Bioactive Molecules for Regenerative Pulp Capping

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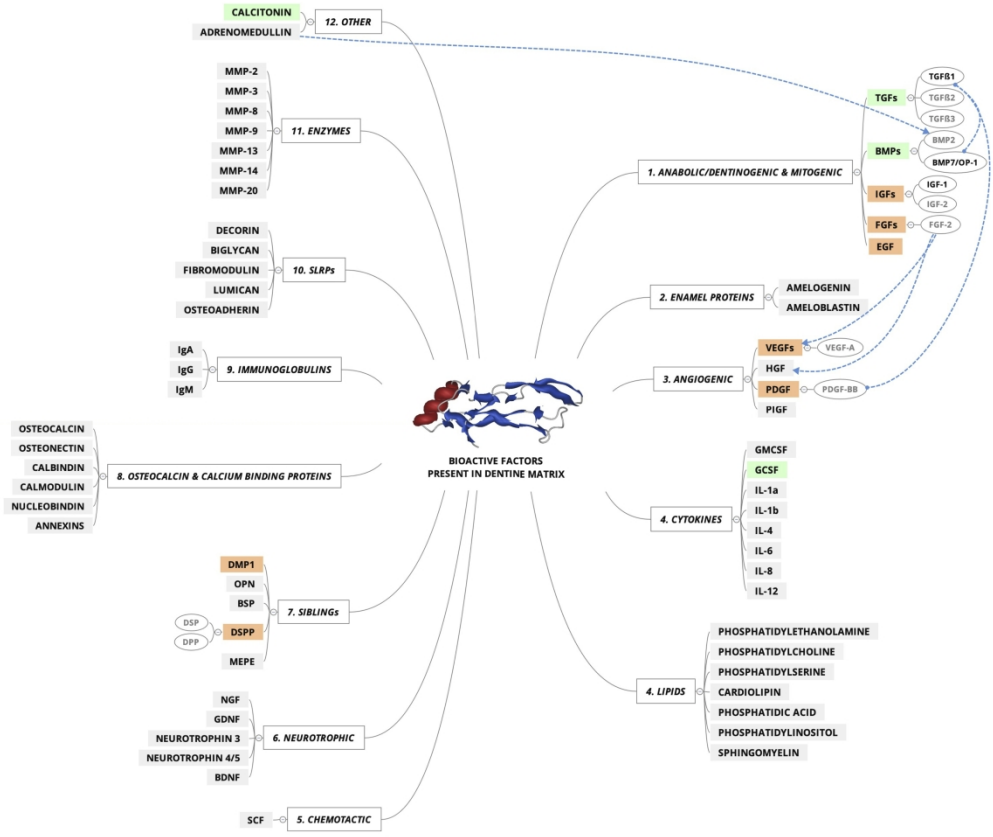
Abstract:	Since the discovery of bioactive molecules sequestered in dentine, researchers have been exploring ways to harness their activities for dental regeneration. One specific area, discussed in this review, is the area of dental pulp capping. Dental pulp caps are placed when the dental pulp is exposed due to decay or trauma in an attempt to enhance tertiary dentine deposition. Several materials currently exist for dental pulp capping, however natural biomimetic scaffolds may offer advantages over existing manufactured materials such as improved aesthetics, better biocompatibility and improved success rates. This review discusses and appraises the current evidence surrounding biomimetic dental pulp capping with a focus on bioactive molecules sequestered in dentine and their associated carriers. Molecules covered most extensively in the literature include; the transforming growth factors (TGF-βs, specifically TGF-β1) and bone morphogenetic proteins (BMPs, specifically BMP-2 and BMP-7), with collagen being the most commonly used scaffold material. Further literature exploring synergistic use of multiple peptides needs to be accomplished in tandem with the development of a tailored scaffold carrier. Further illumination on the roles of some of the molecules identified in dentine needs to be explored before they can be considered as potential bioactive molecules in a biomimetic scaffold for dental pulp capping. Future in vivo work needs to consider the inflammatory environment of the dental pulp in pulpal exposures and comparison against current pulp capping materials.

Table 1: Comparison of primary, secondary and tertiary dentine.

Dentine type	When/Where present
Primary	Produced during initial tooth formation, before the tooth erupts. Produced by odontoblasts.
Secondary	Deposited at a slow rate throughout life after primary dentinogenesis by odontoblasts, reducing the size of the pulp with age. Formed by odontoblasts.
Tertiary	Reactionary: Formed following mild/'slow and chronic' insult to the pulp. Formed by odontoblasts. Tubular structure, similar to primary dentine.
	Reparative: Formed following significant insult to the pulp, resulting in odontoblast death. Formed by odontoblast-like cells derived from resident DPSCs. Atubular structure, often showing some similarities to bone.



Caption : Caption : Figure 1: Schematic diagram summarising the bioactive molecules sequestered in dentine that have been explored in the literature, and this review, for pulp capping dentinogenesis. Molecules with the most evidence supporting their use are in green, amber for intermediate evidence and little evidence in grey. Molecules in grey have been included for completeness. Interactions are shown by arrows. DMP1= Dentine Matrix Protein 1, OPN= Osteopontin, BSP= Bone Sialoprotein, DSPP = Dentine Sialophosphoprotein DSP= Dentine Sialoprotein, DPP= Dentine Phosphoprotein, MEPE= Matrix Extracellular Phosphoglycoprotein, SIBLINGS= Small Integrin-Binding Ligand, N-linked Glycoproteins NGF= Nerve Growth Factor, SCF= Stem Cell Factor, TGF= Transforming Growth Factors BMP= Bone Morphogenetic Proteins, EGF = Epidermal Growth Factor, IGF= Insulin-Like Growth Factor, FGF= Fibroblastic Growth Factor, VEGF= Vascular Endothelial Growth Factor, HGF= Hepatocyte Growth Factor, PDGF= Platelet Derived Growth Factor, PlGF= Placental Growth Factor, GMCSF= Granulocyte Macrophage Colony Stimulating GCSF= Granulocyte Colony Stimulating Factor, IgA = Immunoglobulin A, IgG = Immunoglobulin G, IgM = Immunoglobulin M, SLRPS = Small leucine-rich proteoglycans, GDNF = Glial Derived Neurotrophic Factor, BDNF = Brain Derived Neurotrophic Factor, MMP = Matrix Metalloproteinase

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Abstract

Since the discovery of bioactive molecules sequestered in dentine, researchers have been exploring ways to harness their activities for dental regeneration. One specific area, discussed in this review, is the area of dental pulp capping. Dental pulp caps are placed when the dental pulp is exposed due to decay or trauma in an attempt to enhance tertiary dentine deposition. Several materials currently exist for dental pulp capping, however natural biomimetic scaffolds may offer advantages over existing manufactured materials such as improved aesthetics, better biocompatibility and improved success rates. This review discusses and appraises the current evidence surrounding biomimetic dental pulp capping with a focus on bioactive molecules sequestered in dentine. Molecules covered most extensively in the literature include; the transforming growth factors (TGF-βs, specifically TGF-β1) and bone morphogenetic proteins (BMPs, specifically BMP-2 and BMP-7). Further literature exploring synergistic use of multiple peptides needs to be accomplished in tandem with the development of a tailored scaffold carrier. Further illumination on the roles of some of the molecules identified in dentine needs to be explored before they can be considered as potential bioactive molecules in a biomimetic scaffold for dental pulp capping. Future *in vivo* work needs to consider the inflammatory environment of the dental pulp in pulpal exposures and comparison against current pulp capping materials.

Introduction

Caries (dental decay) is the most prevalent non-communicable human disease and is linked to low quality of life (Åkesson et al., 2016). The Global Burden of Disease Study estimated that 3.58 billion people worldwide have untreated caries (World Health Organisation, 2018). Indirect costs (for example, work absence) due to caries worldwide amount to a loss of US$144 billion/year (Listl et al., 2015), highlighting the significant impact caries has at a global level.

The crowns of teeth are made of enamel, dentine and pulp. Dentine is a living structure, with an inorganic content similar to bone, being predominantly hydroxyapatite, and calcium and phosphate salts (Sloan, 2015). The dental pulp, which is encased by dentine, contains the vessels, nerves and cells that maintain the vitality of the tooth. Dentine and pulp are inextricably linked (termed together 'dentino-pulp-complex' (DPC)). They share embryological origin (both being derived from the ectomesenchyme (Zohrabian et al., 2015)) and both release factors acting on the other (Smith, 2003), facilitating a state of homeostasis and when necessary, regeneration and repair. The DPC is the only part of the tooth with *in vivo* regenerative potential, and is being increasingly studied as a potential for biological scaffold production (Hashemi-Beni et al., 2017; Qu et al., 2015) and dental tissue regeneration.

Dentinogenesis is the production of dentine, and in health this is performed by odontoblasts (dentine producing cells). Odontoblasts are largely inactive after primary dentinogenesis. (Table 1). However, following insult to the pulp, odontoblasts play a role in wound healing in the form of reactionary dentinogenesis, where they lay down dentine to seal the vital pulp from the insult. Should the stimulus be great enough to kill the odontoblasts (such as significant trauma or deep caries), dental pulp stem cells (DPSC) will migrate to the site of injury, differentiate into 'odontoblast-like cells' and begin laying

down dentine, a process called reparative dentinogenesis. This results in a dentine bridge, sealing the precious pulp from further insult (McLachlan et al., 2003).

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Dentine type	When/Where present
Primary	Produced during initial tooth formation, before the tooth erupts. Produced by odontoblasts.
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Tertiary dentinogenesis is driven by dental injury, though the interplay between inflammation and regeneration is a delicate balance. Typically, exposed dental pulps are inflamed and often infected. It is generally accepted that only when infection and inflammation are under control will reparative actions occur in the dental pulp (Cooper et al., 2014). Many of the cytokines and growth factors involved in tertiary dentinogenesis, at high doses, may lead to pulpal death and prevent DPSC differentiation (Cooper et al., 2014). However, certain inflammatory cells (such as macrophages and dendritic cells) have also been shown to stimulate odontoblast differentiation (Goldberg *et al.*, 2008; Saito *et al.*, 2011), and molecules upregulated in caries, such as C5a and stromal cell-derived factor 1/CXCL12 have also been shown to be capable of recruiting immune cells as well as DPSCs (Cooper et al., 2014). These studies highlight the intricate, delicate and vital interplay between inflammation and regeneration in tertiary dentinogenesis and caries.

Reparative dentinogenesis is a complex process, as the DPSCs need to be recruited to the site of injury, undergo differentiation and then begin depositing dentine. Understandably, this is more time-consuming than reactionary dentinogenesis, and because of this it is often ineffective at sealing the pulp from the insult. This process is significantly driven by the release of bioactive molecules (including growth factors, cytokines and other matrix molecules) sequestered within dentine during primary dentinogenesis, and released following dentine damage. These molecules are capable of stimulating reparative and reactionary dentinogenesis (Smith et al., 2012) and summarised in Figure 1.

When a pulp is exposed, through trauma, mechanical action (typically iatrogenic) or caries, the clinician has a choice of whether to attempt to save the pulp (via a direct pulp cap), or not (leading to a root canal treatment (endodontics) or an extraction).

Pulp capping agents (PCAs) attempt to seal the pulp by encouraging tertiary dentine deposition. Direct pulp caps can be technical to perform successfully and correctly, with some studies reporting success rates as low as 9% after pulp exposures at 5 years (Bjørndal et al., 2017). When a pulp is exposed, the tooth needs to be isolated (ideally with a rubber dam), any further caries removed, the area cleaned/wound lavage, haemostasis achieved, the PCA prepared/mixed, placed and allowed to set (if required), and the cavity restored. Clinicians may also decide to remove some of the superficial pulp which has been exposed (termed a 'partial pulpotomy'), in an attempt to remove any inflamed pulpal tissue that may impair healing (Bjørndal et al., 2019). Besides the PCA used, numerous other factors may influence the success rates of a pulp cap, such as; bacterial microleakage from the restoration, operative debris from the cavity preparation, uncontrolled haemorrhage (Murray et al., 2002), the experience of the operator, the type of cavity (interdental/occlusal) (Ritter, 2007), the length of time to permanent

restoration (Mente et al., 2014), the type of exposure (carious/mechanical/trauma) (Ritter, 2007) and the type of lavage/haemostasis agent used (Tüzüner et al., 2012). A pulp cap is deemed successful if at 75-90 days a dentine bridge has formed and the tooth remains vital (Stanley and Pameijer, 1997), though typically a dentine bridge begins to form within 30 days of the original pulp cap and is largely completed by 130 days (Hargreaves and Cohen, 2010). No PCAs currently available are perfect and, considering the prevalence of caries, it is imperative that cost-effective materials with high success rates, desirable clinical outcomes and suitable handling qualities are available for clinical use. Furthermore, the evidence behind the use of many PCAs is generally lacking, with poor quality clinical studies often including; sound teeth, limited follow-up time, lack of histological data, young healthy patients and tooth isolation (Hilton, 2009), which often make comparison to real-life clinical work challenging.

Pulp capping Materials

There are many Pulp capping materials/PCAs available commercially, with $Ca(OH)_2$, and calcium-silicate based materials (Mineral Trioxide Aggregate (MTA) and other tricalcium silicate based cements) being the most commonly used in practice..

Of the more commonly used PCAs, $Ca(OH)_2$ has been used for the longest period of time (having been first explored in the 1920s) and for many years has been considered the 'gold standard' for a direct pulp cap (Hilton, 2009; Qureshi et al., 2014). $Ca(OH)_2$ is often considered as a bench-mark to compare newer PCAs against – *in vivo* and *in vitro* - and there is a wealth of literature supporting its use. The high alkalinity of $Ca(OH)_2$ eliminates micro-organisms, providing a favourable environment for dentinogenesis, but this has negative implications too, making $Ca(OH)_2$ cytotoxic resulting in further pulpal necrosis (Youssef et al., 2019). The exact mode of action for dentinogenesis in a $Ca(OH)_2$ pulp capped tooth remains contentious, with some people believing the irritation, inflammation and necrosis in the pulp caused by the $Ca(OH)_2$ initiates dentinogenesis via an unknown pathway, and others suggesting that it is due to $Ca(OH)_2$ facilitating release of bioactive molecules sequestered in dentine (Bjørndal et al., 2019; Graham et al., 2006; Hilton, 2009), or potentially a mixture of the two. $Ca(OH)_2$ has numerous issues as a PCA, such as; high solubility, lack of adhesion to dentine, and 'tunnel defects' in the reparative dentine formed (Qureshi et al., 2014).

MTA is newer to the commercial market than $Ca(OH)_2$, though has been used for pulp capping since the 1990s (Zafar et al., 2020), and is thought to have better success rates than $Ca(OH)_2$ according to several systematic reviews (Paula et al., 2018; da Rosa et al., 2018b; Zhu et al., 2015). Moisture is required for MTA to set, and when water is added, a colloidal gel composed of calcium oxide crystals is formed (calcium silicate hydrate) and $Ca(OH)_2$ is released, imparting much of MTA's known antimicrobial activity (Zafar et al., 2020). MTA is known to liberate numerous growth factors from dentine during pulp capping (Tomson et al., 2017).

Although MTA would appear to be a good PCA based upon the success rates, there are numerous issues with it, for example; long setting times (> 2 hours for some brands), discolouration of teeth, poor handling characteristics (due to its crumbly nature), risk of pulpal obliteration and being expensive (Linsuwanont et al., 2017; Qureshi et al., 2014; Zafar et al., 2020). Due to the release of $Ca(OH)_2$, MTA also has high alkalinity, which again is cytotoxic (Kim et al., 2019; Youssef et al., 2019). More recent modifications of MTA have attempted to remedy some of these problems (for example, newer generation versions of MTA may have faster setting times, through the addition of calcium sulphate or sodium hypochlorite, the reduction in particle size, or addition of substances to allow for light curing) and reduced tooth discolouration (Zafar et al., 2020), though long term studies demonstrating no reduction in efficacy are lacking.

Newer tricalcium-silicate based cements, (for example, BioDentine™ (Septodont)) have been explored to overcome numerous issues with earlier PCAs (for example, BioDentine™ has a shorter setting time than MTA, and improved handling characteristics (Zafar et al., 2020)), but still problems exist (for

example, tooth discolouration and age-dependent results (Lipski et al., 2018)). Again, due to the high alkalinity of the material, it will have cytotoxic effects on the pulp (Youssef et al., 2019). “No statistically significant differences in the various outcomes” were found in a systematic review when MTA was compared to tricalcium silicate based cements (Paula et al., 2018), though a significant lack of long-term cohort studies and randomised controlled trials were identified as a significant issue, partly due to the newness of the material.

A lack of a highly effective PCA is the driving motivator behind exploration of more biomimetic approaches to pulp capping, with the hope of discovering and utilising key factors present during tertiary dentinogenesis to drive DPC regeneration. Many of the bioactive molecules within dentine can be liberated by current PCAs (Tomson et al., 2017), or through chemical treatment of the dentine (Zhao et al., 2000), though this release is poorly controlled in terms of frequency and volume. By applying these molecules extraneously, it may be possible to exploit their actions in a biomimetic pulp capping agent to actively drive tertiary dentinogenesis, in a controlled manner, for pulp capping, maintaining the vitality of the tooth. This data can be used to inform dentinogenic scaffold design, allowing for the ideal biomimetic environment to be fabricated that can include the bioactive molecules pertinent for reparative dentinogenesis, and provide an ideal environment for DPSC migration and differentiation, with the exclusion of noxious irritants present in many current PCAs and the inflamed pulp; in essence, harnessing the positive reparative factors of tertiary dentinogenesis and excluding the negative factors that drive pulpal necrosis. Moreover, many of the scaffolds designed to carry bioactive molecules also have the ability to also carry medicaments such as anti-inflammatories and antimicrobials which will be of considerable benefit in controlling pulpal inflammation and any residual caries, and many of these scaffolds also swell in the presence of moisture which may be beneficial in providing a seal from the oral environment. In considering the possibility of a biomimetic PCA, one must remember that tertiary dentinogenesis occurs in the pulp, and through creating an environment similar to dental pulp it may be possible to speed up the process of reparative dentinogenesis with fewer unwanted side effects. As such, a scaffold loaded with biomimetic bioactive molecules may prove to be an ideal pulp capping material, fulfilling the key requirements of a PCA according to Bjørndal *et al*., which include; 1) an immediate seal of the dental cavity, protecting the pulp as a dentine bridge is forming, 2) biocompatibility and non-cytotoxic, 3) possess bioactive properties capable of triggering the biological mechanisms for dentine bridge production (Bjørndal et al., 2019).

From the literature, bioactive molecules may be added to scaffolds as gene therapy (DNA) or proteins for dentinogenic pulp capping. Gene therapy eliminates the need for externally produced recombinant factors, and gene transfection can enhance the efficacy of stem cell applications (Yang et al., 2009). Some bioactive molecules (for example Bone Morphogenetic Proteins (BMPs)) have a short half-life and are required in high concentrations at the local site to be effective; therefore the ability for local elevated production of BMP proteins by the patient may be advantageous and could be achieved via gene transfection (Nakashima, 1994a; Nakashima and Reddi, 2003).

Purpose and Scope

The purpose of this review is to explore the literature surrounding extracellular bioactive molecules known to be sequestered in dentine (as per the works by Austah et al., 2019; Schmalz et al., 2017; Smith et al., 2012; Smith et al., 2016; Tomson et al., 2017; Goldberg et al., 2011), and summarise the current evidence behind their utility as PCAs.

It is pertinent in biomaterial engineering to consider the delivery mechanism (or carrier) when considering delivery of any molecules, as spatiotemporal control over the release of bioactive molecules controls the resulting tissue regeneration (Rambhia and Ma, 2015). However, extensive

coverage of carriers for pulp capping is beyond the scope of this review and warrants a review unto its own.

For all molecules discussed, we have focused on literature published in the English language, with a preference for best and most recent evidence behind the use of each molecule, namely *in vivo* studies over *in vitro* work where available and focused on studies specifically addressing pulp capping. However, there is a general lack of large scale works in this field, with most publications being small-scale singular animal or *in vitro* studies, hence this review attempts to accumulate the most pertinent works for each molecule and evaluate the current evidence behind its utility for inclusion within a pulp capping agent.

Extracellular Bioactive Molecules Known to be Sequestered in Dentine

Anabolic/Dentinogenic and Mitogenic

Transforming growth factors (TGF-β)

Transforming growth factors (isoforms TGF-β1, TGF-β2 and TGF-β3) are multifunctional cytokines secreted following initial inflammatory response, are potent modulators of tissue repair and are expressed by all cells in the human body (Vander Ark et al., 2018; Niwa et al., 2018). TGF-βs at a cellular level are known to regulate proliferation, migration, differentiation and apoptosis, and more generally they play a role in regulation of inflammatory responses and both tumour suppression and progression (Vander Ark et al., 2018) . Factors such as pH, integrins and various proteases (e.g. MMP2, MMP9, plasmin) are known to activate TGF-βs (Niwa et al., 2018). TGF-β1, TGF-β2 and TGF-β3 are receptor ligands which bind to TGF-β receptors (TGF-β receptor 1, TGF-β receptor 2 and TGF-β receptor 3) (Vander Ark et al., 2018), with TGF-β1 being the predominant isoform (Niwa et al., 2018). Within pulpal inflammation, TGF-β1 is involved in; chemotaxis of inflammatory cells, angiogenesis, deposition of extra-cellular matrix and the formation of new tissue (About et al., 2000a), and is known to play a role in the differentiation of odontoblasts (Begue-Kirn et al., 1992; Nakashima, 1992). Within the pulp, TGF-β1 is activated through degradation of the TGF-β1 complex, leading to elevated dentin sialophosphoprotein (DSPP) and matrix metalloproteinase 20 (MMP20) levels (Niwa et al., 2018).

TGF-β1 interacts with the extra-cellular matrix (ECM) in a unique way. TGF-β1 is secreted as a homodimer, non-covalently attached to its latency associated pro-peptide (LAP). When bound to LAP, TGF-β1 is prevented from attaching to cell receptors (and is therefore latent (TGF-β1-LAP)). TGF-β1-LAP is bound and stored in the ECM via latent TGF-β1 binding protein (LTBP-1). Mechanical forces within the ECM (from actin and myosin cellular contractions) are transmitted to a binding site in LAP, which induces a hypothetical conformation change, liberating the bound TGF-β1. In essence, a pulling mechanism from a cellular-bound integrin releases the TGF-β1 from the ECM-bound TGF-β1-LAP. Application of the force to the TGF-β1-LAP without a mechanical anchor (provided by the LTBP-1 binding to the ECM), would result in dragging/translocation of the complex and no conformational change facilitating the release of TGF-β1 (Hinz, 2015). This storage and release mechanism from the ECM produces a disjoint between secretion time and action time of TGF-β1 (Hinz, 2015).

There are numerous *in vitro* studies within the literature proving the ability of TGF-β1 for inducing odontogenic differentiation of isolated dental pulp stem cells (DPSC)s (Begue-Kirn et al., 1992; Begue-Kirn et al., 1994). However, this review is focused on the potential of bioactive molecules being used as pulp capping agents and currently there is only one human *in vivo* study within the literature.

Therefore, most of the current best evidence for the use of TGF-β1 as a bioactive molecule comes from *in vivo* animal models; the evidence for use of TGF-βs as a bioactive molecule for pulp capping are discussed below.

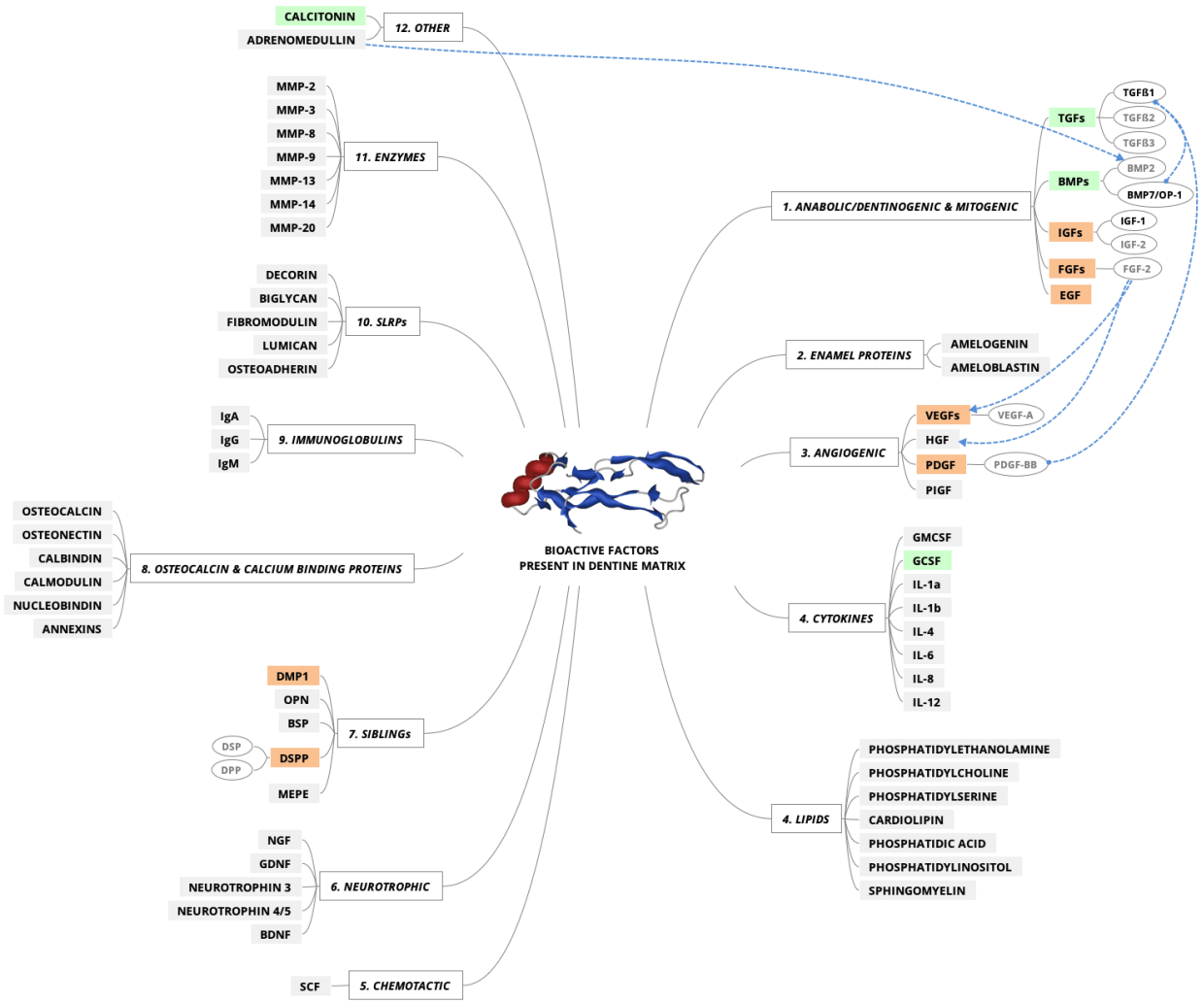
In the only *in vivo* human study, it has been demonstrated that TGF-β1 loaded onto collagen membranes (20 ng/ml, 5 µl) can act as a suitable pulp capping agent, producing similar effects to $Ca(OH)_2$, but with earlier induction of angiogenesis (Kunarti, 2008). However, this study used sound premolars scheduled for extraction due to orthodontic reasons, with a follow up of only 21 days, making comparison to routine clinical use (i.e. a carious tooth with a long-term restoration) difficult, though the initial results appeared promising.

Using a dog model, Li *et al* (Li et al., 2014) formed a chitosan bilayer membrane, containing TGF-β1 loaded chitosan microspheres and compared the effectiveness of this loaded biomaterial for pulp capping against an unloaded chitosan scaffold, no pulp cap, and $Ca(OH)_2$. They found that all agents and the control group induced an initial inflammatory response, but only the $Ca(OH)_2$ and loaded chitosan groups produced reparative dentine – and of these, the loaded chitosan scaffold produced 3-6 times more reparative dentine than $Ca(OH)_2$. In an earlier dog model study by Tziafas *et al*., (Tziafas et al., 1998), TGF-β1 loaded filters formed odontoblasts and dentine, and were more successful than basic fibroblastic growth factor (FGF) and Insulin growth factor (IGF) in producing dentine. Similar findings were also identified by Zhang *et al.,* (Zhang et al., 2008) using a goat model with TGF-β1 loaded onto poly(lactic-co-glycolic acid) microspheres used for pulp capping. They found a higher concentration of TGF-β1 (400 ng) formed a better tertiary dentine bridge than lower concentrations (20 ng): all of their negative controls produced no dentine.

Conversely, in a rat study by Oliva-Rodríguez *et al* (Oliva-Rodríguez et al., 2011), TGF-β1 loaded onto alginate microparticles, specifically designed for controlled release, produced a similar result to $Ca(OH)_2$ for pulp capping. However, with the addition of BMP-7 to the TGF-β1 loaded alginate, the effects were improved (Oliva-Rodríguez et al., 2011).

When comparing TGF-β1 to other bioactive molecules, TGF-β1 has been shown to outperform BMP-7 and WNT signalling protein (WNT-1). In a porcine model study, a calcium silicate based material was soaked in a solution containing BMP-7, TGF-β1 or WNT-1, and each bioactive molecule was assessed for pulp capping. Each calcium silicate scaffold was soaked in 30 µL of a 300 mg/mL porcine albumin solution containing 30mg of the bioactive molecule. TGF-β1 instigated odontoblastic differentiation and more consistent reactive dentine formation of an appropriate depth and structure for effective pulp capping (Tziafas et al., 2017). Hu *et al*., (Hu et al., 1998) have also shown that TGF-β1 loaded on a collagen scaffold can outperform Platelet Derived Growth Factor (PDGF) BB, Basic Fibroblastic Growth Factor, Epidermal Growth factor (EGF), Insulin-like Growth Factor (IGF) and $Ca(OH)_2$ in a rat animal model. In contrast to the studies supporting the use of TGF-β1, Nakashima reported no dentine formation and impaired pulpal healing with TGF-β1 (2 µg) loaded on collagen and implanted into a dog model (Nakashima, 1994a).

Based upon these studies, it is possible that the release frequency and carrier may have an effect on the activity of TGF-β1 in inducing tertiary dentinogenesis and reparative pulpal changes. However, It is also clear that TGF-β1 may be as good, if not better than $Ca(OH)_2$ as a PCA. Modification of the delivery mechanism to incorporate synergistic growth factors could be promising for improved results. The wealth of literature supporting TGF-β1 makes it a front-runner for inclusion in a biomimetic PCA, despite it being more than 10 years since the *in vivo* human study. It is not clear why exploration of this bioactive molecule for use as a PCA has slowed when it shows such promise. Further clinical trials are needed to forward research on this growth factor.



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Bone Morphogenetic Proteins (BMPs)

Morphogens are extracellularly secreted signals governing morphogenesis and control craniofacial patterning. Morphogens are subdivided into four evolutionarily conserved protein families; BMPs, Fibroblast Growth Factors, hedgehog proteins and wingless-and int-related proteins (Nakashima and Reddi, 2003). BMPs are a family of 22 secreted extracellular matrix-associated proteins involved in numerous functions within the human body, such as muscle development, stem cell and organ formation, bone and cartilage formation, iron metabolism and vascular biology (Brazil et al., 2015; Nakashima and Reddi, 2003). BMPs are considered part of the TGF-β family, with both acting on Smad intracellular signalling (TGF-β utilising Smad1/5/8, BMPs utilising Smad2/3) (Brazil et al., 2015; Nakashima and Reddi, 2003).

In the literature, BMPs have been added to scaffolds as gene therapy (DNA) or proteins for dentinogenic pulp capping. BMPs have a short half-life and are required in high concentrations at the local site to be effective; therefore the ability for local elevated production of BMP proteins by the patient may be advantageous and could be achieved via gene transfection (Nakashima, 1994a; Nakashima and Reddi, 2003).

Again, similar to TGF-β1, there is a wealth of literature confirming the role of BMPs in dentinogenesis, and their ability to induce odontogenic differentiation (About et al., 2000b; Chen et al., 2008; Iohara et al., 2004; Suzuki et al., 2011). However, there is considerably less literature covering the use of BMPs specifically as pulp capping agents for *in vivo* applications and for this, one must look to animal models again, as there are no clinical trials within the literature covering their use. The main BMPs explored within the literature are BMP2 and BMP7.

Some of the earliest work on BMPs for pulp capping was carried out by Nakashima (Nakashima, 1994b; Nakashima, 1994a), who explored BMPs for pulp capping in a dog model. Briefly, across two studies they explored the use of recombinant BMP-2 and BMP-4 carried on a collagen matrix (Nakashima, 1994a) and mixed with inactivated, demineralized dentine matrix powder (particle size 200-500 µm size) (Nakashima, 1994b). When used in conjunction with collagen, BMP-2 (2 µg) and BMP-4 (4 µg) induced osteodentine formation, however tubular dentine was lacking, and interestingly, the unloaded collagen carrier control produced a very minimal amount of osteodentine (Nakashima, 1994a). When used in conjunction with inactive, demineralised powered dentine, 2 µg of BMP-2 and BMP-4 demonstrated osteodentine towards the superficial aspect of the cavities and tubular dentine towards the deeper radicular aspects (Nakashima, 1994b).

BMP-2 has been explored as a pulp capping agent in conjunction with MTA; 1 g MTA mixed with 1 µg BMP-2 was compared to MTA alone and no difference in the quality and quantity of reparative dentine was seen between the rat groups, and in both scenarios the reparative dentine had a bone-like morphology (Ko et al., 2010).

An example of BMP gene therapy being used can be found in the study by Yang et al (Yang et al., 2009), who used an adenoviral vector containing human *BMP-2* transfected into DPSCs and loaded onto a sintered ceramic scaffold; *in vitro* and *in vivo* experiments demonstrated mineralised tissue formation, but the appearance again was more in-keeping with bone than dentine. In an attempt to explain the formation of bone over dentine, the authors suggested that BMP-7 may be a more appropriate candidate for dentine production, and that the scaffold structure and environment (at a micro and macro level) may play a role in the formation of bone over dentine from cells of dental pulp origin. Subsequently, BMP-7 carried on nanofibrous poly(L-lactic acid) scaffolds has been used successfully *in vitro* and *in vivo*, to push odontoblastic differentiation and dentine-like hard tissue formation (Wang et al., 2010).

When BMP-7 was compared to MTA as a pulp capping agent in rats, MTA out-performed BMP-7, with a more impervious dentine bridge being formed in the MTA group and increased DSP activity. The group found that the material produced by BMP-7 also resembled bone more than dentine (Andelin et al., 2003). However, in this study BMP-7 was added to the pulp exposures without the adjunct of a scaffold, highlighting the potential importance of the scaffold in BMP delivery, likely for timely and sustained release, and potentially structural direction in influencing tubular dentine deposition over osteodentine.

For pulp capping, BMP-7 and Bone Sialoprotein (BSP) (each delivered on a gelatin carrier) have been shown to produce more mineralised tissue than $Ca(OH)_2$ in a rat model (Goldberg et al., 2001). The BMP-7 group produced more tissue towards the apical and radicular areas of the pulp, whereas the BSP group produced dentine more in the coronal area, and the dentine produced by the BSP group was more like tubular dentine than the BMP-7 group and less pervious (Goldberg et al., 2001). The preferential production of osteodentine in the radicular area of the pulp was also found by Six *et al* (Six et al., 2002b) exploring BMP-7 on collagen scaffolds as pulp caps.

Rutherford *et al*., explored the use of BMP-7 using primate animal models (Rutherford et al., 1993; Rutherford et al., 1994). Using a powdered collagen matrix mixed with recombinant human BMP-7 (2.5 µg/mg of collagen), they compared BMP-7 to $Ca(OH)_2$ (Rutherford et al., 1993) and explored the tertiary dentine formation at different time points post-operatively (Rutherford et al., 1994). In summary, BMP-7 outperformed $Ca(OH)_2$ in the short term (Rutherford et al., 1993) and demonstrated 75% tertiary dentine bridge formation at 1 month and 95% at 4 months (Rutherford et al., 1994). Similar to other studies, the unloaded collagen carrier failed to produce a dentine bridge. From the descriptions in these studies, the dentine produced seems to be a mix of tubular and osteodentine.

More recent studies have attempted to discover the optimum dosage of unloaded BMP-7 to push odontogenic differentiation in culture of DPSCs and maintain DPSC proliferation: 50 ng/mL and 100 ng/mL were the most successful (Zhu et al., 2018).

One issue with many of these studies is that sterile, uninflamed teeth were used. This is an issue Rutherford and Gu attempted to resolve by exploring BMP-7 delivered on collagen scaffolds to inflamed ferret pulps. The results showed no reparative dentinogenesis, possibly in relation to elevated BMP antagonistic binding proteins present in inflamed dental pulps inactivating the protein (Rutherford and Gu, 2000). Rutherford (Rutherford, 2001) went on to explore whether *in vivo* gene transfection of *BMP-7* in an adenovirus within a collagen hydrogel carrier could induce reparative dentine formation in inflamed ferret pulps and compared this to autologous fibroblasts transfected with *BMP-7 ex vivo*, also carried in a collagen hydrogel into the inflamed pulps. The direct transfection group did not produce reparative dentine, however the group with pulps capped using the *ex vivo* transfected fibroblasts did produce good quality reparative dentine with associated odontoblasts. The authors postulated that this may be due to sustained secretion by the fibroblasts of BMP-7.

Recombinant BMP-2 is marketed commercially for bone regeneration (InductOs ™, Medtronic BioPharma, The Netherlands) as well as recombinant BMP-7 (Osigraft ™, Olympus Biotech, Ireland), however both of these systems have variable success rates and little long-term clinical evidence supporting their use (Ayoub and Gillgrass, 2019; Calori et al., 2015; Chevet-Noël et al., 2020; Corinaldesi et al., 2013; Sailhan et al., 2010; Vincentelli et al., 2019) . No research has been undertaken using these materials off-label for dental pulp capping, though the systems used are similar to the experimental work carried out using recombinant BMP-2 and BMP-7 and this could be a specific area of interest moving forwards.

Blended scaffolds have also been used to successfully deliver plasmid vectors coding for *BMP-7.* In the study by Yang *et al.,* (Yang et al., 2012) chitosan/collagen scaffolds (one containing a plasmid coding for human BMP-7 and one control) were seeded with DPSCs and implanted subcutaneously in mice. They were reviewed after 4 weeks and upregulation of DSPP was observed (suggesting odontoblastic differentiation) in both groups, but this was significantly higher in the *BMP-7* group.

In summary, BMP-2 and BMP-7 have been used to varying degrees of success as pulp capping agents in animal studies. It is clear from the literature though that a bone phenotype may result in the hard tissue produced, and this seems to occur more readily when the BMP is not delivered on a scaffold. This is not ideal for pulp capping as bone is more porous than dentine and can therefore be pervious, preventing a suitable pulp seal. It is not currently known what causes a bone phenotype over a dentine phenotype; the carrier, the environment, the use of proteins or DNA may all play a role, and further work is needed to resolve this issue should BMPs be pursued as a potential pulp capping bioactive molecule. The limitations of requiring a high concentration of BMPs required to produce dentine, the possibility of inflammation impeding their action and the short half–life of these molecules are significant hurdles. Although there is considerable literature exploring BMPs as PCAs and clinical application of these molecules has reached the commercial market for bone regeneration, dentine is not the same as bone, and until the issue of bone production over dentine production is resolved and controlled BMPs may prove less suitable for PCAs than TGF-β1, and other molecules (discussed below) may show more promise.

Insulin-like growth factor

The IGF (insulin-like growth factor) system is complex, being comprised of two ligands (IGF-I and IGF-II), two cell surface receptors, at least six IGF binding proteins (IGFBP) and multiple proteases (Catón et al., 2007). IGF-1 is covered most widely in the literature; a cell surface receptor tyrosine kinase that binds to IGF-I and IGF-II receptors, to activate various downstream signalling pathways, such as AKT/Protein Kinase B (a signal transduction pathway) and Mitogen-Activated Protein Kinase (MAPK) (Teng et al., 2018). IGF-1 has been implicated in the differentiation and proliferation of dental pulp stem cells (DPSC) along odontogenic and osteogenic lineages (via the MAPK pathway) (Lv et al., 2016; Nakashima, 1992). IGF-1 has also been shown to play a role in tertiary dentine formation; a mouse study by Matsumura et al (Matsumura et al., 2017) showed that tertiary dentine volume was reduced following odontoblast-specific IGF-I ablation. IGF-I signalling is also implicated in cell stemness, including cancer cell stemness, and resistance to chemotherapy, which has raised concerns over its use (Teng et al., 2018).

Limited *in vivo* work has been completed to explore the use of IGF-1 as a bioactive molecule in pulp capping. In a rat study by Lovschall, Fejerskov and Flyvbjerg (Lovschall et al., 2001), the efficacy of IGF-1 as a potential pulp capping bioactive molecule was proven; recombinant IGF-1 (400 ng) was loaded onto methylcellulose gels and compared against methylcellulose gels loaded with saline, and $Ca(OH)_2$. In all of the teeth examined histologically, dentine bridges covered more than 50% of the pulp, however significantly more tertiary dentine was identified in the IGF-1 loaded group.

When looking at IGF-2 for pulp capping in a rat model, Hu et al (Hu et al., 1998) found fibrous tissue repair only for IGF-2, and found that by comparison TGF-B1 proved to be more effective for pulp capping.

With little experimental animal data, IGF-1 and IGF-2 need considerably more evidence regarding its efficacy before it is widely considered as a potential bioactive molecule for pulp capping. As very little recent work has been done on IGF-1 for pulp capping, it would seem that this bioactive molecule has largely been side-lined in favour of other molecules, such as TGF-β1. Concerns over carcinogenesis may be limiting the exploration of this growth factor for use as a pulp cap.

Fibroblastic Growth Factor 2

There is sparse literature surrounding the use of Fibroblastic Growth Factor 2 (FGF-2) for pulp capping to produce a dentine bridge, despite it being implicated as a potential bioactive molecule in DPSC differentiation (Nakashima, 1992). FGF-2 is known to be a promoter of stem cell homing, stemness. proliferation and angiogenesis (Lim et al., 2017; Smith et al., 2016).

Very few *in vivo* studies demonstrating pulp capping with FGF-2 exist, despite the fact that it has been long established that FGF-2 can elicit tertiary dentinogenesis (Hu et al., 1998) (though admittedly to a lesser extent than TGF-β1 in this study). One *in vivo* study exploring this further is by Kikuchi *et al*.,(Kikuchi et al., 2007) where collagen sponges mixed with FGF-2 loaded gelatin hydrogels were implanted into the exposed upper molars of rats. This provided a controlled release of FGF-2, and was compared against pulps capped with free FGF-2 and the collagen sponge/gelatin hydrogel mixture alone. The free FGF-2 group demonstrated tertiary dentine formation in the residual pulp only, whereas with controlled release of FGF-2, the tertiary dentine formation was over the pulpal exposure. This demonstrates that the control release of FGF-2 is pertinent to the formation of dentine in a pulp cap. In later work by the same group, different concentrations of FGF-2 were explored as PCAs loaded in their collagen sponge/gelatin hydrogel carrier and implanted into exposed rat pulps: DPSCs and vessels invaded the scaffold and dentine was produced. A concentration of 0.5mg/ml of FGF-2 was found to produce the highest volume of tertiary dentine (Ishimatsu et al., 2009).

FGF-2 has been (like many of the bioactive molecules sequestered in dentine) more widely explored for bone regenerative purposes and proved to be successful in animal models (e.g. Behr et al., 2012; Hong et al., 2010; Kigami et al., 2013), and it is likely only a matter of time before exploration of FGF-2 picks up speed in the field of pulp capping and the limited current evidence is very encouraging. It does seem that controlled release of this molecule is key to its efficacy in sealing the pulp, so careful consideration of the scaffold onto which it is loaded is required and further work on identifying the ideal scaffold for this molecule's release into the pulp.

Epidermal Growth Factor

Epidermal Growth Factor (EGF) was first isolated from mouse salivary glands and is part of an EGF family of ligands, and when bound to their high-affinity receptors, these ligands have powerful mitogenic activities. After binding, EGF is known to activate two major intracellular pathways to invoke cell proliferation and cytoprotection (Berlanga-Acosta et al., 2009), and EGF is implicated in DPSC migration (Howard et al., 2010). Although important for repair and regeneration of tissues, the actions of EGF are also implicated in tumour progression (Xu et al., 2017).

Very little work has been completed on exploring EGF in dental pulp capping – possibly (similar to IGF-1) due to its known links to tumour progression. Most of the research on EGF for pulp capping is over twenty years old. As an example, Hu *et al*., explored Platelet Derived Growth Factor (PDGF) and EGF (5 ng/µl and 2 ng/µl respectively) soaked onto a collagen membrane and inserted into exposed rat incisor. They found a combination of PDGF-BB and EGF to out-perform $Ca(OH)_2$ and the unloaded collagen membrane (Hu et al., 1997). Hu *et al*., went on to explore EGF, again on collagen membranes in a rat model, in comparison to other bioactive molecules (TGF-β1, PDGF-BB, IGF II, basic fibroblastic growth factor (bFGF)) and compared it to the unloaded collagen carrier and $Ca(OH)_2$. TGF-β1 proved to be the best at producing a dentine bridge with a tubular quality and limited pulpal inflammation. However, the authors noted that the other molecules may have therapeutic utility when used in unison, though this required further exploration (Hu et al., 1998).

The fact that other bioactive molecules have been shown to out-perform EGF limits the exploration of this molecule as a sole agent in pulp capping. However, its utility as a powerful mitogen, ability to increase cell proliferation and role in cytoprotection may prove incredibly valuable in the typically inflamed environment where pulp caps are used. For these reasons it may be beneficial to consider EGF as beneficial adjunct in PCAs, to be used with more established molecules such as TGF-β1.

Angiogenic

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF), has been explored considerably in pulp regeneration (Al-Hassiny et al., 2019), likely due to its angiogenic properties in potential revascularisation of the pulp, though little work has been done covering this bioactive molecule for pulp capping.

VEGF is well known for its angiogenic properties, and this has been demonstrated to also be the case for dental pulp tissue (Al-Hassiny et al., 2019; Zhang et al., 2011), but for successful dental pulp capping, a hard dentine bridge is required. VEGF, when bound to its receptor has several functions; it stimulates endothelial cell proliferation, increases blood flow, facilitates chemotaxis and increases capillary hyperpermeability (Al-Hassiny et al., 2019; Matsushita et al., 2000).

VEGF has a comparatively short half-life (Zhang et al., 2014) and is expressed throughout the DPC in both mature and immature teeth, and has been shown to have an effect on mineralisation and odontoblastic differentiation in the early stages of DPSC differentiation (Aksel and Huang, 2017). It is capable of causing odontoblastic differentiation of DPSCs via gene transfection *in vitro* (Zhang et al., 2014) demonstrating its potential use as a PCA. The short half-life means, that without gene transfection, VEGF recombinant protein will require a carrier for sustained release. A chitosan/β-glycerophosphate scaffold carrying 100 ng/ml of VEGF protein has been used successfully *in vitro* to achieve odontoblastic differentiation of DPSCs, and the use of a carrier facilitated controlled release with better mineralisation than VEGF alone (Wu et al., 2019).

Much of the work on VEGF for pulp capping has only been accomplished in the last five years, possibly due to dental researchers focusing solely on its angiogenic role (as implied by its name) and failing to consider the other roles this bioactive molecule may have and subsequent therapeutic gains to be developed by exploring it further. To date, no *in vivo* studies on animals or humans have been carried out to explore VEGF as a PCA. The short half-life means that controlled and sustained release may be necessary to have any significant effect. Its role in odontoblastic differentiation warrants further exploration. .

Hepatocyte Growth Factor

It has long been established that pulpitis increases Hepatocyte Growth Factor (HGF) expression in the dental pulp (Ohnishi et al., 2000; Ohnishi and Daikuhara, 2003), and that HGF is a potent mitogen and morphogen, and has angiogenic and cellular motility effects (Ye et al., 2006).

In vitro work has also demonstrated that HGF can increase DPSC proliferation and odontoblastic differentiation (Ye et al., 2006). As HGF is also released from dentine matrix, it is thought to contribute to cellular signalling events in the repair of the DPC (Tomson et al., 2013). FGF-2 is thought to stimulate the secretion of both HGF and VEGF, leading to an increase in angiogenesis (Gorin et al., 2016) in the dental pulp. As HGF has demonstrated multiple roles *in vitro* in the healing of the DPC, it could be a powerful additive to a biomimetic PCA. To date HGF has not been explored as a PCA *in vivo* – it will be interesting to see how our knowledge of this peptide and its role in tertiary dentinogenesis increases with time, though the initial work completed on this molecule is promising.

Platelet Derived Growth Factor

PDGF is known to stimulate pulp cells and push their differentiation into odontoblasts (Nakashima, 1992; Zhang et al., 2017b). PDGF is present in dentine matrix (Roberts-Clark and Smith, 2000), and consists of a family of 5 polypeptides (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD), with PDGF-BB the most widely explored as it interacts with all three PDGF receptors (Zhang et al., 2017b). PDGF is known more generally to be a powerful mitogen, with potent angiogenic effects, and is a key mediator in wound healing and tissue regeneration (Zhang et al., 2017b). It is also known to be a powerful chemoattractive agent for mesenchymal stem cells and DPSCs (Zhang et al., 2017b). Positive synergistic effects have been noted *in vitro* for PDGF with TGF-β1 and dentine non-collagneous proteins, leading to increased cell viability and proliferation when compared to each of these factors alone (Tabatabaei and Torshabi, 2016).

PDGF-BB has been shown to be capable of stimulating tertiary dentinogenesis (Hu et al., 1998), though in the study by Hu *et al*., its effects were not as great as TGF-β1, possibly limiting the exploration of this molecule more widely in the literature. Similar to HGF, the multiple roles this molecule likely plays in the healing of the DPC necessitates a better understanding of its potential therapeutic role in dentistry. More *in vivo* work is needed to establish the role of PDGF in dentinogenesis for pulp capping, though presently it would seem that this peptide would most likely play a synergistic role in a biomimetic PCA, likely coupled with another molecule.

Placental Growth Factor

Placental Growth Factor (PlGF), present in dental matrix (Roberts-Clark and Smith, 2000), is thought to play a role in osteoblastic differentiation (McCoy et al., 2013) and angiogenesis (Kinnaird et al., 2004). As of yet, PlGF has not been explored fully to elucidate its role in tertiary dentinogenesis, and no *in vivo* work has been accomplished. Because of this, it is currently very difficult to see what role PlGF may have in a biomimetic PCA, and is included in the review only as a matter of completeness.

Inflammatory Cytokine

Interleukins

Inflammatory cytokines have been shown to be present in dentine components sequestered in pulp capping and caries. Evidence also indicates towards a reparative role of low grade inflammatory processes (such as those induced by dental injury), contributing to DPC repair (Tomson et al., 2017). Interleukins IL-1α, IL-1β, IL-4, IL-6, IL-8 and IL-12 are sequestered in dentine, with IL-8 having the most abundant expression (Cooper et al., 2010).

Interleukins are secreted proteins, capable of binding to specific receptors, and are known to play a role in the communication among leukocytes (Akdis et al., 2011). Although interleukins are traditionally considered inflammatory molecules, they are known to be present in dentine, and their associated inflammatory response is widely considered an integral part of the regenerative process of dental pulp capping (Cooper et al., 2010; Smith et al., 2012). However, careful modulation and resolution of the inflammatory process is key to facilitating regeneration and covered in a comprehensive review by Smith *et al*., (Smith et al., 2012). The balance between mild inflammation facilitating the eradication of carious bacteria and their toxins, and regeneration is delicate and complex. If the inflammatory response is too great, the tooth will lose vascularity and become necrotic; if it is too little, deleterious stimuli will remain, leading to increased tissue damage. It is perhaps because of this refined interplay between inflammation and regeneration that interleukins have not been widely explored in the literature as a potential bioactive molecule to add to a PCA. As exogenous interleukins may add to and affect the balance of inflammation/regeneration, it would be

better to avoid their usage as additives to PCAs until we can better understand therapeutic levels of these proteins.

Colony Stimulating Factors (GMCSF and GCSF)

Granulocyte Macrophage Colony Stimulating Factor (GMCSF) and Granulocyte Colony Stimulating Factor (GCSF) are members of the 'Colony Stimulating Factor' group of glycoproteins, and are known to be present in the inflamed pulp (Tomson et al., 2017). The Colony Stimulating Factor group of glycoproteins stimulate the senescence, proliferation, and differentiation of haematopoietic cells (Nakashima and Iohara, 2017; Trapnell and Abe, 2006).

The secretion of GMCSF (and osteopontin) by immunocompetent cells at the dentine-pulp junction, locally induces maturation of dendritic cells, thus encouraging increased activity of odontoblasts and their differentiation from pulpal resident progenitors, causing speculation over the potential role of GMCSF in odontoblastic differentiation (Saito et al., 2011). GCSF has been shown to be effective at mobilising DPSCs (Nakashima and Iohara, 2017), which is essential for the replacement of apoptosed odontoblasts at the dentine-pulp junction, necessary for tertiary dentinogenesis.

GCSF with autologous isolated DPSCs, on a collagen scaffold, has been shown to be capable of regenerating a DPC in a dog model. For this study, numerous control groups were used, though unfortunately none with GCSF alone, making it difficult to attribute the regeneration to solely GCSF (Iohara et al., 2013). . GCSF has also been shown to be beneficial in inducing mineralisation for dentinogenesis *in vitro* (Takeuchi et al., 2015).

Little work has been done to explore the roles that GMCSF and GCSF may play in *in vivo* pulp capping, and the potential that harnessing these bioactive molecules may have in aiding DPSC recruitment (possibly even to a suitable acellular scaffold that is capable of being colonised), and differentiation for reparative dentinogenesis. Although both molecules are currently used therapeutically in haematological malignancies, more *in vitro* and animal work is required before they can be considered suitable for human clinical trials in pulp capping and until we have a better understanding of the potential role of these molecules in biomineralisation, it is unlikely that they would be used as sole agents in any PCA that is designed in the near future

Immunoglobulins

Immunoglobins, also known as antibodies, are Y-shaped glycoproteins predominantly produced by plasma cells. Immunoglobulins IgA, IgG and IgM and believed to be sequestered in dentine (Schmalz et al., 2017). It is likely that they would play a role in the defence against cariogenic bacteria (Smith et al., 2012). The role of immunoglobulins in production of a hard tissue dentine bridge is entirely speculative, with no evidence of their inclusion in experimental PCAs in the literature (Smith et al., 2012), making the case for inclusion of them in an experimental PCA difficult. Nevertheless, their role in combatting any residual caries would prove beneficial in a PCA, if an alternative more broad-spectrum anti-microbial was not included, and they are included in this review only for completeness.

Chemotactic

Stem Cell Factor

Stem Cell factor (SCF) is a chemokine and homing agent for progenitor cell recruitment and can cause a significant increase in DPSC proliferation (Pan et al., 2013), and has a potential role in the differentiation of DPSCs to become odontoblasts (Ruangsawasdi et al., 2017). SCF is known to be liberated from dentine during pulp capping (Tomson et al., 2017). No *in vivo* pulp capping experiments have been identified in the literature and most of our current knowledge on SCF has emerged in the

last five years. Exploration and potential exploitation of this bioactive molecule for DPC regeneration is still very much in its infancy and without any evidence supporting its direct role in hard tissue barrier formation, it is likely that SCF would only have a supportive role in DPC regeneration from a PCA. Due to the lack of information surrounding this molecule for dental pulp capping, it is included only as a matter of completeness.

Neurotrophic Proteins

Neuropeptides have been identified in dental extra-cellular matrix, and are thought to play various roles in pain transduction (Smith et al., 2012). It is widely accepted that neuropeptides are associated with pulp regeneration, neural differentiation and angiogenic events (Li and Wang, 2016; Smith et al., 2016; Zhang et al., 2017a), but specifically tertiary dentinogenesis and pulp capping are less widely explored. Nerve Growth Factor (NGF), Glial Derived Neurotrophic Factor (GDNF), neurotrophin 3, neurotrophin 4/5, and brain-derived neurotrophic factor (BDNF) are all known to be sequestered in dentine, with GDNF and neurotrophin 4/5 expressed at the highest levels (Austah et al., 2019; Tomson et al., 2017). The role these molecules may play in pulp capping is poorly understood and an area of recent exploration (Austah et al., 2019). No *in vivo* work has been completed exploring the role of neurotrophic proteins as part of a pulp capping system, though GDNF, BDNF and neurotrophin 4/5 are thought to affect DPSC migration (Xiao et al., 2018; Xiao et al., 2020). Nerve growth factor (NGF) has been shown to play a potential role in differentiation of odontoblasts (Arany et al., 2009; da Rosa et al., 2018a), thereby facilitating dentinogenesis, though no *in vivo* work has been done to confirm this.

Currently, the evidence supporting the use of neurotrophic proteins in an experimental PCA is too weak for them to be considered a viable option. It is likely these molecules play a role in the inflammatory pulpal response based upon the literature but there is no evidence that they directly assist with hard tissue barrier formation in the pulp. With further work exploring their actions *in vitro*, and eventually *in vivo*, they may prove useful as an additive to assist in odontoblastic differentiation, but it is difficult to see how they could play a role as a sole agent in a PCA.

Small Integrin-Binding Ligand, N-linked Glycoproteins

Small Integrin-Binding Ligand, N-linked Glycoproteins (SIBLINGs) are, after collagen, the most abundant peptides in dentine. They are capable of binding to integrin receptors, facilitating cell attachment and signalling. The SIBLING family includes osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE) (Bleicher et al., 2015). Members of the SIBLING family are often used as markers for odontoblast differentiation, though it is important to note that their expression can overlap with osteoblasts and be associated with bone (Smith et al., 2012).

DMP-1 is known to be capable of inducing odontoblastic differentiation of DPSCs and stimulating mineralised tissue deposition (Hao et al., 2002; He et al., 2003; Narayanan et al., 2001). DMP-1 is also thought to play a role in activating pulpal fibroblasts as part of the inflammatory-regenerative process following pulpal damage (Abd-Elmeguid et al., 2012). Work has been achieved in exploring its use for endodontic perforation repair (Alsanea et al., 2011), which found a collagen scaffold loaded with DPSCs and DMP-1 to be capable of repairing a perforation with newly deposited dentine. DMP-1 has been hypothesised to be cleaved by matrix metaaloproteinase 2 (MMP-2) into two forms, with the C-polypeptide form having an effect on the differentiation of DPSCs to odontoblasts. The same researchers went on to explore the use of this cleaved form of DMP-1 for pulp capping when loaded onto agarose beads in a rat model and found that the cleaved DMP-1 produced a dentine bridge more quickly than unloaded agarose beads and of a higher quality(Chaussain et al., 2009).

DSPP is immediately cleaved after production, giving rise to Dentine Sialoprotein (DSP) and dentine phosphoprotein (DPP) (Bleicher et al., 2015; Smith et al., 2012). DSP is thought to play a role in DPSC differentiation and hard tissue formation (Li et al., 2017). It is also thought to be involved with migration and activation of immune cells (da Rosa et al., 2018a). DPP is also involved in mineralisation and plays a role in the initial formation of hydroxyapatite crystals (da Rosa et al., 2018a). Very few *in vivo* studies focused on exploring the effects of DSPP, DSP or DPP as PCAs are present in the literature. One of these few studies explored a DSP synthetic peptide as a PCA in a dog model (Kim et al., 2009), and compared this to MTA and $Ca(OH)_2$. The study found more inflammation and less hard tissue deposition in the synthetic DSP group compared to both the MTA and $Ca(OH)_2$ group, which both had similar results. In a study exploring DPP cross-linked into the fibrils of a collagen scaffold (0.5 µg of DPP to 29.5µg type I atelocollagen fibrils), better quality reparative dentine was found in the experimental group than the control groups of collagen alone and $Ca(OH)_2$ in a rat model (for example, a lack of tunnel defects and more complete coverage of the pulp) (Koike et al., 2014). Koike *et al.,* also found the rate of dentine deposition to be quicker in the experimental group than the control groups (Koike et al., 2014). Because the DSP synthetic peptide performed so badly compared to other PCAs, it is likely not a key molecule to pursue in the design of a biomimetic PCA. However, the DPP results are encouraging and it may be that out of the two molecules cleaved from DSPP, DPP is the more pertinent for tertiary dentine formation; further work needs to be accomplished exploring DSPP and its products to better understand the roles they play in dentine bridge formation and DPC repair.

BSP is expressed in tertiary dentine and, similar to DPP, is involved in the initial formation of hydroxyapatite, though following initial production it can also act as an inhibitor (da Rosa et al., 2018a; Smith et al., 2012), therefore it may be better considered a regulator of hydroxyapatite formation. In a study by Six *et al*., (Six et al., 2002a) the effects of BSP and BMP-7 (both within a gelatin carrier) for pulp capping were compared – as previously discussed. BMP-7 predominantly elicited osteodentine deposition in the coronal and radicular areas of the pulp leading to near total pulpal obliteration, whereas BSP produced more atubular dentine, filling only a third of the crown. Like many of the SIBLINGS, the role of BSP in dentinogenesis has been explored to some degree, but very few studies have been completed assessing its potential role as a PCA. The work by Six *et al*., (Six et al., 2002a) raises the possibility that BSP may be a better bioactive molecule for inclusion in a PCA than BMP-7, partially due to its more controlled mineralisation effects and also due to a better, more impervious, hard tissue barrier. BSP may indeed be a key component for any future PCAs, and as has been shown, can outperform the more commonly considered BMP group of molecules and warrants further investigation.

OPN is another molecule poorly researched for pulp capping, though this may be in part due to its perceived action of inhibiting hydroxyapatite crystal growth (da Rosa et al., 2018a). However, OPN has also been shown to play an essential role in the collagen formation of tertiary dentinogenesis from new odontoblast-like cells (Saito et al., 2016). Although inhibition of hydroxyapatite propagation may seem like the opposite effect to what is required for a PCA, careful and controlled moderation of dentine is essential for a successful clinical outcome – some PCAs (such as MTA (Agamy et al., 2004)) can cause pulpal obliteration from excessive dentine deposition, demonstrating a lack of hydroxyapatite inhibition. Further *in vitro* work is required to better understand the role OPN may play in tertiary dentinogenesis prior to consideration of this bioactive molecule in a PCA, though it could be argued that the inhibitory role this molecule plays could be pivotal in controlling the dentine deposited.

The hypothesised functions of MEPE within tertiary dentinogenesis are primarily those of mineralisation regulation and inhibition, and phosphate metabolism (Bleicher et al., 2015; da Rosa et al., 2018a). In further work by Six *et al*., (Six et al., 2007), they rebuke the idea that MEPE inhibits mineralisation by exploring the actions of Dentonin, a synthetic derivative of MEPE, as a PCA when

loaded onto agarose beads. Dentonin was found to have a rapid action on the initial stages of pulpal repair (cell recruitment and cell proliferation). No evidence for mineralisation inhibition was detected in their rat model. As the exact role of MEPE in tertiary dentinogenesis is poorly understood, further work is needed to better understand the potential of this bioactive molecule as a PCA, though it is possible that is plays a regulatory role similar to the other SIBLING molecules.

Small leucine-rich proteoglycans (SLRPS)

SLRPs are a family of numerous proteoglycans, which can be subdivided into five classes. They may be extracellular, pericellular (basement membrane zone), cell surface or intracellular, they may also be divided into canonical and non-canonical (Listik et al., 2019). Five SLRPs have been identified in dentine matrix and predentine; Decorin, Biglycan, Fibromodulin, Lumican and Osteoadherin (Orsini et al., 2009).

Decorin and Biglycan have several hypothesised roles in dentinogenesis; i) collagen stabilisation, ii) collagen fibrillogenesis, iii) calcium binding, iv) hydroxyapatite interaction, v) hydroxyapatite growth inhibition (Orsini et al., 2009). Specifically, Decorin and Biglycan are believed to play a role in the organisation of the collagen matrix during dentinogenesis, are thought to regulate other molecules such as TGF-β1, influence cell cycle progression and calcium binding (Baker et al., 2009; Embery et al., 2001). Fibromodulin, Osteoadherin and Lumican are believed to play roles in mineralisation of dental tissues (Orsini et al., 2009). Fibromodulin may also play a role in the fibrillogenesis of collagen in predentine (Goldberg et al., 2006). Fragmented SLRPs are also thought to potentially influence signalling to odontoblasts in tertiary dentine deposition (Stankoska et al., 2016).

To date, no experimental PCAs have explored the use of the SLRPs in *in vivo* models. Because their breakdown may help to signal to odontoblasts to begin dentine deposition, fragmented versions of these proteoglycans may be beneficial for consideration of inclusion in a PCA, but as previous studies have shown that dentine deposition can be elicited without the inclusion of these molecules, they are not a priority for consideration. Further work on the roles of these molecules in dentinogenesis is required, including further clarification of their roles in carious and regenerative settings, before they can be considered as reasonable candidates for inclusion in a PCA.

Other

Adrenomedullin

Adrenomedullin (ADM) is a cleaved and processed protein from preproadrenomedullin, and is highly conserved across species and is part of the Calcitonin Gene-related Peptide (CGRP) family (Musson et al., 2010).

ADM is thought to upregulate the expression of BMP-2, enhancing odontogenic differentiation of DPSCs (Zhu et al., 2017). This fits with our current understanding of the localisation of ADM to epithelial cells during initial dental development and later to mineralised secretory cells (including odontoblasts) (Musson et al., 2010). ADM has also been shown to be expressed at higher levels in carious over healthy extracted human teeth, and inhibit apoptosis and enhance proliferation of DPSCs *in vitro* (Zhu et al., 2016). ADM is thought to be involved in tertiary dentinogenesis, via p38, eliciting a process of odontoblastic differentiation and mimicking primary dentinogenesis (Simon et al., 2010), though only *in vitro* work currently supports this hypothesis.

ADM has only a small body of work exploring the role of this protein in tertiary dentinogenesis. No human or animal studies were found in the literature exploring the use of this bioactive molecule as a PCA. Its effect of inhibiting apoptosis of cells *in vitro*, could raise concerns over the use of this peptide

due to the link of immortal cells to tumour cell theorems. Indeed, the molecule was first identified in a pheochromocytoma (a tumour of adrenal glands) (Kitamura *et al.*,1993). Considering the current body of work covering ADM, it is not presently a candidate for use in a PCA, though further work eliciting the actions of this molecule in tertiary dentinogenesis may yet yield results to the contrary.

Calcitonin

Calcitonin is a hypocalcaemic hormone. Calcitonin has been explored as a pulp capping agent as far back as 1982; Smith and Soni used a rat model for comparing pulp capping of $Ca(OH)_2$ and calcitonin, and they discovered that calcitonin produced a similar response in terms of dentine deposition to $Ca(OH)_2$ (Smith and Soni, 1982). Similarly, in a dog model, Cullum and Kline found that although calcitonin was able to induce pulp capping dentinogenesis, it did not outperform $Ca(OH)_2$, though less inflammation was identified in the calcitonin group (Cullum and Kline, 1985). More recently, calcitonin has also been shown to stimulate osteodentine deposition in ferrets (Kline and Yu, 2009), adding to the growing literature supporting this bioactive molecule as a potential candidate for inclusion in a biomimetic PCA. Since the early work on calcitonin, little progress has been made in exploring the best way of utilising this peptide in PCAs.

Lipids

No work has been done to date using lipids sequestered in dentine for pulp capping and these are included solely for completeness.

Lipids make up a tiny component of dentine matrix (0.26-0.36%)(Goldberg et al., 2008a). Lipids found in dentine include; phosphatidyl inositol, sphingomyelin, phospatidylcholine, phospatidylethanolamine, phosphatidyl serine, phosphatidic acid and cardiolipin (Goldberg et al., 2011). Of these, sphingomyelin is the most widely explored lipid found in dentine and experimental mouse work has demonstrated that cleaving via neutral sphingomyelinases produce ceramide and phosphocholine, and this process is considered important for normal dentine mineralisation (Aubin et al., 2005; Goldberg et al., 2008a). No work has been done to date exploring whether lipids are released from dentine on application of existing PCAs (such as MTA), nor has any work been done exploring whether these lipids are present in reparative dentine or indeed what their role may be in reparative dentine formation. For these reasons, it is difficult to see what role, if any, these molecules may play in being added to a PCA, and as such they are not a priority for consideration for inclusion in a PCA, though further work on their roles in dentine may be of benefit to the wider dental community.

Enamel Proteins

Enamel matrix has long been known to support hard tissue formation in dental structures and have been used for dental pulp capping in animal models (Nakamura et al., 2002), however without knowing the exact composition of the material it is difficult to know or hypothesise the roles individual molecules have on tertiary dentine deposition.

Amelogenin and ameloblastin are well known enamel proteins, however they are also present in dentine and believed to play a role in odontoblast differentiaiton (Goldberg et al., 2011), which fits with the potential role these moelcules may play in hard tissue formation during pulp capping.

Although amelogenin is not present in healthy, mature adult dentine, predentine or pulp, it has been found to be present in injured and carious dentine at sites of injury and in newly differentiated odontoblasts and is known to be present during primary dentinogenesis (Mitsiadis et al., 2014). Leading on from this work, researchers have explored the potential role that amelogenin may have in

dentine hard tissue formation for therapeutic effects, for example, it has been explored successfully for apical closure of root canals in dogs (Mounir et al., 2018). Amelogenin exists as several different isotypes, and each may have differing and varying effects on dentinogenesis. Frasheri et al., 2016 have found that no mineralized tissue is produced when DPSCs are exposed to full length amelogenin, and they suggest that amelogenin may not act as an inducer for dentine production but as an enhancer.

Ameloblastin is thought act as a signalling molecule in primary dentinogenesis and dental-pulp complex regeneration (Spahr et al., 2002) and one study has explored recombinant ameloblastin (without a carrier) as a PCA in rats and out-performed $Ca(OH)_2$(Nakamura et al., 2006). The researchers did however find fibrosis in some of the teeth capped, which they feel could be due to 'overdosing' with ameloblastin leading to rapid regenerative activity or related to chronic inflammation caused by the recombinant protein production methods – either way this would need addressing and exploring further before ameloblastin can be considered as a definite candidate for a PCA.

We are beginning to understand the role that some of the enamel proteins may play in reparative dentine formation and as such their potential use for a PCA. The fact that ameloblastin and amelogenin have both successfully produced hard tissue barriers in animal models places these molecules as true potential candidates for a PCA, though we need a better understanding of the potentially dose-dependent results for ameloblastin and to better understand how the different amelogenin isotypes affect dentine deposition.

Osteocalcin and Calcium Binding Proteins

Osteocalcin is expressed in differentiating odontoblasts (Goldberg et al., 2011) and is often used in the literature as a marker for this process.

Osteocalcin is deposited in dentine by odontoblasts, but it is found in bone and cementum too. Although inhibition of osteocalcin is known to inhibit bone production (Ducy et al., 1996), inhibition of osteocalcin (by warfarin) in mice produced no obvious difference in the structure of dentine (Gorter de Vries et al., 1991), and excess osteocalcin did not seem to produce any effects on dentine structure either (Bronckers et al., 1998). The purpose of osteocalcin in dentine is not fully elucidated. Some studies have shown that it plays a role in glucose metabolism, and may therefore be important in facilitating dentine matrix secretion in highly metabolically active odontoblasts (Ferron et al., 2008), though this needs further clarification.

Osteonectin (also known as Secreted Protein, Acidic and Rich in Cysteine/SPARC) is expressed in secretory odontoblasts. Osteonectin contains a calcium binding domain and as such is believed to potentially play a role indirectly in dentine formation, however further work is needed to confirm this (Goldberg et al., 2011).

Other calcium binding proteins include; calmodulin, calbindin, annexines and nucleobindin. Nucelobindin, calbindin and some of the annexines are believed to play a role in transporting calcium into the ECM during dentine deposition (Goldberg et al., 2011).

Insufficient work has been completed on osteocalcin and the calcium binding proteins found in dentine to fully understand what their role is in dentinogenesis, let alone specifically reparative dentinogenesis. The fact that inhibition of osteocalcin has little to no effect on dentine production suggests that either the role of osteocalcin is too minor to affect dentine production, or that other pathways are able to compensate for inhibition of this molecule. No work has been done on using

osteocalcin or the calcium binding proteins for pulp capping and as such this section has been included solely for completeness.

Enzymes

Matrix Metalloproteinases (MMPs) are calcium dependent, zinc containing enzymes that are sequestered in dentine and play an integral role in development and normal tissue turnover, but also pathological events too.

The main MMPs identified in dentine are; MMP-8 (a collagenase), MMP-2 and MMP-9 (gelatinases), stromelysin-1 (MMP-3 or proteoglycanase), MMP-14 (MT1-MMP, an MMP-2 activator), MMP-13, and enamelysin (MMP-20) (Chaussain et al., 2013).

It has been suggested that during carious invasion, these entrapped enzymes may be re-exposed or even activated, leading to increased demineralisation and matrix breakdown (Chaussain et al., 2013). Based upon this, MMPs may be an unusual candidate for inclusion in PCAs, however they may play a role in releasing and activating bioactive molecules from dentine and so have an overall therapeutic effect on dental-pulp complex regeneration (Chaussain et al., 2013), or indeed play a role in activating certain molecules (Chaussain et al., 2009). However, much experimental work has also been completed on the use of MMP inhibitors for slowing down the progression of caries (Gendron et al., 1999; Sulkala et al., 2001; Tjaderhane et al., 1999), highlighting the multi-faceted effects these molecules have in the carious tooth.

Based upon our current understanding of these molecules, it may be best to consider these molecules as complicit in carious progression as the literature proving their role in caries (through exploring MMP inhibitors) currently outweighs the evidence that they may be beneficial in dentine regeneration. As such, it is difficult to ascertain the role these enzymes would play in any PCA.

Discussion

Most *in vivo* work from the literature has explored the use of single proteins as bioactive agents within various carriers. Little *in vivo* work has been done exploring the use of multiple synergistic bioactive molecules for pulp capping. Indeed, the synergistic effect of the different peptides may yield greater tertiary dentinogenesis and better mimic the physiological conditions of tertiary dentinogenesis and from exploring the literature it is clear that the bioactive molecules sequestered in dentine do not work in solo but as a collaborative mixture with specific roles in cell migration and cycling and hard tissue regulation and deposition. Because of this, we should be considering using these molecules as a finely tuned and discrete cocktail of the most essential components for hard tissue formation, without causing pulpal obliteration and facilitating an impervious seal to the pulp.

When considering what would be an ideal mix of molecules to use in PCA, careful deliberation of the role of each molecule needs to be taken into account – for example, the inclusion of TGF-β1 may negate the need for inclusion of SIBLING molecules due to its role in release of DSPP. We would recommend the inclusion of molecules that encourage the migration, proliferation and differentiation of DPSCs in conjunction with a molecule that will inhibit the reparative dentinogenic process, thereby preventing pulpal obliteration. Careful consideration of the spatio-temporal release of these molecules needs to be considered in any PCA design to ensure the correct molecules are released at the correct concentration at the correct time for a suitable period. Preference should be given to molecules reliably capable of producing a dentine hard tissue phenotype over a bone phenotype to give a more impervious dentine bridge. Some molecules are capable of potentiating the effects of others (for example, the role of PDGF on TGF-β1 (Tabatabaei and Torshabi, 2016)), which may help to accelerate the production of a dentine bridge and may too be considered for inclusion. Until the safety

of some molecules is confirmed – particularly those associated with neoplastic properties – these are best avoided for inclusion in a PCA.

When the pulp gets exposed to toxins from caries (for example lactic acid), it becomes inflamed. There is generally a lack of studies exploring the use of bioactive molecules for pulp capping in inflamed, *in vivo*, pulp environments. As mentioned earlier in this review, some authors (such as Rutherford and Gu, 2000), have attempted to address this problem. For animal studies to be comparable to clinical work, the experimental setup needs to reflect clinical practice, including; carious teeth/inflamed or infected pulps, isolation of the tooth, suitable wound lavage, haemostasis and isolation control and a suitable restoration. Going forwards, it would be sensible to explore this further with any bioactive molecule considered for inclusion within a pulp cap, to ensure the efficacy of direct pulp capping within an inflamed pulp environment.

From the literature, the peptides with the most supporting evidence are TGF-β1, and from the BMP group; BMP-2 and BMP-7. More work is required for consideration of the other peptides found sequestered in dentine before they can be considered appropriate candidates for a PCA, with a focus on *in vivo* and clinical work. It may be necessary to compare different bioactive molecules, or even biomimetic PCAs, in the same animal models to compare their efficacy. The work by Hu *et al.,* (Hu et al., 1998) is a stand out study in this instance, where EGF, IGF, basic FGF, TGF-β1, PDGF, $Ca(OH)_2$, a collagen carrier control, and a procedure control were compared in the same rat models. Their work demonstrated that TGF-β1 had significantly better hard and soft tissue healing compared to the other groups.

It is likely that as work continues on the biology of dentine, more bioactive molecules sequestered in dentine will come to light. However, many questions on the release of bioactive molecules in dentine remain unanswered, for example are they released in standard cavity preparation?, how long is release sustained for?, are they chemically or physically bound in dentine (or both)?, and whether caries influences their release dynamics? Understanding this may help in the design of a biomimetic PCA. It is of course entirely possible that not all molecules sequestered in dentine have a role to play in dentine regeneration and may simply be entombed during the process of primary dentinogenesis, and an understanding of all of the molecules sequestered in dentine will help us to better determine which are actually pertinent to tertiary dentine production.

Although some peptides have been explored as PCAs without the use of a carrier, it would be prudent to consider a carrier as an integral part of the creation of a biomimetic PCA. Carriers can allow sustained and timely release of molecules and indeed some have been demonstrated to elicit tertiary dentinogenesis without the need of additional encapsulated factors (Njeh et al., 2016) and some carriers used in the literature are commercially successful PCAs in their own right. The carrier can provide some degree of mechanical and structural support, facilitate the migration of DPSCs, and affect odontogenic differentiation and mineral deposition (Wang et al., 2011). Some of the carriers explored for bioactive molecules have shown to be capable of eliciting tertiary dentine bridges unloaded (ie when they are used as a control without a bioactive molecule added) – for example Goldberg et al., 2001, using gelatin and Koike et al., 2014 using collagen. These studies raise certain questions and ideas – for example, the potential role of a carrier being an active part of the PCA rather than passively releasing and holding the bioactive components, and the possibility that the bioactive molecules sequestered in dentine and subsequently released in cavity preparation may be sufficient for dentine bridge formation without additional exogenous molecules.

A short term area of interest may be to explore the use of existing PCAs coupled with added bioactive molecules, as has been explored to some extent already in the literature, to see if we can overcome some of the limiting factors of commercially available PCAs. Another area of potential exploration

would be to consider utilising existing materials used for bone regeneration in a pulp capping environment as many of the molecules cross over to see what effect these may have and how these may be modified to facilitate dentine production over bone.

Comparing *in vivo* animal studies is difficult without a clear clinical model comparison. It is important for *in vivo* work to include a suitable control to compare pulp capping efficacy against. Without agreeing upon a clinically standardised control for use in clinical studies, a suitable control will remain contentious. With many different PCA available commercially, it may be advantageous to compare any experimental PCA against multiple different commercially available materials acting as 'controls' for what is currently achievable in the clinic, though experimentally this may be difficult. Indeed, numerous aspects of the management of a direct pulp cap are open to debate, such as the order of the procedures, the agent used for wound lavage, the agent used for haemostasis, the preferred restorative material, and excavation technique (Bjørndal et al., 2019; Chisini et al., 2015; Munir et al., 2020); without consensus upon these, agreeing upon a standardised control for animal work will be difficult.

It has been 13 years since the *in vivo* human work comparing TGF-β1 to $Ca(OH)_2$ in sound premolars (Kunarti, 2008). Since then, newer and more effective PCAs have become available and more widely used, which may draw into question whether this is still an avenue that should be explored. With more populations requiring a functional dentition for longer (due to an increased life expectancy), a success rate of 71% at 6 years (Çalışkan and Güneri, 2017) as for MTA for example, may not be considered a good enough outcome for a surgical procedure in an age of burgeoning regenerative medicine. Dentistry, and specifically regenerative endodontics, could lead the field of regenerative medicine providing more predictable, reproducible and successful treatment outcomes for patients. However, we are falling behind our medical colleagues who are pushing further with bone and cartilaginous regeneration. Harnessing the innate power of the dental pulp for regeneration may prove a useful template in broader fields of regenerative medicine, where creation of scaffolds designed around the body's own physiological repair mechanisms may be of benefit. However, considerable optimisation is going to be needed before such a material is ready for clinical trials and commercial marketing.

Conclusion

As there are numerous issues with currently available PCAs, the need for improved physical characteristics and better outcomes when using PCAs is paramount. The utilisation of bioactive molecules in a PCA has the potential to facilitate quicker, more efficient and more successful pulp capping. Tailoring the correct mix of bioactive molecules with the most appropriate carrier is an area that needs greater exploration *in vivo* and the area that we feel requires the most exploration; not only would this potentially provide a better outcome for pulp capping, but it may also elucidate the synergistic roles of many of the sequestered molecules in dentine in tertiary dentinogenesis. Work to date has focused on the formation of a dentine bridge, with less work on the regulation and inhibition of this process once successfully completed, which needs consideration to prevent pulpal obliteration, for example through inclusion of molecules such as SLRPs and SIBLINGS. Successful design of a PCA incorporating a suitable bioactive molecule with a suitable scaffold would allow the pulp's own native stem cells to migrate, differentiate and successfully create a tertiary dentine bridge and the best possible outcome clinically likely involves a mixture of certain molecules.

TGFB-1, BMP-7 and BMP-2 have the most evidential support from the literature with regards to potential pulp capping bioactive molecules, though there are numerous limitations to using the BMP family of molecules and other molecules may prove to be more suited. Considerable optimisation of

the various factors and carrier is required to create the most suitable biomimetic PCA, along with exploration of their use in inflamed pulpal tissue, and this is only achievable through high-quality animal and clinical trials, which to date are lacking in the literature.

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