that the formation of mineral could be attributed instead to fibroblasts [54] or fibrocytes [65]. The traditionally accepted view was that cellular differentiation of migrating progenitor cells occurred under the influence of bioactive molecules liberated from damaged dentin, which formed new odontoblast-like cells [52,62]. It is also possible that fibroblasts produce mineral in the reparative response, but this would also occur through a cyto-differentiation as these cells do not produce mineral in their basal state, but can be induced to form mineral under certain induced conditions [66–69].

It is important to note, that the origin of the cell type that produces the repair is central to the notion that the dentin-pulp complex can be regenerated [70]. The extensive heterogeneity in hard tissue morphology may indicate that both repair and regeneration of dentin are possible with evidence that the quality of the healing response can be influenced by the environmental niche with the presence or absence of caries [71], type of capping material [6] or the operator's technical skill [9]. In order to control confounding factors, experimental pulp capping studies that histologically analyze the response of pulp cells to pulp capping use disease-free, unrestored teeth and short recall periods [72]. However, in the clinic pulpal injuries are affected by a host of events including infectious, inflammatory and operator variable, and as a result the interplay between pulp injury, infection, pulpitis and altered host defense responses over several months will be critical to the final outcome [2]. This type of experimental pulp capping studies also tends to focus on the choice of capping material [6,72,73] rather than other critical aspects including wound disinfection [74], bacterial leakage [75] or technical aspects of the procedure itself. Indeed, the bulk of studies investigating the comparative quality of pulp capping materials do not comment on the technical aspects of the procedure or verify the histological absence of microorganisms associated with failed or poor healing outcomes [76].

***Opinion*** Although regeneration of dentin has been shown to occur after experimental pulp capping with HCSCs in 'sound' teeth (Fig. 2), the clinical reality is that the pulp is likely to be inflamed and tissue responses are less predictable. New improved materials could potentially improve the quality of hard tissue deposited at the wound interface, both in terms of quality and volume of dentin. Furthermore, current pulp

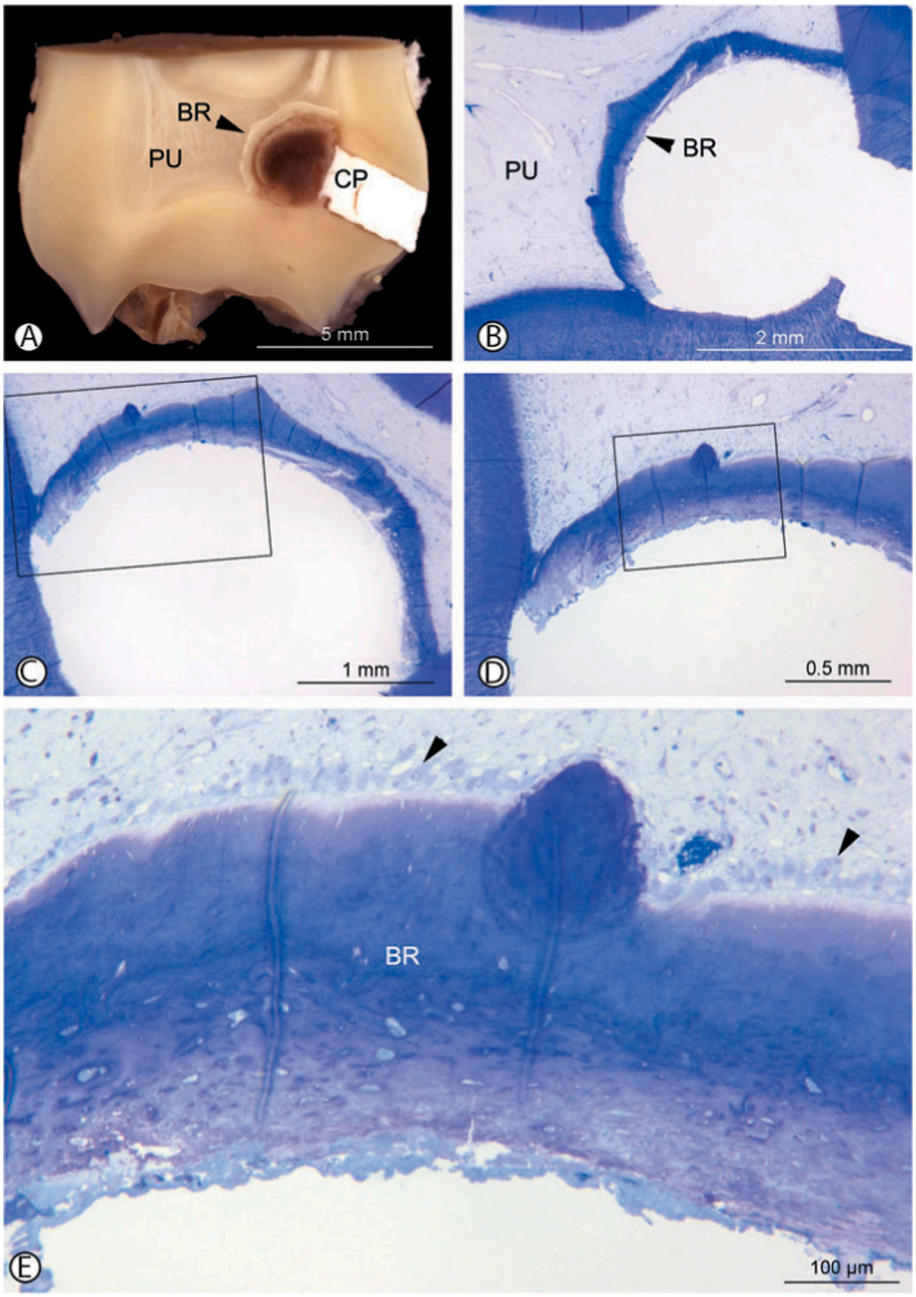


**Fig. 2. Pulpal response to MTA capping after 3 months.** Pulpal response to mineral trioxide aggregate (MTA) capping after 3 months observation. Distal microphotographic view **(A)** of the mesial half of a maxillary left third molar (tooth 28) shows the remnants of the restorative and capping material (CP) and a distinct hard tissue bridge (BR) across the exposed pulp (PU). The photomicrograph **(B)** is part of a histological section of the specimen in **(A).** Note the mineralized hard tissue barrier (BR), stretching across the full length of the exposed pulp **(B, C).** The rectangular areas demarcated in **(C)** and **(D)** are magnified in **(D)** and **(E)** respectively. Note the cuboidal pulpal cells (arrowheads) lining the bridge (BR) and absence of pulpal inflammation in **(E).** Original magnifications: a ·6, b ·8, c ·23, d ·200. Adapted with permission from Ref. [6].

capping and pulpotomy techniques involve placement of a setting dental material directly against the pulp tissue, which stimulates a hard tissue reparative barrier, but by its physical presence prevents any possible regeneration of dentin, as would be possible if a bioactive scaffold or collagen sponge were placed instead of hard setting material. However, the later tissue engineering-based approach would require a rethink in the way dentistry is currently provided as well as the development of a new set of approved dental biomaterials. There is little evidence at present that there is much development on commercially-developed tissue engineering strategies, in spite of evidence that there is considerable academic research activity in this area.

### *2.4. To address the deficiencies of current pulp capping materials*

Successful VPT relies upon and is influenced by the pulp capping material. Over the years, countless capping materials have been used including; gold foil [77], calcium hydroxide [78,79], resin-based composites [80], corticosteroid/antibiotic medicaments [81], resin-modified glass ionomer [82], and HCSCs [83]. Misconceptions regarding the role of a pulp capping material, have suggested that preventing microleakage is the only concern [75]; which perhaps led to the use of resin-bonded solutions that irritated pulp tissue and impaired its defensive reaction [84,85]. Although the seal of the capping material is important and should be provided by either the pulp capping material or the overlying restoration, other factors such as biocompatibility, pro-mineralization, anti-inflammatory and or anti-microbial properties are also key, as well as practical considerations related to handling, setting time, cost and radiopacity [86]. As the aim of the pulp capping material is to help maintain a viable pulp, the promotion of mineralization through tertiary reparative dentinogenesis increases the thickness of dentin between the pulp and the deepest part of any cavity and therefore the proximity of the threat to the pulp is distanced.

Calcium hydroxide materials in non-setting or hard-setting forms have been considered the gold standard material for many years [79,

87–89]. Indeed, calcium hydroxide application directly onto the pulp can be successful clinically [90] and forms a mineral barrier [91]; however, the barrier is often porous and of poor quality [6,51]. Notably, from a mechanistic perspective the action of calcium hydroxide remains unclear [92] and generally forms low quality mineralized osteodentin when applied to the exposed pulp [6,51]. As a result, it has largely been superseded by the use of new materials such as HCSCs [83], which have demonstrated improved results histologically [15] with evidence of some tubularity over time [6], as well as clinically compared with calcium hydroxide [16- Hilton et al., 2013]. HCSC use; however, has been attributed to occurrences of postoperative tooth discoloration [93], an issue which subsequent HCSCs such as Biodentine do not pose to the same extent [94], while also favorably inducing mineralization [95] and cellular differentiation *in vitro* [96]. In addition to the biological effects of calcium hydroxide and HCSCs on DPCs, they also have the ability to induce the release of the aforementioned bound DMCs from dentin [60,61]. Current pulp capping materials including HCSC's, however, are limited by their lack of specificity as they are not designed to directly target regenerative or inflammatory processes, which is unfortunate as GFs [97], drugs [29] and lyophilized DMCs [98] have all been shown to improve mineralization and reparative processes *in vitro* and *in vivo*.

***Opinion*** The introduction of HCSCs over 30 years ago has improved pulpal tissue responses and clinical outcomes in VPT. However, current VPT materials are limited by low-quality tertiary dentin formation, non-specific action, and the absence of targeted constituents focused on tissue regenerative strategies or targeting inflammation [12,24]. Therefore, although there is an urgent need to develop next-generation capping material, there is a need to consider not only the bioactivity of the material but also the ability of the material to prevent leakage in the long-term, so physical properties (although not necessarily strength) remain important.

## 3. Epigenetic therapeutic solutions – opportunities and challenges

As previously described, there is a need to develop smarter pulp capping biomaterials that contain pharmacological inhibitors or endogenous morphogens and are focused on pulp regenerative processes, thereby overcoming the limitations of existing materials. Several potential solutions have been investigated including the direct application of GFs [99], Wnt-signaling pathway inhibitors [100], anti-oxidants [101], anti-hypertensive drugs [26] and recently epigenetic-modifiers [27,102,103]. In the latter so-called, 'epigenetic therapeutic approach', drugs or epigenetic modifying agents are used to treat medical and dental conditions [104].

Targeting epigenetic machinery has exciting potential, as epigenetic modifications play an essential role in cell development and differentiation by regulating gene expression without altering the DNA sequence [105]. Recently, the critical role of epigenetic influence on embryonic stem cell (ESC) regulation and DPSC fate, as well as the therapeutic potential of orchestrating self-renewal and differentiation has been underlined [70,106,107]. The epigenetic regulatory mechanisms that have been well researched remain DNA methylation and post-translational histone modifications, although the emerging role of ncRNAs in the epigenetic regulation of gene expression has been the subject of intensive research in medicine and dentistry [108,109]. It is evident that epigenetics can control multiple transcriptional mechanisms central to the maintenance of health and response to disease, so there is an opportunity to target epigenetic modifications as diagnostic biomarkers or as part of a dental therapeutic strategy.

Dental pulp research in this area has largely focused on DNA-associated histone acetylation [103], a nuclear process balanced by histone-acetyltransferases (HATs) and histone-deacetylases (HDACs), as it has been shown that modifying this balance with pharmacological inhibitors can accelerate DPC mineralization and improve wound healing *in vitro* [28,29] as well as *in vivo* [110]. Targeting HDAC enzymes with HDACi seems to be the most promising way to therapeutically alter this balance, with a resulting modification of cellular transcription. Pan-HDACis target all Zn-dependent HDAC-isoforms and include suberoylanilide hydroxamic acid (SAHA) and Panobinostat, which are EU and FDA-approved to treat certain forms of cancer [111,112], and have been investigated in bone engineering [113] and inflammatory modification [114]; however, their use in VPT is novel [103] and not commercially approved.

Several different types of HDACis have shown considerable promise in the field of dental pulp regeneration, including Trichostatin A (TSA), SAHA and Valproic acid (VPA) (Table 1). In multiple studies low concentrations of these HDACis have been demonstrated to promote differentiation and migration in DPC with minimal toxicity *in vitro*, suggesting a potential *in vivo* role and translation towards clinical treatment of exposed dental pulp [115–119]. The first proof of principle study using a DPC culture showed that although TSA, VPA reduced DPC proliferation they also increased mineralization dose-dependently in a dental-pulp derived murine cell line only negatively affecting cell viability and cell cycle at the higher concentrations [27]. Although this work highlighted the potential of HDACi-enhanced DPC mineralization, it raised other questions relating to different responses in primary cultures, which have been demonstrated to react differently to HDACi than transformed cell lines [120,121].

Subsequent DPC experimentation using primary rodent DPCs [28] as well as human DPSC populations have consistently highlighted that pharmacological HDAC inhibition induced reparative cellular responses (increased cellular mineralization, pro-mineralization gene and protein markers) at concentrations which did not stimulate significant cytotoxic effects. Generally, pan-HDACi, including TSA, SAHA and VPA have been used experimentally [27,28,110,115,116], but recently isoform-specific HDACi have shown promising results targeting HDAC2/3 inhibition using MI192 [119] and HDAC4/5 inhibition using LM-235 [117], with resulting increases in calcific nodule formation *in vitro*. *In vitro* experimentation has also raised the issue of dose and concentration of HDACi with continuous HDACi administration (14 days), resulting in an inhibition of differentiation [28]. One *in vivo* study examined the effects of HDACis on dental pulp with the developmental effect of HDACi on dental tissues after TSA injection into the tails of pregnant mice. Subsequent histological analysis revealed that the volume of dentin

**Table 1**
A selection of studies that have investigated the effects of HDACis and DNMTis on mineralization and differentiation in dental pulp cell populations. TSA = Trichostatin A; SAHA= Suberyolanilide hydroxamic acid; VPA= Valproic acid; BMP= Bone morphogenetic protein; OPN= Osteopontin; ALP = Alkaline phosphatase. DMP = dentin matrix acidic phosphoprotein; DSPP = dentin sialophosphoprotein. Adapted from Ref. [109].

| Reference | Cell Population | Specific HDACi or DNMTi | Mineralization-associated Gene Expression Changes |
|---|---|---|---|
| [27] Duncan et al., 2012 | DPC line (MDPC-23) | TSA, VPA | **Up**: BMP4, DMP1, TGF-β1 |
| [115] Kwon et al., 2012 | DPC line (MDPC-23) | SAHA | **Up**: DMP1, DSPP, ALP, Nestin |
| [28] Duncan et al., 2013 | Rodent Primary DPCs | TSA, VPA | **Up**: BMP2, BMP4, DMP1, DSPP, Nestin |
| [110] Jin et al., 2013 | Human DPSCs | TSA | **Up**: BSP, DMP1, DSPP, **Down**: OCN |
| [116] Paino et al., 2014 | Human DPSCs | VPA | **Up**: BSP, OPN **Down**: OCN |
| [117] Liu et al., 2018 | Human DPSCs | LMK-235 | **Up**: DSPP, ALP, RUNX2 |
| [118] Lee et al., 2020 | Human DPSCs | MS-275 | **Up:** DMP1, ALP, DSPP and RUNX2 |
| [119] Man et al., 2021 | Human DPSCs | MI192 | **Up:** BMP2, OCN, ALP |
| [102] Zhang et al., 2015 | Human DPSCs | 5-AZA-CdR | **Up**: DSPP, DMP1, OSX, RUNX2, DLX5, ALP |

deposited was thicker and the number of odontoblasts in the area was higher in the HDACi group than in the control group [110]. From a translational perspective, the stimulation of mineralization in DPCs without toxicity after short-term exposure to the HDACi supported the potential chairside application of these materials as pulp capping agents within VPT or even ostensibly as part of a tissue engineering strategy to replace lost pulp tissue (Fig. 3).

Mechanistically, researchers have attributed the action of HDACi to stimulate an increase in transcription, with one high-throughput study highlighting that SAHA promoted mineralization and cell migration in rodent DPCs by inducing the expression of matrix metalloproteinase 13 (*Mmp13*). Importantly, cell proliferation was not compromised when low concentrations of SAHA were applied [29]. More recently, a variety of HDACis including TSA, VPA, sodium butyrate (NaB) and MS-275 were applied to a rodent odontoblast-like cell line, MDPC-23 [122]. All of the HDACi employed increased the expression of a range of mineralization-associated genes such as *bone morphogenetic protein*-2 (*Bmp2*), *Bmp4, Osteocalcin (Ocn), dentin matrix acidic phosphoprotein (Dmp-1); dentin sialophosphoprotein (Dspp)* and *Runt-related transcription factor 2 (Runx2)*, albeit to varying extents. MS-275, VPA and NaB all increased mineralization of the cell line, as determined by Alizarin Red S staining. Notably, TSA did not significantly increase mineralization at any concentration ranging from 1 nM to 100 nM [122], which is consistent with the findings of the Duncan et al. [27], and supports the previously mentioned observation that transformed cell lines are less susceptible to the effects of HDACis.

To improve the clinical relevance of HDACi application specific to the tooth, the interaction between HDACis and dentin matrix was investigated in order to replicate the interface between dental restorative materials and pulp capping biomaterials after pulp exposure [63]. A range of restorative and pulp capping materials have previously been shown to release bioactive DMCs, which promote reparative events and guide tertiary reparative dentinogenesis [60]. HDACis released DMCs consisting of GFs previously identified as being released from dentin by endodontic irrigants and dental materials [21,57,60], as well as novel GFs [63]. Notably, the HDACis extracted GFs less efficiently than the well-characterized extractant EDTA for certain GFs (*e.g.*, TGF-β1), but more effectively for others (*e.g.*, GDF15, BDNF), while in comparison different HDACi had differing extraction profiles.

From a pulp capping perspective, the promotion of mineralization and the control of inflammation are both crucial to the success of the treatment [2]. In addition to the studies focused on the mineralization response (Table 1), HDACi are also known to exhibit anti-inflammatory properties [12], by reducing pro-inflammatory cytokine production *in vitro* and *in vivo* [114]. Indeed, lipopolysaccharide (LPS) stimulated cells cultured in the presence of SAHA exhibited reduced cytokine production for tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-1-beta (IL-1β), in a dose-dependent manner [114]. Furthermore, several HDACi including SAHA have been shown to reduce inflammatory related pain in a rat model [123]; this is a finding which could be beneficial for a pulp capping material in the treatment of symptomatic pulpitis or classic 'toothache'. As HDACi appear to have a pro-mineralization and anti-inflammatory effect in DPC populations, future research should focus on the effect on reparative effects under pulpitic conditions or in conditions of high glucose or oxidative stress. These environmental stressors will affect cell differentiation and likely reparative response. At present, in order to limit the influence of confounding factors current studies tend to examine mineralization or

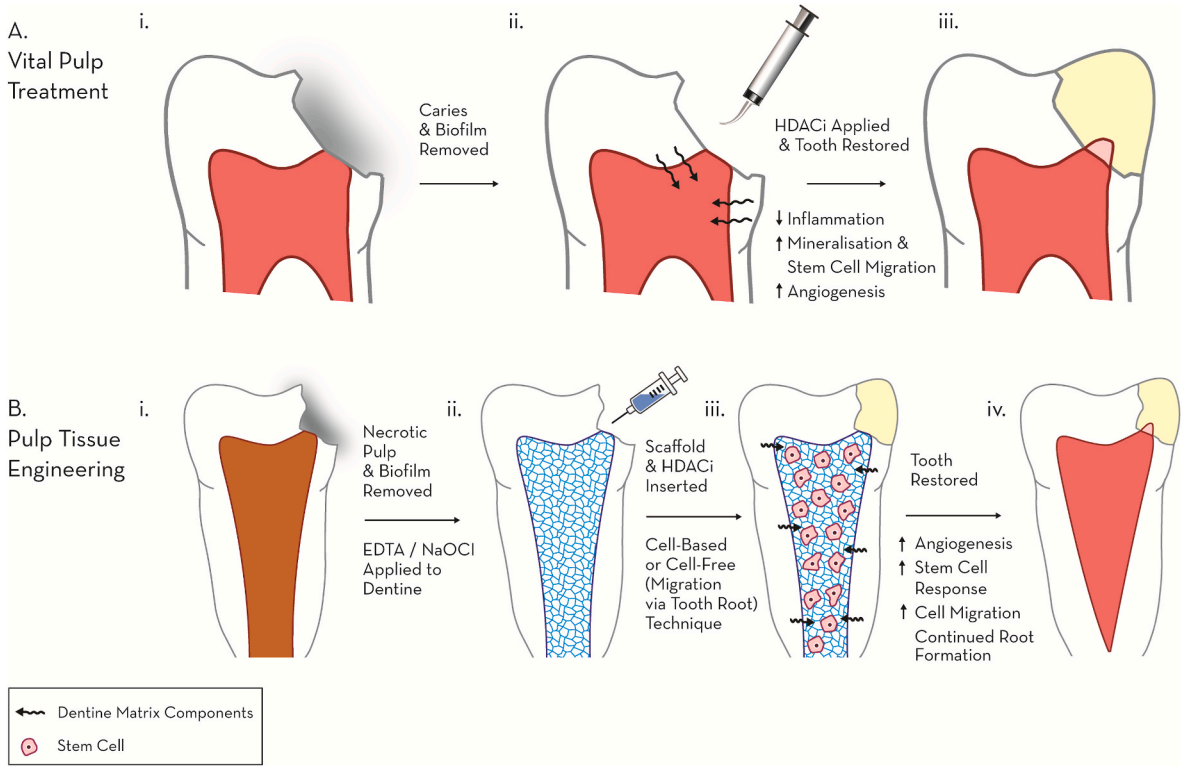


**Fig. 3.** Schematic diagram highlighting the therapeutic potential of HDACi in regenerative endodontics. **(A)** Vital pulp treatment. (i) Deep carious lesion exposes pulp tissue. (ii) HDACi topically applied to exposed pulp, potentially as a component of a dental restorative material [e.g., mineral trioxide aggregate (MTA), calcium hydroxide, resin-based composite (RBC)] promotes tissue-repair processes (mineralization, modulated inflammation and cell migration). (iii) Tooth permanently restored with amalgam and mineralized bridge formation evident under dental pulp capping material. **(B)** Pulp tissue engineering. (i) Pulp necrosis (ii) Necrotic tooth chemo-mechanical debrided and EDTA, NaOCl applied to dentine to release matrix components. (iii) HDACi applied within a cell or non-cell based scaffold stimulates further growth factor release, cell migration and differentiation. (iv) Tooth restored and new pulp-like tissue formed promoting continued root growth and restoring tooth tissue vitality. Reproduced from Ref. [109].

inflammation in isolation rather than examining the effect of HDACi in inflamed tissue or after carious exposure *in vivo*. This represents a limitation of the current literature in this area [103].

Notably, a solitary study analyzed the effects of the DNMTi 5-AZA-CdR on DPCs [102] and demonstrated that although mineralization- and dentinogenic-associated gene expression, including *DSPP*, *DMP1*, Osterix (*OSX*) and *RUNX2,* were all upregulated and calcific nodule formation was accelerated (Table 1) it was accompanied by a significant reduction in cell viability at all concentrations.

***Opinion*** A series of studies have highlighted the potential benefit of developing new dental biomaterials targeted towards epigenetic processes. The promise of HDACi stems from their dual action of possessing pro-mineralization and anti-inflammatory properties. These effects have been demonstrated using rodent and human cells as well as *in vivo* models. However, in order to translate these potential next-generation therapies into clinical material for dentists, clinical trials on human subjects must be planned and funded. This has not to date been straightforward in dentistry with notable reticence of dental manufacturers to get involved in what they describe as 'early-phase' translational research (preferring to enter product development at a later stage) coupled with increasing regulatory hurdles, which makes the development of these smart restorations costly and time consuming. Unfortunately, these challenges risk holding back conservative treatment of carious induced disease, one of the most prevalent diseases globally.

## 4. What are the specific materials used for potential drug delivery in VPT?

### 4.1. Unintended therapeutic effects in traditional pulp-dressing materials

Historically, pulp capping materials have been an area of VPT in which new innovations have been investigated with the subsequent response to the new material analyzed [124]. As a result of the capping material directly contacting dental pulp tissue, the material represents the perfect vehicle to possess potentially beneficial pharmacological additives. Notably, traditional pulp capping materials were not doped but rather were 'unintentionally' equipped with the pharmacological effects that were beneficial to pulp tissue.

For example, the classic pulp capping material, calcium hydroxide, causes necrosis of the superficial layer of the pulp tissue by its high pH, which subsequently induces pulp defense and repair processes [125]. It has been postulated that the beneficial effect of calcium hydroxide is due to the calcium ions it releases [126] as well as an inherent ability to extract GFs from dentin [60], which can subsequently stimulate odontoblast-like cell differentiation of progenitor cells in the pulp tissue. MTA, which has largely replaced calcium hydroxide as the pulp capping material of choice, has succeeded and expanded the favorable characteristics of calcium hydroxide [127]. MTA application upregulates BMP expression in DPCs *in vitro* [128], which can stimulate subsequent matrix mineralization. MTA also induces DPC expression of the transcription factor Runx2, as well as other pro-mineralization molecules including OCN, alkaline phosphatase, dentin sialoprotein, and vascular endothelial growth factor (VEGF) [129]. Furthermore, MTA has been shown to release TGF-β1, adrenomedullin (ADM) and hepatocyte growth factor (HGF) from dentin [21,130]. Presently, a variety of bioactive molecules, including SCF, M-CSF, GM-CSF, IGF-1, IGFBP-1, NGF, and GDNF, are known to be liberated from dentin by MTA's effect [131,132]. The beneficial effects of HCSC pulp materials have been integrated into newer products, such as tricalcium silicate-based cement, Biodentine (Septodont, Saint-Maur-des-Fosses, France) or the resin-modified calcium silicate, TheraCal (Bisco, Schaumburg, IL, USA) [133- Kim et al., 2021]. Biodentine has been shown to induce TGF-β1 release from human DPCs and stimulate early dental pulp mineralization [134 – Laurent et al., 2012], while DPSCs incubated with TheraCal exhibited higher Runx2 expression [133]. Notably, although materials such as calcium hydroxide and MTA induce reparative processes, there is no controlled release of bioactive components or targeted therapeutic action.

The critical features of a vital pulp capping material are that they should provide a seal to microbes, be biocompatibility, pro-mineralization, anti-inflammatory and or anti-microbial, as well as practical considerations related to handling, setting time, cost and radiopacity. Furthermore, if possible particularly in the case of scaffolds the material should be capable of promoting regeneration [86].

### 4.2. Early attempts to incorporate bioactive reagents into pulp capping materials

Attempts to incorporate bioactive compounds into pulp capping materials is not new with documented experiments recorded in the 1970s. Anneroth and Bang capped the exposed pulp tissue of a Java monkey with demineralized dentin matrix and observed subsequent hard tissue formation in the teeth at the exposure site [135]. This experiment implemented the previously demonstrated ability of demineralized dentin matrix to induce ectopic bone formation [136], which later led to the discovery and purification of BMP from rat dentin [137,138]. The finding of BMPs in dentin stimulated scientists to apply these proteins to dental pulp, expecting the formation of reparative tertiary dentin. Nakashima and other groups implanted crude or purified BMPs on the surface of amputated pulp of dog or monkey and observed reparative dentin formation [139–141]. From a materials perspective these experiments utilized collagen matrix or the mixture with chondroitin as the carrier of BMPs and highlight the potential for scaffolds to act as vehicles to deliver bioactive components to the injury site with the tooth.

### 4.3. Recent attempts to incorporate drug delivery in VPT materials

Polymeric substrates, as a carrier of bioactive reagents, have distinctive advantages as for several reasons [142]: (i) they protect the bioactive reagents from degradation; (ii) they enable the localized and sustained release of active reagents, ensuring bioavailability of critical concentrations of the reagent; (iii) they serve as extracellular matrix (ECM)-mimicking scaffold supports allowing tissue ingrowth during tissue regeneration. Polymeric substrates are currently applied in various forms, such as scaffolds, hydrogels, and nanoparticles, as well as the combinations of these forms [142].

#### 4.3.1. Naturally-derived polymeric materials

Naturally derived materials use molecules derived from the cells themselves. Their characteristics and functionality are preferable for drug delivery and occur as a result of years of natural selection. The advantage of natural materials cannot be overemphasized or readily be surpassed by any synthetic material, which can be tested for suitable characteristics over a relatively short time. A further advantage of natural materials is that they are relatively easily obtained due to their abundancy and production without strict reaction control or complicated steps. Presumably due to these advantages, naturally derived materials have been preferred for endodontic procedures and regenerative strategies for many years (Table 2). Several important natural materials are reviewed below.

*4.3.1.1. Collagen/gelatin.* Collagen/gelatin has been used as a drug carrying material in endodontic research for many years [143,144]. Collagen/gelatin is the principle component of natural ECM and as a result shows low cytotoxicity [145], prominent biocompatibility [146], and low immunogenicity in humans [147]. Additionally, collagen/gelatin can be used in solid form such as a sponge or matrix, as well as hydrogel when enzymatically processed (Table 2). Recently, the usage of collagen/gelatin continues to be high; with workers employing a gelatin methacrylate (GelMA), collagen molecule modified with reactive side

**Table 2**
Natural polymeric materials used for *in vivo* transplantation as well as human studies with multiple patients are listed. Reports using inorganic materials, *in vitro* research, and case reports describing single patient were not included. For individual studies with #, normal, and **bold** entries indicate ectopic, orthotopic transplantation, and human studies, respectively. The modification to the reagents used is briefly explained in parenthesis when necessary.
BMC = bone marrow cell; BMSC = bone marrow stem cell; BMMSC = bone marrow mesenchymal stem cell; DFSC = dental follicle stem cell; DMC = dental mesenchymal cell; DPC = dental pulp cell; DPSC = dental pulp stem cell; SCAP = stem cell from the apical papilla; SHED = stem cell from human exfoliated deciduous teeth; UCMSC = umbilical cord mesenchymal stem cell; ECM = extracellular matrix; GelMA = gelatin methacrylate; PEG = polyethylene glycol; PRP= platelet-rich plasma.

| Material | Reagents used | Cell type | First author | Year | Ref. |
|---|---|---|---|---|---|
| Collagen/gelatin | | | | | |
| <fiber/membrane/sponge> | BMP2, BMP4, TGF-β1 | - | Nakashima | 1994 | [143] |
| | OP-1 (BMP7) | - | Rutherford | 1994 | [144] |
| | BMP7 | - | Jepsen | 1997 | [180] |
| | BMP7 | - | Rutherford | 2000 | [181] |
| | BMP7 | - | Six | 2002 | [182] |
| | DMP-1 | - | Almushayt | 2006 | [183] |
| | #(collagen sponge) | swine DPC | Sumita | 2006 | [147] |
| | #(collagen sponge) | hDPSC | Zhang | 2006 | [184] |
| | FGF-2 | - | Kikuchi | 2007 | [185] |
| | #DMP-1, ceramic powder | hDPSC | Prescott | 2008 | [146] |
| | (collagen I/III sponge) | dog CD31(−)/CD146(−) DPC | Iohara | 2009 | [186] |
| | FGF-2 | - | Ishimatsu | 2009 | [187] |
| | (collagen sponge) | - | Inuyama | 2010 | [188] |
| | #nano-hydroxyapatite | rat DPSC | Yang | 2010 | [189] |
| | **(collagen membrane), PRP (+collagen sponge)** | **-** | **Goyal** | **2011** | [190] |
| | SDF-1 | dog CD105+ DPC | Iohara | 2011 | [191] |
| | SDF-1 | dog CD31-/CD146- DPC, CD105+ DPC | Nakashima | 2011 | [192] |
| | #(chondroitin sulfate, hyaluronic acid) | rat DPSC | Zhang | 2012 | [193] |
| | SDF-1 | dog $CD31^{-}$ DPC, CD105+ DPC, BMC | Ishizaka | 2012 | [194] |
| | #BMP4, FGF2 | rat DPC | Srisuwan | 2012 | [195] |
| | (collagen sponge) | dog DPSC | Wang | 2013 | [196] |
| | #BMP7 | hDPSC | Yang | 2012 | [197] |
| | G-CSF, (atelocolagen) | dog DPSC, DPC | Iohara | 2013 | [198] |
| | #SCF | hDPC | Pan | 2013 | [199] |
| | G-CSF, (atelocolagen) | dog mobilized DPSC | Iohara | 2014 | [200] |
| | (intrafibrillar-silicified collagen) | hDPSC | Niu | 2014 | [201] |
| | #(nanofibrous-gelatin), Magnesium phosphate | hDPSC | Qu | 2014 | [202] |
| | EDTA-treated swine dentin matrix, (swine dental pulp ECM), PLGA-Gelatin sheet | transduced swine DFSC | Chen | 2015 | [164] |
| | #(nanofibrous-gelatin) | hDPSC | Qu | 2015 | [203] |
| | #Exosome | hDPSC, hBMSC | Huang | 2016 | [204] |
| | G-CSF | dog DPSC | Iohara | 2016 | [205] |
| | (gelatin sponge), simvastatin | dog DPSC | Jia | 2016 | [206] |
| | **(collagen membrane)** | **-** | **Sharma** | **2016** | [207] |
| | (collagen membrane), (gelatin foam) | - | Fahmy | 2017 | [208] |
| | **(collagen membrane)** | **-** | **Jiang** | **2017** | [209] |
| | **G-CSF, (atelocollagen)** | **mobilized hDPSC** | **Nakashima** | **2017** | [210] |
| | preameloblast-conditioned medium | - | Bucchi | 2019 | [211] |
| | leptin | - | Choi | 2019 | [212] |
| | (collagen sponge) | - | Zaky | 2020 | [213] |
| | (collagen sponge), β-Defensin 4 | - | Zhai | 2020 | [214] |
| | **blood clot** | **-** | **Shetty** | **2021** | [215] |
| | **(collagen membrane)** | **-** | **Jiang** | **2022** | [216] |
| | #(nanofibrous gelatin) | amphiregulin-treated hDPSC | Li | 2022 | [217] |
| | lithium chloride, Wnt3a | - | Sukarawan | 2023 | [218] |
| <gel> | FGF-2 | - | Kikuchi | 2007 | [185] |
| | FGF-2 | - | Ishimatsu | 2009 | [187] |
| | #(collagen/chitosan blend) | HAT-7 epithelial cell, hDPSC | Ravindran | 2010 | [219] |
| | #FGF-2, VEGF, PDGF, NGF, BMP-7 | - | Kim | 2010 | [220] |
| | #SDF-1, bFGF, BMP-7 | hDPC | Suzuki | 2011 | [221] |
| | #FGF-2, VEGF, PDGF -containing gelatin beads | rat DPC | Srisuwan | 2013 | [222] |
| | FGF-2, VEGF, PDGF -containing gelatin beads | rat DPC | Srisuwan | 2013 | [222] |
| | #G-CSF | mobilized hDPSC, $CD31^{-}$ hDPC, hDPSC, hiPSC | Murakami | 2013 | [223] |
| | #(recombinant human collagen type I) | transduced SHED | Rosa | 2013 | [224] |
| | #(Collagen TE®) | mobilized hDPSC, hDPSC | Horibe | 2014 | [225] |
| | **bFGF** | **-** | **Nagy** | **2014** | [226] |
| | (type I collagen) | indium-111-oxine-labeled rat DPC | Souron | 2014 | [227] |
| | #bFGF, G-CSF | - | Takeuchi | 2015 | [228] |
| | #SDF-1 | - | Zhang | 2015 | [229] |
| | #PDGF-BB, NGF, BDNF | - | Li | 2016 | [230] |
| | #(GelMA) | hDPSC, HUVEC | Khayat | 2017 | [231] |
| | #(rat tail collagen-I) | ECM-treated hDPSC | Zhang | 2017 | [232] |
| | (Collagen TE®) | swine DPSC | Zhu | 2018 | [233] |

(*continued on next page*)

**Table 2** (*continued*)

| Material | Reagents used | Cell type | First author | Year | Ref. |
|---|---|---|---|---|---|
| | **(collagen granule)** | **-** | **Mittal** | **2019** | [234] |
| | #bioactive glasses, FGF2 | - | Washio | 2019 | [235] |
| | #exosome-like vehicle | rat dental papilla cell | Zhang | 2020 | [236] |
| | **(synthetic collagen)** | **-** | **Mittal** | **2021** | [237] |
| | #(GelMA) | hDPSC, HUVEC | Liang | 2022 | [148] |
| <microsphere/particle> | FGF-2 | - | Kikuchi | 2007 | [185] |
| | FGF-2 | - | Ishimatsu | 2009 | [187] |
| | #FGF-2, VEGF, PDGF | - | Srisuwan | 2012 | [195] |
| | FGF-2, VEGF, PDGF | - | Srisuwan | 2012 | [195] |
| | #VEGF | hDPSC | Li | 2016 | [238] |
| | #(GelMA) | hDPSC | Yang | 2021 | [239] |
| Alginate | | | | | |
| <fiber/membrane/sponge> | **-** | **-** | **Machado** | **2020** | [240] |
| <gel> | #- | rat DPC | Fujiwara | 2006 | [241] |
| | #- | hDPC | Kumabe | 2006 | [242] |
| | emdogain, (propylene glycol alginate) | - | Orhan | 2012 | [243] |
| | #- | hSCAP cell line RP89 | Lambricht | 2014 | [168] |
| | emdogain, (propylene glycol alginate) | - | Matsumoto | 2014 | [244] |
| | #VEGF, laponite, (RGD-alginate) | hDPSC | Zhang | 2020 | [154] |
| | #(GelMA) | hDPSC, HUVEC | Liang | 2022 | [148] |
| <microsphere/particle> | OP-1 (BMP7), TGF-β1 | - | Oliva-Rodríguez | 2011 | [245] |
| | #calcium chloride, thrombin | ferret DPSC | Verma | 2017 | [246] |
| | #TGF-β1, dexamethasone | - | Shrestha | 2019 | [247] |
| | #VEGF, laponite, (RGD-alginate) | hDPSC | Zhang | 2020 | [154] |
| | #(GelMA) | hDPSC, HUVEC | Liang | 2022 | [148] |
| Chitosan | | | | | |
| <fiber/membrane/sponge> | #(carboxymethylcellulose) | - | Chen | 2016 | [161] |
| | #BMP7 | hDPSC | Yang | 2020 | [197] |
| | **-** | **-** | **Mittal** | **2019** | [234] |
| | dexamethazone corticosteroid, bone matrix | - | Abbas | 2020 | [248] |
| | **-** | **-** | **Machado** | **2020** | [240] |
| | #simvastatin, calcium hydroxide | - | Soares | 2021 | [249] |
| | #ciprofloxacin, IDR-1002, (Polyvinyl alcohol/Chitosan) | hSCAP cell line RP89 | Gonçalves da Costa Sousa | 2022 | [250] |
| <gel> | #(collagen/chitosan blend) | HAT-7 epithelial cell, hDPSC | Ravindran | 2010 | [219] |
| | silver-doped bioactive glass | - | Zhu | 2019 | [251] |
| | (photobiomodulation therapy) | - | Moreira | 2021 | [252] |
| <microsphere/particle> | #TGF-β1, dexamethasone | - | Shrestha | 2019 | [247] |
| Acemannan | | | | | |
| <fiber/membrane/sponge> | (Acemannan) | - | Jittapiromsak | 2010 | [253] |
| | **(Acemannan)** | **-** | **Songsiripradubboon** | **2016** | [254] |
| | (Acemannan) | - | Songsiripradubboon | 2017 | [254] |
| Hyaluronic acid | | | | | |
| <fiber/membrane/sponge> | (dried hyaluronic acid sponge) | - | Inuyama | 2010 | [188] |
| <gel> | #(Colgel®) | hSCAP cell line RP89 | Lambricht | 2014 | [168] |
| | #TGF-β1 | swine DMC | Tan | 2015 | [255] |
| | TGF-β1 | swine DMC | Tan | 2015 | [255] |
| | #platelet lysate, cellulose crystal | hDPC | Silva | 2018 | [256] |
| | #- | swine DPSC | Zhu | 2018 | [233] |
| | - | swine DPSC | Zhu | 2018 | [233] |
| Fibrin | | | | | |
| <fiber/membrane/sponge> | #extraction of dentin matrix proteins, TGF-β1, (fibrin sealant) | (homing hDPSC) | Widbiller | 2018 | [257] |
| <gel> | #(PEGylated fibrin) | SHED | Galler | 2011 | [258] |
| | #- | - | Ruangsawasdi | 2016 | [259] |
| | #SCF | - | Ruangsawasdi | 2017 | [260] |
| | #dentin matrix proteins | hDPSC | Galler | 2018 | [261] |
| | #extraction of dentin matrix proteins, TGF-β1 | (homing hDPSC) | Widbiller | 2018 | [257] |
| <microsphere/particle> | #calcium chloride, thrombin | ferret DPSC | Verma | 2017 | [246] |
| Demineralized dentine matrix | | | | | |
| <fiber/membrane/sponge> | #- | rat DPSC | Zhang | 2012 | [193] |
| Small intestinal submucosa | | | | | |
| <fiber/membrane/sponge> | #- | rat DPSC | Zhang | 2012 | [193] |

(*continued on next page*)

**Table 2** (*continued*)

| Material | Reagents used | Cell type | First author | Year | Ref. |
|---|---|---|---|---|---|
| Hydroxyethylcellulose (Natrosol®) | | | | | |
| <gel> | **Chlorhexidine gluconate, Calcium hydroxide** | **-** | **Nagata** | **2014** | [262] |
| ECM | | | | | |
| <fiber/membrane/sponge> | #(swine dental pulp ECM) | - | Chen | 2015 | [164] |
| | EDTA-treated swine dentin matrix, (swine dental pulp ECM), PLGA-Gelatin sheet | transduced swine DFSC | Chen | 2015 | [164] |
| | #dentin matrix | hDPSC | Tran | 2015 | [263] |
| | #(swine dental pulp ECM) | hDPSC | Hu | 2017 | [264] |
| | (swine dental pulp ECM) | - | Alqahtani | 2018 | [166] |
| | #(DPSC ECM, HUVEC ECM) | hBMMSC, hDPSC | Huang | 2018 | [265] |
| | #(bovine dental pulp ECM) | - | Bakhtiar | 2021 | [167] |
| | #(human amniotic membrane ECM) | hDPSC | Bakhtiar | 2022 | [266] |
| <gel> | #(Matrigel®) | SCAP cell line RP89 | Lambricht | 2014 | [168] |
| | #(Matrigel®) | hUCMSC, HUVEC | Zhang | 2020 | [169] |
| | #(Matrigel®) | hUCMSC, VEGF-induced hUCMSC | Zhang | 2020 | [170] |
| | **(treated dentin matrix)** | **-** | **Holiel** | **2021** | [267] |
| Silk fibroin | | | | | |
| <fiber/membrane/sponge> | #SDF-1α | hDPSC | Yang | 2015 | [268] |
| | SDF-1α | - | Yang | 2015 | [268] |
| | #bFGF | hDPSC | Yang | 2015 | [269] |
| Glycerin | | | | | |
| <gel> | propolis | - | Pagliarin | 2016 | [270] |
| Cotton pellet | | | | | |
| <fiber/membrane/sponge> | BMP9 | - | Li | 2021 | [271] |

group, for the scaffold harboring DPSC [148]. GelMA can also be crosslinked with UV irradiation (photo-crosslink) to add a high melting temperature to the gel, overcoming the drawbacks of a gelatin gel.

*4.3.1.2. Alginate.* Alginate is a heteropolysaccharide mainly produced from the cell wall of brown algae. It is biocompatible, biodegradable [149] and non-toxic material [150]. Ultimately, alginate capacity to form gel is the major reason for its use in tissue engineering and wound healing [151], indeed, many endodontic studies have used alginate in the gel form (Table 2). However, they have deficiencies such as poor mechanical stiffness, uncontrolled degradation rates *in vivo* [152], and no ability to support cell adhesion. These drawbacks have been counteracted by adding materials to the alginate structure, producing a more robust structure [151], or by modification with cell adhering residue such as RGD [153]. Excellent results have been shown in regenerating pulp-like tissue *in vivo* using a scaffold based on modified alginate [154].

*4.3.1.3. Chitosan.* Chitosan is a deacetylated form of chitin, an amino polysaccharide polymer produced mainly by arthropods. Chitosan is completely biodegradable, non-antigenic, biocompatible, and even antimicrobial [155,156]. Furthermore, it can be readily fabricated to a desirable shape and has been shown to induce osteogenic differentiation, making it a desirable scaffold material for bone tissue engineering [157,158]. Chitosan does possess some drawbacks for use as a scaffold, with a low mechanical strength and high degradation speed in human tissue [159]; however, these deficiencies have been addressed by adding other functional materials such as hyaluronic acid or collagen [160]. In endodontics, Chen et al. added carboxymethylcellulose to chitosan scaffolds and applied them to a pulp regeneration model [161]. Addition of carboxymethylcellulose decreased the pore diameter and increased internal porosity of the scaffold. The pulp cells co-cultured in the scaffolds show improved adhesion, spreading, cell capacity, and three-dimensional configurations [161]. Da Costa Sousa et al. added poly(vinyl alcohol) to produce nanofiber, in which they incorporated the antibiotic and immunomodulatory peptide IDR-1002 [162]. This scaffold with SCAP was subsequently embedded in tooth fragment and subcutaneously implanted in mice, resulting in pulp-like tissue generation within the scaffold [162].

*4.3.1.4. Extracellular matrix (ECM).* Native ECM, produced by decellularization of native tissue generally using appropriate detergents such as Triton-X, has a potential to be an ideal scaffold because it includes all functional and structural molecules to support DPC activity [163]. Furthermore, ECM scaffold is biodegradable, biocompatible, and has preferable mechanical structure for seeded DPCs. Utilizing these merits of ECM scaffold in endodontic research, decellularized dental pulp ECM has been frequently used to harbor stem cells (Table 2) [164–167]. Also, commercially available gel-form ECM (Matrigel®) has been used for pulp regeneration [168–170]. One significant drawback of native ECM is its immunogenicity mainly caused by incomplete decellularization and damage-associated molecular patterns (DAMPs) [171]. To bypass this problem, apoptosis-assisted decellularization, efficient antigen removal, crosslinking to restore protein distortion caused by decellularization, have been proposed as solutions (reviewed in Ref. [171]).

### *4.3.2. Synthetic polymers*

Design flexibility when using synthetic polymers enables their physicochemical properties, such as degradation rate, microstructure, and mechanical strength [172]. However, due to the inherent absence of bioactive components, DPCs cannot readily proliferate, differentiate, or migrate in synthetic polymers [173]. Furthermore, there remains a need to establish their harmlessness before advocating their use in human teeth. Indeed, although there have been multiple synthetic polymers investigated, only three endodontic clinical studies using synthetic polymer scaffolds have been reported so far (Table 3). Notably, chemical modification and the incorporation of bioactive molecules into synthetic polymers can allow them to mimic the native environment [173].

Polyglycolic acid (PGA), poly-l-lactic acid (PLLA), polylactic-glycolic acid (PLGA), and poly(ε-caprolactone) (PCL) are repeatedly used in pulp regeneration research (Table 3). These polymers are used due to them being nontoxic, biodegradable, and their physicochemical properties such as mechanical stiffness, degradation speed, porosity, and microstructure can be controlled [174]. With these advantages, these four materials are approved by the FDA for some kind of medical use (PGA for absorbable suture, PLLA as a hydrophobic aliphatic polyester in different biomedical applications, PLGA for drug delivery, and PCL for use as an implant polymer material) [175]. However, local

**Table 3**
Synthetic polymeric materials used for *in vivo* transplantation as well as human studies with multiple patients are listed. Reports using inorganic materials, *in vitro* research, and case reports describing single patient were not included. For individual studies with #, normal, and **bold** entries indicate ectopic, orthotopic transplantation, and human studies, respectively. The modification to the reagents used is briefly explained in parenthesis when necessary. Materials approved by the FDA for some medical purpose are shown in underlined.
BMMSC = bone marrow mesenchymal stem cell; DFSC = dental follicle stem cell; DMC = dental mesenchymal cell; DPC = dental pulp cell; DPSC = dental pulp stem cell; HDMEC = human dermal microvascular endothelial cells; HUVEC = human umbilical vein endothelial cell; SCAP = stem cell from the apical papilla; SHED = stem cell from human exfoliated deciduous teeth; ECM = extracellular matrix; MTA = mineral trioxide aggregate; PEG = polyethylene glycol; PGA = polyglycolic acid; PLGA = poly(lactic-co-glycolic acid); PLLA: poly-L-lactic acid.

| Material | Reagents used | Cell type | First Author | Year | Ref. |
|---|---|---|---|---|---|
| PGA | | | | | |
| <fiber/membrane/ sponge> | #- | hDPC, human gingival fibroblast | Buurma | 1999 | [272] |
| | #- | swine DPC | Sumita | 2006 | [147] |
| | #TGF-β1, (+10% PLLA) | swine DMC | Tan | 2015 | [255] |
| | #(+3% PLLA) | rat tooth bud cell | Duailibi | 2004 | [273] |
| | #(+3% PLLA) | swine tooth bud cells | Young | 2002 | [274] |
| PLLA | | | | | |
| <fiber/membrane/ sponge> | #- | SHED, HDMEC | Cordeiro | 2008 | [275] |
| | #- | SHED | Casagrande | 2010 | [276] |
| | #- | hDPSC | Demarco | 2010 | [277] |
| | #- | SHED | Sakai | 2010 | [278] |
| | #dexamethasone, ascorbic acid, β-glycerophosphate, BMP7 | hDPSC | Wang | 2010 | [279] |
| | #- | hDPSC | Wang | 2011 | [280] |
| | #VEGF | hDPSC | Li | 2016 | [238] |
| | #BMP2 | hSCAP | Wang | 2016 | [281] |
| | #Matrigel® | transduced hDPSC | Zhang | 2016 | [282] |
| | Matrigel® | rat BMMSC | Ito | 2017 | [283] |
| | #- | transduced hDPSC | Silva | 2017 | [284] |
| | Matrigel® | rat BMMSC,rat endothelial cell | Sueyama | 2017 | [285] |
| | #simvastatin | hDPC | Soares | 2018 | [26] |
| | Matrigel® | LacZ-labeled rat BMMSC | Kaneko | 2019 | [286] |
| | Matrigel® | rat DPSC | Zaw | 2022 | [287] |
| | #- | transduced hDPSC | Zhang | 2022 | [288] |
| <microsphere/particle> | #- | hDPSC | Kuang | 2015 | [289] |
| | - | hDPSC | Kuang | 2016 | [290] |
| | #BMP2 | hSCAP | Wang | 2016 | [281] |
| PLGA | | | | | |
| <fiber/membrane/ sponge> | #- | hDPSC, hSCAP | Huang | 2010 | [291] |
| | #- | rat DPSC | Zhang | 2011 | [193] |
| | #hydroxyapatite, tricalcium phosphate, calcium carbonate hydroxyapatite | hDPSC, rat tooth bud cells | Zheng | 2011 | [292] |
| | #- | hDPSC | Sun | 2014 | [293] |
| | EDTA-treated swine dentin matrix, (swine dental pulp ECM), PLGA-Gelatin sheet | transduced swine DFSC | Chen | 2015 | [164] |
| | - | - | **Sharma** | **2016** | [207] |
| | #- | rat tooth bud cell | Duailibi | 2004 | [273] |
| | #- | hDPSC grown in microgravity condition | Li | 2017 | [294] |
| | #- | swine tooth bud cells | Young | 2002 | [274] |
| <gel> | collagen, risedronate, lornoxicam | - | Shamma | 2017 | [295] |
| PCL (poly(ε-caprolactone)) | | | | | |
| <fiber/membrane/ sponge> | SDF-1, BMP7 | - | Kim | 2010 | [296] |
| | #nano-hydroxyapatite | rat DPSC | Yang | 2010 | [189] |
| | MTA | - | Lee | 2011 | [297] |
| | #NGF | mouse tooth germ cells | Eap | 2013 | [298] |
| | #DMOG | hDPC | Yoo | 2018 | [299] |
| Self assembling peptide (SAP) | | | | | |
| <gel> | #FGF-2, TGF-β1, VEGF, (MMP2-cleavable peptide with RGD motif) | hDPSC | Galler | 2011 | [178] |
| | #FGF-2, TGF-β1, VEGF, (MMP2-cleavable peptide with RGD motif) | hDPSC | Galler | 2012 | [300] |
| | #(RADA16-I) | SHED | Rosa | 2013 | [224] |
| | #(RADA16-I) | hDPSC, HUVEC | Dissanayaka | 2014 | [301] |
| | (RADA16-I) | swine DPC | Mangione | 2017 | [302] |
| | #dentin matrix proteins, (MMP2-cleavable peptide with RGD motif) | hDPSC | Galler | 2018 | [261] |
| | extraction of dentin matrix proteins, TGF-β1, (MMP2-cleavable peptide with RGD motif) | - | Moon | 2018 | [303] |
| | #extraction of dentin matrix proteins, TGF-β1, (MMP2-cleavable peptide with RGD motif) | (homing hDPSC) | Widbiller | 2018 | [257] |

(*continued on next page*)

**Table 3** (*continued*)

| Material | Reagents used | Cell type | First Author | Year | Ref. |
|---|---|---|---|---|---|
| | #- | gene-modified hDPSC | Zhu | 2019 | [304] |
| | (RGD- and VEGF-mimetic peptide) | hDPSC | Xia | 2020 | [305] |
| | #(angiogenic hydrogel), FGF4, FGF9 | hCNC-like cell | Kobayashi | 2021 | [306] |
| | #(angiogenic hydrogel) | - | Siddiqui | 2021 | [179] |
| | (angiogenic hydrogel) | - | Siddiqui | 2021 | [179] |
| PEG <gel> | Chlorhexidine gluconate | - | Rodríguez-Benítez | 2014 | [307] |
| | metronidazole, ciprofloxacin, minocycline | - | Rodríguez-Benítez | 2014 | [307] |
| | metronidazole, ciprofloxacin, minocycline | - | Pagliarin | 2016 | [270] |
| | #dentin matrix proteins | hDPSC | Galler | 2018 | [261] |
| | **metronidazole, ciprofloxacin, minocycline** | **-** | **Neelamurthy** | **2018** | [308] |
| Polydioxanone <fiber/membrane/sponge> | #VEGF | - | Yadlapati | 2017 | [309] |
| | metronidazole, ciprofloxacin, minocycline | - | Bottino | 2019 | [310] |
| Poly-N-isopropylacrylamide <fiber/membrane/sponge> | #- | hDPSC | Itoh | 2018 | [311] |
| Polyvinyl alcohol <fiber/membrane/sponge> | #chlorhexidine gluconate | - | Kalyan | 2019 | [312] |
| | **chlorhexidine gluconate** | **-** | **Kalyan** | **2019** | [312] |
| | #ciprofloxacin, IDR-1002, (Polyvinyl alcohol/Chitosan) | hSCAP cell line RP89 | da Costa Sousa | 2022 | [162] |
| Vitrogel 3D <gel> | #SDF-1α, BMP-2 | hSCAP | Xiao | 2019 | [313] |

accumulation of acidic degradation products of these polymers can evoke a strong inflammatory response, which limits their biomedical applications [176].

Self-assembly peptides (SAP) are used in endodontic research principally as a hydrogel (Table 3). SAP monomers bind to each other via the hydrophobic sequence or by hydrogen bonds to form a nanofiber consisting of a β-sheet, β-helix, or α-helix structure, resulting in a hydrogel by way of their entanglement [177]. Modifications of the monomer sequence have added functionality to each gel; for example, the addition of RGD-motif and MMP2 digestion site, enabled the gel to be more biocompatible and biodegradable [178]. This hydrogel successfully induced hDPSC to differentiate into odontoblast-like cells [178]. Further, SAP with VEGF motif have been used to induce angiogenesis into a scaffold embedded in dog's teeth, which enhanced cell survival in the scaffold [179].

***Opinion*** A plethora of research studies have been carried out in recent years to investigate and develop new scaffold materials principally related to a tissue engineering approach (Tables 2 and 3). These approaches allow the incorporation of bioactive components or drugs which may target aspects of the tissue inflammation and repair. Existing materials such as calcium hydroxide or MTA adopt a different approach, being solid in nature and directly contacting the exposed pulp tissue. Although these materials, exhibit pro-reparative effects they are not designed to allow tissue expansion or replacement of lost tissue, so are by their very nature not regenerative materials. By contrast synthetic and non-synthetic materials offer the prospect of tissue outgrowth and the regeneration of lost tissue. Several natural and synthetic materials have been investigated *in vitro* and *in vivo* and have the potential when doped with bioactive GFs or HDACi to improve the healing response. Notably, however, no scaffold either doped or not doped with bioactive components is currently available for dental practitioners to use. In the next section we explore what the potential reasons are for this.

## 5. Are epigenetic-based or other 'smart' next-generation dental materials likely to be developed?

Even if extensive preclinical laboratory biological testing of novel biomaterials addresses pertinent issues, for example in the case of HDACi-augmented pulp capping materials including off-target effects, delivery mechanisms, release kinetics, potentially altered mechanical properties as well as esthetic aspects relevant to dentistry [109], other 'clinical' hurdles have to be addressed. This testing is becoming more complex and has significant cost and time implications even for academic-industry research collaborations attempting to introduce new bioactive therapies. These regulatory obstacles can delay even strong ideas as industrial partners may question the likelihood of profit after several years of preclinical and clinical development. To take the example used throughout the current article, despite strong *in vitro* and *in vivo* biological evidence supporting the use of HDACi as part of a targeted dental restoration, much work remains to develop a next-generation dental product that is available for dentists to use. So why is this the case and is it likely to become easier for innovative dental products to evolve in the future?

Dentistry is an unusual area, because it rarely attracts the levels of funding (exchequer, international or industrial-based) evident in related medical disciplines, despite containing some of the most prevalent global human diseases including caries and periodontal disease and the 5th most prevalent human condition in dental trauma [43,314,315]. Furthermore, dental materials are the most commonly placed biomaterial in the human body with an estimated 175 million placed per annum in the United States alone [316], yet mysteriously there is not a sophisticated targeted dental biomaterial available for routine use by dentists in operative dentistry and endodontics, despite evidence to suggest that these new materials may provide better outcomes for patients [317]. Obtaining regulatory approval for a new dental material is becoming more complex, and was modified at least with Europe in May 2021, by the introduction of new regulation on medical devices [318]. Within EU law, medical devices regulation, which includes dental filling materials, has classified devices to class I, IIa, IIb and III according to their intended purpose and any potential associated-risks [318]. For pulp capping materials, which contact the dental pulp inside a tooth the categorization is clearly now stated as Class IIa without a drug substance and Class III if a drug (or likely active additive component is added). If we examine more closely the regulations as they pertain to dentistry and

examine Annex VIII of the Medical Device Regulation rule 8 [318], which suggests that 'All implantable devices and long-term surgically invasive devices are classified as class IIb unless they: are intended to be placed in the teeth, in which case they are classified as class IIa'; evidently, existing pulp capping materials such as calcium hydroxide or MTA are considered in this IIa category. Closer examination of pulp capping agents within Annex VIII rule 14 [318 - Regulation (EU) 2017/746 of the European Parliament] states that 'All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product, including a medicinal product derived from human blood or human plasma, and that has an action ancillary to that of the devices, are classified as class III.' suggests that GFs, and even natural products contained within blood or extracellular matrix may be considered a drug or medicinal products and therefore class III. Note that a medicinal product has previously been defined within Europe [319] 'as any substance or combination of substances presented as having properties for treating or preventing disease in human beings or any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis'. A substance is further explained (Table 4). In the USA, a similar division exists within the FDA using three, not four categories. Generally, the higher the risk of the medical device, the higher the medical device classification with the result that Class III including pulp capping materials are considered the highest risk. With a higher classification comes more stringent data requirements to demonstrate the device's safety, effectiveness, and performance in a clinical biological environment and by extension more time to evaluate and more financial cost. One fundamental difference between class IIa and class III is the need for more clinical data for example, a clinical trial to be carried out for class III, which inevitably adds both time and cost into a project that ultimately affects a company's return to investment. For that reason, considerable legal debate is ongoing with regulators and the dental industry about the definition of what constitutes an 'active component', whether components are 'leached' out of the material as well as considerations about whether the effects of the added substances are direct or indirect.

EU Class III medical devices will involve more testing than IIa with objective clinical testing (by way of a clinical trial), an assessment of benefit and risk, safety and an analysis of whether existing clinical evidence from other medical uses can be extrapolated to the new purpose, so called 'off-label' use [318]. This type of regulation is difficult for academics to navigate and time consuming even for industrial partners with significant associated administrative burden. That said, the new regulation also present opportunities in that the addition of drugs or bioactive substances into a dental material will mean Class III testing rather than a separate testing route for medicinal products. There is an argument that at least for targeted pulp capping material development, this path has now become more straightforward in Europe.

**Table 4**
Definition of substance that could be considered as a medicinal product Adapted from point 2 of Article 1 of [129 - Directive 2001/83/EC of the European Parliament].

| Type of substance (any matter irrespective of origin which may be): | For example: |
|---|---|
| **Human** | Human blood and human blood products; |
| **Animal** | Micro-organisms, whole animals, parts of organs, animal secretions, toxins, extracts, blood products; |
| **Vegetable** | Micro-organisms, plants, parts of plants, vegetable secretions, extracts; |
| **Chemical** | Elements, naturally occurring chemical materials and chemical products obtained by chemical change or synthesis; |

The challenges of dental biomaterial development are shared in medicine where the rapid increase over the last 20 years in miRNA-related patents for diagnosis or targeted therapy has not yet resulted in an (FDA)-approved miRNA-based therapeutic [320]. This highlights not only the obstacles associated with development of a clinical product, but also regulatory issues, with product development taking time [321], albeit less time for medical devices than medicinal products [322,323]. In the past, new dental materials requiring simpler testing routes may have been prioritized over materials that require a medicinal product development for reasons of cost and expediency; however, within Europe the new legislation may imply that at least for VPT this is no longer an abbreviated route, which could provide the stimulus required for companies to support new 'smart' product development. Within the new EU regulation, the insertion of an epigenetic-modifying agent, the dental material remains a device, albeit now at class III. These development costs as before may have a 'knock-on' effect to the patient as they have to be incorporated into the product price which often in dentistry is borne by the patient rather than a third-party insurance company. So, the question manufacturers may ask themselves is, will the costs of biomaterial development result in a product that a significant number of high street dentists will avail of, albeit at a modestly but not significantly increased price?

Other groups have discussed the use of other pharmacological inhibitors, focused on the stimulation of Wnt/β-catenin pathway and used at low concentrations in VPT [317]. The use of these inhibitors, which have been prescribed therapeutically for diseases in other parts of the body, is an example of what the authors called 'drug repurposing' for dentistry [324], and is similar to the dental use of HDACi where FDA and EU-approval is available for treatment of myeloma [12]. The repurposing as part of a dental material offers the hope that a candidate drug can be immediately tested *in vivo* to investigate tertiary dentinogenesis responses [325]. Although the authors concluded that this approach bypasses the high level of economical and time investment that are usually required in novel drug discoveries, this is only partly true. In order to develop a dental material, the inhibitor will still need to undergo significant investigation in an animal pulp capping model before embarking on extensive human testing. This will require considerable funding and industrial partnerships, but potentially less than other new drugs.

Other indirect techniques within VPT have proposed using a modified glass ionomer dental restoration containing lithium to promote the Wnt-signaling pathway [326]. Unfortunately, this suggestion has not been adopted by dental materials manufacturers, partly due to the fact that glass ionomer for biological reasons is not recommended as a direct pulp capping agent [327].

***Opinion*** Dental materials are classed as medical devices, which are subject to legal regulatory control in Europe and the United States. Recent changes in EU legislation have introduced new rules and obligations in this area, which classifies pulp capping agents as Class IIa or Class III if they contain a bioactive substance, medicinal product oDMCr drug. The definition for these substances is broad, with the result that new pulp capping materials including 'smart' vital pulp biomaterials including those with added 'natural' morphogens or in this example epigenetic modifiers will come under Class III. Although this will increase the experimental and regulatory burden for the development of these materials, it also provides genuine opportunity for new targeted material to develop. Development of such next-generation materials can only realistically occur in collaborations between industry and research partners or by 'spin-put' companies formed as a result of academic endeavors. Up to this point, dental companies have not been keen to fund the type of 'early-stage' development required to foster the type of relationships required to shift materials from proof of concept towards product development. Within the regenerative medicine space at least this type of partnership has worked in medical disciplines, but has been much less evident within dentistry. Potentially for this type of important partnership to blossom, governmental intervention and legislative

change is required to incentivize companies to work with translational research units to move the discipline and eventually patient care forward.

## 6. Conclusions and opinion

Position statements from global international organizations have described the difficulties of predictably treating deep caries and as a result the conservative management of the exposed pulp has been designated a priority area for research activity, in order to promote better biomimetic solutions. Recent research has highlighted a range of substances, including pharmacological inhibitors or morphogens, that could supplement existing restorations or contribute bioactivity to new pulp capping dental materials to improve the regenerative healing response. These next-generation materials could encompass epigenetic therapeutic drugs such as HDACi that promote mineralization, reduce inflammation and stimulate reparative processes, with considerable volumes of recent research highlighting the potential of using these EU and FDA-approved epigenetic modifiers to work as part of a next-generation pulp capping material. Other smart restorations could include the use of antioxidants, growth factors, Wnt-signaling (GSK-3) inhibitors or simvastatin amongst other bioactive components all of which have been shown to have potential in improving biological responses in the dental pulp. In order for robust academic research to translate to the clinic, however, academic/industrial partnerships are required which aim to develop strong science into new dental products. Positively, as a result of new European legislation, the clinical development of new pulp capping materials now seems more likely, as all new pulp capping agents whether a bioactive substance is added or not will be designated in the same category. This in combination with repurposing of drugs within dentistry that are approved for other biological functions provides opportunity for new smart dental restorations in the future. The need for new pulp capping to address the deficiencies of existing restorations is evident, and the development of new targeted dental materials should be considered a priority area for dental material research in the next 10 years.

## Authorship contribution statement

**Henry F Duncan** planned and wrote sections of and edited the manuscript. **Yoshifumi Kobayashi**: wrote sections of the manuscript and edited the manuscript. **Michaela Kearney**: wrote sections of and edited the manuscript. **Emi Shimizu** planned and wrote sections of and edited the manuscript.

## Ethics approval and consent to participate

This opinion-led review paper did not require ethical approval. All authors consented to the final version of the manuscript.

## Funding and conflict of interest

This research as supported by the following grants to Emi Shimizu, National Institute of Dental and Craniofacial Research (NIDCR), grant number R01DE025885, R01DE031812 and a Fulbright Health Impact award 2019 to Henry F. Duncan. The authors have no conflicts of interest to declare.

## Data availability

The authors do not have permission to share data.

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