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Review

Post-mitotic odontoblasts in health, disease, and regeneration

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ABSTRACT

Objective: Description of the odontoblast lifecycle, an overview of the known complex molecular interactions that occur when the health of the dental pulp is challenged and the current and future management strategies on vital and non-vital teeth.**Methods:** A literature search of the electronic databases included MEDLINE (1966–April 2019), CINAHL (1982–April 2019), EMBASE and EMBASE Classic (1947–April 2019), and hand searches of references retrieved were undertaken using the following MESH terms ‘odontoblast*’, ‘inflammation’, ‘dental pulp*’, ‘wound healing’ and ‘regenerative medicine’.**Results:** Odontoblasts have a sensory and mechano-transduction role so as to detect external stimuli that challenge the dental pulp. On detection, odontoblasts stimulate the innate immunity by activating defence mechanisms key in the healing and repair mechanisms of the tooth. A better understanding of the role of odontoblasts within the dental pulp complex will allow an opportunity for biological management to remove the cause of the insult to the dental pulp, modulate the inflammatory process, and promote the healing and repair capabilities of the tooth. Current strategies include use of conventional dental pulp medicaments while newer methods include bioactive molecules, epigenetic modifications and tissue engineering.**Conclusion:** Regenerative medicine methods are in their infancy and experimental stages at best. This review highlights the future direction of dental caries management and consequently research.

1. Introduction

The National Institute of Dental and Craniofacial Research defines that regenerative medicine harnesses the body's growth and healing properties to repair or replace damaged cells, tissues, or organs. Researchers are drawing on the fields of stem cell and developmental biology, bioengineering, material science, and gene editing, among others, to develop safe and effective regenerative therapies (NIDCR, 2018).

Currently in dentistry, the main cells that are targeted in regenerative dental medicine are the odontoblasts and dental pulp stem cells. The growth and healing properties of the vital dental pulp complex is assisted by the application of bioactive materials. The replacement of damaged dental pulp complex is the research related to tissue engineering. Regenerative endodontics is ‘biologically based procedures designed to replace damaged tooth structure, including dentine and root structure, as well as cells of the pulp-dentine complex’ (Murray, Garcia-Godoy, & Hargreaves, 2007).

Odontoblasts are post-mitotic cells that are maintained throughout

the life of a tooth until cell death occurs by either trauma, disease or apoptosis. Their primary role is the deposition of primary, secondary and tertiary dentine in teeth. However, they also play a critical role in physiological maintenance of the pulp, and in the event of injury, have the ability to trigger a defensive immune response assisting the tooth to heal and repair (tertiary dentine). This timely review provides an overview of current knowledge in the odontoblast life-cycle and their role in innate immunity that will help clinicians and researchers develop reproducible chairside methods so as to diagnose dental pulp disease accurately. Subsequently, to utilise this knowledge for the application of current regenerative medicine approaches to assist odontoblasts in healing and repair after removal of the causative factor of the disease. The trends and potential methods using bioactive molecules, epigenetic modifications and tissue engineering will be reviewed.

2. Methods

A literature search was conducted using the following electronic databases MEDLINE via OVID (1966–April 2019), CINAHL (1982–April

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2019), EMBASE and EMBASE CLASSIC (1947-APRIL 2019) and hand searches of references retrieved. The search strategy used was replicated for all electronic databases, was first conducted and up to and including April 2019. The search strategy used the following MESH keywords; 'odontoblast*', 'inflammation', 'dental pulp*', 'wound healing', 'regenerative medicine', 'odontoblast*[AND]inflammation [AND]dental pulp*', 'odontoblast*[AND]dental pulp* [AND]wound healing', 'odontoblast*[AND]inflammation[AND]dental pulp*[AND]regenerative medicine', 'odontoblast*[AND]dental pulp* [AND]wound healing[AND]regenerative medicine', 'odontoblast*[AND]inflammation[AND]dental pulp*[review]' and 'odontoblast*[AND]dental pulp* [AND]wound healing[AND]review'. After removal of duplicates and unrelated research, 367 publications were reviewed. A narrative literature review of the analysis is provided.

2.1. The lifecycle of odontoblast cells

Odontoblasts originate from ectomesenchyme-derived dental papilla cells from the cranial neural crest. These cells are stimulated to differentiate into pre-odontoblasts by the inner enamel epithelium cells through a series of complex signalling cascades and pathways (Nanci, 2013). The original lifecycle staging was introduced by Couve (Couve, 1986) where four functional phenotypes were described: pre-odontoblast, secretory, transitional and aged. However, this was recently updated to pre-odontoblast, secretory, mature and old odontoblast phenotypes (Couve, Osorio, & Schmachtenberg, 2013) (Fig. 1).

In the pre-odontoblast stage cells are short, cylindrical, and 15 µm in height (Couve, 1986). The cells lack polarity at this stage. The centrioles are located near the poorly defined Golgi apparatus, forming the primary cilium. Rough endoplasmic reticulum (RER) is observed throughout the cell as the intracellular mechanism for the production of protein is developing. The cell continues to elongate as it transitions from the pre-odontoblast stage into the secretory stage.

Secretory stage odontoblasts (Fig. 1A) form the classical single layer of columnar cells 50 µm in height which are highly polarised and connected to each other via gap junctions and junctional complex (Couve, 1986). The gap junctions connect the odontoblast to each other and dental pulp fibroblasts at the subodontoblast region at the base of odontoblasts. These gap junctions facilitate movement of ions and molecules, and allow 'communication' between cells while the gap

complexes are found at the apical pole between the odontoblast cell body and odontoblast processes that progress into the pre-dentine area where active transportation of secretory molecules are discharged during secretory stage (Sasaki & Garant, 1996). The RER is observed in the terminal end of the cell, close to the nucleus, where proteins are synthesised and sent to the Golgi apparatus for packaging and distribution. Mitochondria are scattered throughout the cell to assist by supplying energy for the transportation of secretory molecules within and towards the apical pole of the odontoblastic process. During the secretory stage, the odontoblasts have been reported to actively produce primary dentine at the rate of 4 µm per day (Farges et al., 2015) or 4–8 µm per day (Bleicher, 2014; Couve, 1986; Simon et al., 2009) for 2–3 years continuously. During primary dentinogenesis (before tooth eruption), high expression of phosphoproteins and in particular dentine matrix acidic phosphoprotein (DMP)-1 has been observed (Balic & Mina, 2011). The pre-dentine is rich in extracellular matrix (ECM) such as Type 1 collagen (90%), non-collagenous proteins-glycoproteins, proteoglycans and dentine phosphoproteins DMP-1 and dentin sialophosphoprotein (DSPP) (Kawasaki & Weiss, 2008). DMP-1 and DSPP are the biomarkers for active dentine production (Goldberg, Kulkarni, Young, & Boskey, 2011) indicating the odontoblast is in the secretory stage.

The odontoblast transforms from the secretory (primary dentinogenesis) to mature stage (Fig. 1B) (secondary dentinogenesis) when crown formation is complete and the tooth erupts. The changes are observed in cell phenotype and reduction in dentine secretion rate to 0.4–0.5 µm per day (Bleicher, 2014; Couve, 1986; Farges et al., 2015; Simon et al., 2009). Odontoblast cells become narrower and less polarised. Autophagy is involved in cellular homeostasis where pathogens, damaged organelles and proteins are recycled (Couve et al., 2013; Pei, Wang, Chen, & Zhang, 2016; Qian, Fang, & Wang, 2017; Zhang & Chen, 2018). This is an essential process affecting development, differentiation, immunity and aging in post mitotic cells that is lysosome-mediated. Due to the increased autophagic activity, a reduction in organelles (RER, Golgi apparatus and mitochondria) with increased autophagic vacuoles is observed (Couve, 1986). Similarly, there is a down-regulation of DMP-1 production (Balic & Mina, 2011). This process continues as odontoblasts age.

During the old odontoblast stage (Fig. 1C), cells are shorter (45 µm in height), crowded and pseudostratified in appearance. The cytoplasm

Fig. 1. Adapted from Couve et al. (2013), which summarises the rate of deposition, cell height and shape at the various stages; Secretory [A], Mature [B] and Old [C]. Abbreviations: N = nucleus, SG = secretory granules, RER = rough endoplasmic reticulum, GC = Golgi complex, L = lysosomes, AV = autophagic vacuoles, M=mitochondria, LF = lipofuscin deposits, 1° = primary, 2° = secondary and 3° = tertiary.

has significant reduction of organelles, no secretory granules and has large lipid filled vacuoles (lipofuscin accumulation) due to decreased lysosomal activity. Lipofuscin deposits tend to accumulate due to decrease efficiency of the autophagic-lysosomal system in the old odontoblast (Couve, 1986) and can be used as an age-marker (Rubinsztein, Marino, & Kroemer, 2011).

Under pathological conditions, a mature odontoblast can upregulate dentine production by reverting to the secretory stage (Couve, 1986). Increased dentine deposition can occur in the intratubular, peritubular areas and tertiary dentine in an attempt to decrease dentine permeability and reduce insult to the dental pulp (Mjor, 2009). The tertiary dentine produced has two distinct types: reactionary and reparative dentine (Farges et al., 2015; Smith et al., 1995). The p38-mitogen-activated protein kinases (MAPKs) pathway, responsible for basic cellular functions such as proliferation, differentiation, motility, stress response, apoptosis and survival, may be involved in the transition from secondary to tertiary dentinogenesis activity through Transforming growth factor (TGF) super family release (Simon, Smith, Berdal, Lumley, & Cooper, 2010). TGF- β 1 and TGF- β 3 actively promote odontoblast differentiation and tertiary dentinogenesis (repair/healing conditions) (Simon et al., 2010). Hence, members of the TGF super family are markers for odontoblast differentiation (Simon et al., 2010; Smith, Smith, Shelton, & Cooper, 2012).

Reactionary dentine is produced by existing odontoblast cells exposed to mild external stimuli. The tubular dentine secreted has similar features as primary and secondary dentine. However, if the stimuli continue with increased severity, the bacterial by-products may cause irreversible damage to the odontoblast cells. The perivascular region is rich in dental pulp stem cells (DPSC) that play a role in postnatal homeostasis and repair (Shi & Gronthos, 2003). The activated complement system provides C5a fragments (consists of 74 amino acid) that help increase DPSC recruitment (Chmielewsky, Jeanneau, Laurent, Kirschfink, & About, 2013) while C3a fragments (consists of 77 amino acid) increase DPSC proliferation and guide their migration to the site of infection (Rufas, Jeanneau, Rombouts, Laurent, & About, 2016). DPSC undergo a complex cascade of cellular responses that involves differentiation into odontoblast-like cells secreting reparative dentine in an attempt to block off offending stimuli from reaching the dental pulp (Farges et al., 2015). There is a lack of organised structure in the reparative dentine, the tubular dentine feature is lost and a more amorphous calcified material or calcified bridge-like material is deposited to prevent bacterial invasion into the dental pulp space. The progression of caries is a complex interaction within the dental biofilm where the net balance of aciduric, acidogenic and neutralising bacteria affects the caries activity shift to demineralisation, stability or remineralisation (Marsh, 2016). The predominant microorganisms are *Streptococcus* spp., *Lactobacillus* spp., *Bifidobacterium* and *Actinomyces* genera (Chávez de Paz & Dahlén, 2017; Marsh, 2016; Takahashi & Nyvad, 2016). The acidic bacterial metabolites cause demineralisation of dentine that releases mediators and biomolecules trapped within dentine into the dental-pulp complex (Simon et al., 2009). Hence once reactivated, the odontoblast will trigger the immune response commensurate with the infection faced. In mild situations, when the infection is neutralised, the odontoblast returns to its mature state. Under more severe conditions, the odontoblasts may be in a state of flux between mature and secretory states. Currently there is no agreement (Simon et al., 2009) whether the calcio-traumatic line, a hypercalcified layer of dentine produced when a physio-pathological event occurs (Schour, Chandler, & Tweedy, 1937), is pathognomonic of change in odontoblast stage or function (Goldberg et al., 1995; Lesot et al., 1993).

2.2. Odontoblast role with innate immunity

The immune response has four stages: i) detection and identification of pathogen; ii) dissemination of information to other cells; iii) recruitment and organisation of a coordinated attack; iv) destruction or

neutralisation of the pathogen. The odontoblast is the first cell to sense bacterial invasion, hence, the first line of defence to bacterial acid-driven demineralisation of enamel and dentine (Farges et al., 2015). Biologically active molecules are released from the demineralised dentine, detected by the odontoblasts initiating pulp immune and inflammatory responses within the dentine-pulp complex (Farges et al., 2015). Bacteria and their by-products stimulate the odontoblast membrane activating the Toll-like receptor (TLR) family that initiates innate immunity and upregulates production of antimicrobial agents such as β -defensins (BD) and nitric oxide (NO), chemokines (CCL2, CXCL1, CXCL2, CXCL8 and CXCL10) and proinflammatory cytokines (IL-1 α , IL-1 β , TNF α , IL-6 and IL-8) by the odontoblast. The antimicrobials, BD and NO, are released via odontoblast processes into the dentinal tubules to the source of infection in an attempt to neutralise the invading bacteria, and lipopolysaccharide-binding proteins (LBP) released to attempt to reduce bacterial pathogenicity at the site of carious dentine (Farges et al., 2015). Simultaneously, odontoblasts release chemokines and proinflammatory cytokines into the pulp (sub-odontoblast area) triggering non-specific innate immunity. An increase in leucocytes, NK (natural killer) and dendritic cells (DC) from both local and circulatory system migrate to the site of infection. Neutrophils are recruited to engulf and destroy pathogens. Pathogens that aren't destroyed are neutralised or deactivated by neutrophils, continue the infective process. To assist with the influx of cells to the site of the injury, angiogenesis occurs in tandem with detection of the pathogen at the early stages of innate immunity (Farges et al., 2015).

Angiogenesis and neurogenesis are critical components in the healing and repair process in any part of the body, including the dental pulp complex. Demineralised dentine releases pro-angiogenic growth factors that contribute to increasing vascularity at the site of injury (Smith, Duncan, Diogenes, Simon, & Cooper, 2016). Blood vessels also release inflammatory mediators, such as histamine, neuropeptides, prostaglandins and NO, that increase the permeability and vascularity of the affected pulp allowing recruitment of immune cells (Farges et al., 2015). The dental pulp is richly innervated and when stimulated expresses potent neuropeptides such as calcitonin gene-related peptide, substance P and neuropeptide Y (Byers & Narhi, 1999) that stimulate sprouting of nerve endings at the site of injury and repair (Byers, Suzuki, & Maeda, 2003; Rajan et al., 2014). The interstitial space expands and becomes rich in cytokines, leukotrienes, proteases and complement factors. The complement system is believed to be first activated in the pulp fibroblast cells via classical, alternative and Lectin pathways to promote adaptive immunity primarily by anaphylatoxins C3a and C5a fragments (Ehrencruber, Geiser, & Deranleau, 1994; Hartmann et al., 1997; Nataf, Davoust, Ames, & Barnum, 1999). These fragments initiate vascular and cellular changes. As the inflammatory process progresses macrophages, DC, NK cells trigger the activation of adaptive immunity composed of T and B cells that are specifically targeted at the invading pathogen (Farges et al., 2015).

Persistence of the noxious stimuli results in the immune system becoming fully activated and causing significant damage to both host and pathogen. Low grade dental pulp inflammation has an important role in stimulation of repair. Autophagy is activated to protect cells from damage. Odontoblast differentiation is upregulated via suppression of NF(nuclear factor)-kappaB signalling pathway (Couve et al., 2013; Pei et al., 2016; Zhang & Chen, 2018). However, as the dental pulp is in an enclosed space, uncontrolled inflammation can contribute to dental pulp necrosis (Cooper, Holder, & Smith, 2014; Goldberg, Njeh, & Uzunoglu, 2015). Therefore, the production of immune regulators is critical in achieving a balance between immune signalling or inhibition of repair and regeneration (tertiary dentinogenesis). Inflammatory regulators such as IL-10, IL-4, IL-13, iTreg and adrenomedullin (ADM) are critical as they help to regulate and control excessive immune response within the dental pulp tissue, limiting damage to the host. The rich innervation also coordinates protective nociception and regulates inflammation and odontoblast function (Smith et al., 2016). When

odontoblasts are under continuous stress (late stage inflammation), autophagy helps control inflammation by inducing cell apoptosis promoting survival of remaining odontoblasts (Pei et al., 2015). There is an interdependent relationship between inflammation, angiogenesis and regeneration (Smith et al., 2016) in mineralised tissue repair and the healing process.

There is growing evidence that epigenetic mechanism may also contribute to regulation of the inflammatory process in the dental pulp (Cardoso et al., 2010; Hui et al., 2014, 2017). Bacterial infection is the primary aetiological factor that influences dental pulp inflammation (Kakehashi, Stanley, & Fitzgerald, 1965; Trope, 2008), hence it may have an environmental effect in stimulation of inflammatory genes (Hui et al., 2017; Lindroth & Park, 2013). The genes regulate inflammation through cytokines IL-1, IL-2, IL-6, IL-8, IL-10 and IL-12 (Fitzpatrick & Wilson, 2003; Wen, Schaller, Dou, Hogaboam, & Kunkel, 2008), interferon gamma (IFN- γ) (White, Watt, Holt, & Holt, 2002), TLR-2 and TLR-4 (Shuto et al., 2006; Takahashi, Sugi, Hosono, & Kaminogawa, 2009).

2.3. Role of biological markers in dental pulp disease and healing

Clinical diagnosis based on a history of pain, clinical signs and symptoms, radiographic and sensibility tests, has been found unreliable by some researchers (Dummer, Hicks, & Huws, 1980; Garfunkel, Sela, & Ulmansky, 1973; Seltzer, Bender, & Ziontz, 1963) and accurate by others (Pigg, Nixdorf, Nguyen, Law, National Dental Practice-Based Research Network Collaborative, & G., 2016; Ricucci, Loghin, & Siqueira, 2014). Therefore, researchers have attempted to use biological markers for pulp inflammation to help improve diagnosis of pulp status as accurate diagnosis will guide the management of the pulp and the subsequent prognosis of treatment. A recent systematic review analysed available biomarkers in relation to pulp inflammation (Rechenberg, Galicia, & Peters, 2016). Based on 57 of 847 studies that fulfilled the criteria for qualitative analysis, they reported 64 biological markers in irreversibly inflamed pulps that were statistically different from healthy pulps while 19 biological markers were not. The biological markers associated with pulp status can be grouped into the following: **1) Cytokines** (IL-1 α , IL-1 β , IL-1 γ , IL-2, IL-4, IL-6, IL-7, IL-8, IL-12p40, IL-13, IL-15, IL-18, TNF- α , TNF- β , MIP-1 α , MIP-1 β , MIP-3 α , CCR6, TGF- α , TGF- β 1, CXCL10, SDF-1, Oncostatin M, GM-CSF, GFO, MCP-1, MCP-3, MDC, INF- α , G-CSF, Eotaxin, fli3ligand, Fractalkine, CD40 L, sIL-2 α , IP-10, PDGF-AA, PDGF-AB/BB, RANTES, Osteocalcin); **2) Protease and other enzymes** (MMP-1, MMP-2, pro-MMP-2, MMP-3, MMP-9, t-PA, SOD, Cu, Zn-SOD, Mn-SOD, MDA, Elastase, Capthesin-G, Alkaline phosphatase, Aspartate aminotransferase, Catalase, NADPH-diaphorase, eNOS, iNOS, cGMP PDE, cAMP PDE, TIMP-2, MPO); **3) Inflammatory mediators** (cAMP, cGMP, PGE2, PGF2 α , α -2 M, 6-K-PGF1 α , TXB2, Endotoxins, COX-2, Substance P, Neurokinin A, CGRP, Neuropeptide Y, VIP, NOD2, VEGF, FGF); **4) Antimicrobial peptides** (hBD-1, hBD-2, hBD-3, hBD-4); **5) Others** (Substance P receptor, AAM ϕ CD163 + expressing CGRPr, NaV1.8, NaV1.9, miRNAs, EphA7) (Rechenberg et al., 2016).

In 42% (21/50) of studies histology using Hematoxylin and Eosin staining was used to confirm the diagnosis. Other methods used in the study included investigation into detection of antigen and antibody (via i.e multiplex assay, microarray, western blot, flow cytometry, radio-immunoassay, immunohistochemistry), RNA expression (via reverse transcription polymerase chain reaction (RT-PCR)), enzyme detection (via enzyme-linked immunosorbent assay, zymography, specific enzyme assays) and detection and quantification of bacterial endotoxins (via limulus amoebocyte assay) (Rechenberg et al., 2016). Current experimental options for chairside molecular pulpal diagnostics involve non-invasive and invasive methods (Rechenberg et al., 2016). Most of these studies are related to periodontal disease and inflammation. The difficulty with pulpal diagnosis is obtaining a sample (ie pulp tissue or pulpal blood) for analysis that will involve invasive procedures to

access the pulp. The method used in non-invasive procedure is through sampling gingival crevicular fluid or dentinal fluid (post cavity preparation) while invasive methods involve accessing the pulp chamber by obtaining periapical fluid through the root canal using sterile paper points or coronal pulpal blood with sterile cotton pellet (Rechenberg et al., 2016). Contamination, invasiveness of the procedure and quantity of the sample obtained are major difficulties faced for accurate pulpal diagnosis. It is hoped that in future an accurate non-invasive chairside diagnostic method may be available as this will help clinicians determine treatment with the best prognosis (Kearney, Cooper, Smith, & Duncan, 2018).

Hard tissue formation in humans is genetically regulated. Genes of the secretory calcium-binding phosphoprotein (SCPP) are involved in the mineralisation of enamel and dentine. The SCPP gene has five acidic SCPP genes and two subfamilies (Kawasaki & Weiss, 2008). The genes encoding five acidic SCPP proteins consist of DSPP, DMP-1, IBSP (integrin binding sialoprotein), MEPE (matrix extracellular phosphoglycoprotein) and SPP1 (secreted phosphoprotein 1). The first subfamily encodes Pro and Gln (P/Q)-rich SCPP deposited by ameloblasts producing enamel (George & Veis, 2008). The second subfamily encodes acidic SCPP (aka SIBLINGs-Small Integrin Binding Ligand N-linked Glycoproteins) proteins that are secreted by mesenchyme-derived cells, osteoblasts, osteocytes and odontoblasts. The SCPP gene family is found on Chromosome 4 that forms two cluster genes that consisting of five acidic SCPP genes and 16 P/Q-rich SCPP proteins separated by a 17 megabase intergenic region. The *Amelogenin* gene (*AMEL*) is found in the sex chromosome. The 16 P/Q-rich SCPP genes encode enamel, milk casein and salivary proteins, namely Casein alpha S1 (CSN1S1), Casein beta (CSN2), Statherin (STATH), Histatin 3 (HTN3), Histatin 1 (HTN1), LOC401137, Odontogenic, ameloblast-associated (ODAM), Follicular dendritic cell-secreted peptide (FDCSP), Casein kappa (CSN3), Proline-rich 5 (PROL5), Proline-rich 1 (PROL1), Mucin 7 (MUC7), Amelotin (AMTN), Ameloblastin (AMBN) and Enamelin (ENAM) (Kawasaki & Weiss, 2008). There are two other related genes to SCPP: Secreted protein acidic cysteine-rich (*SPARC*) and SPARC-like 1 (*SPARCL1*) which encodes extracellular matrix (ECM) proteins (Yan & Sage, 1999).

The *SPARCL1* and SCPP-Pro-Gln-rich 1 (*SCPPPQ1*) genes regulate expression of DSPP that stimulates the production of dentine matrix in odontoblast cells and bone matrix in osteoblast and osteocytes (Kawasaki, 2011). In the dentine pulp complex, DSPP is secreted by odontoblasts during the active secretory phase (Simon et al., 2009). DMP-1 is a highly phosphorylated extracellular matrix protein, in the SIBLINGs family that has a role in dentine mineralisation and regulation of odontoblast differentiation (Martini et al., 2013). Researchers have identified DMP-1 as a phenotypic marker for odontoblast differentiation (Butler, 1995; Unterbrink, O'Sullivan, Chen, & MacDougall, 2002) and maturity (Simon et al., 2009). Hence, both DMP-1 (Goldberg et al., 2011; Narayanan, Gajjaraman, Ramachandran, Hao, & George, 2006; Simon et al., 2009) and DSPP (Begue-Kirn, Krebsbach, Bartlett, & Butler, 1998; Narayanan et al., 2006; Goldberg et al., 2011) could therefore be used as specific odontoblast markers.

There are several pathways involved in tertiary dentinogenesis. The Wnt signalling pathway regulates many developmental processes and can stimulate the pro-survival/anti-apoptotic signal in stem cells to promote healing (Hunter et al., 2015). This pathway plays an important role in the development of many self-renewing organs, such as the bone, gut and skin, and is needed for the maintenance of homeostasis in these organs (Yoshioka et al., 2013). β -Catenin is the main component of the Wnt/ β Catenin pathway and is expressed in odontoblasts, odontoblast-like cells and dental pulp cells (Han et al., 2011). It is a dual function protein controlling the coordination of cell-to-cell adhesion and gene transcription. It is a subunit of the cadherin protein complex and acts as an intra-cellular signal transducer in the Wnt signalling pathway (Yoshioka et al., 2013). Odontoblasts are Wnt responsive and have the ability to enhance dentine regeneration (Hunter et al., 2015). Both post-mitotic odontoblasts and odontoblast-like cells

are activated via the β -Catenin/Wnt signalling pathway (Han et al., 2011). Therefore, odontoblast activity can be assessed through β -Catenin marker that indicates activation of β -Catenin/Wnt signalling pathway.

Nuclear Factor (NF)- κ B is another pathway that is activated, it has both pro- and anti-inflammatory roles that help initiate inflammation, and has the ability to modulate inflammation through regulation of apoptosis of leucocytes in a feedback mechanism to control the intensity and duration of the inflammatory response (Lawrence, 2009). When initiated, pro-inflammatory cytokines such as IL-1 and TNF α are released.

The MAPK (Mitogen-Activated Protein Kinase) family and PI3K/AKT/mTOR (Phosphatidylinositol 3-Kinase/Protein Kinase B/Mechanistic Target of Rapamycin) pathways are implicated in cell proliferation, apoptosis, adhesion and migration. The MAPK family consists of three subfamilies namely p38 MAPK, ERK (Extracellular Signal-Regulated Kinase) and JNK (c-Jun N-terminal Kinase) responsible for cell differentiation and proliferation, odontoblast secretory activity and inflammatory responses. The TGF- β /Smad (Transforming Growth Factor β /Small Mother Against Decapentaplegic) signalling pathways are triggered by dental pulp cells exposed to bioactive molecules and TGF- β 1. A detailed review of the pathways have been provided by da Rosa, Piva, and da Silva (2018).

Odontoblasts are also able to respond to environmental stimuli such as thermal, mechanical and chemical as they express Transient Receptor Potential (TRP) family (da Rosa et al., 2018). Low temperatures at the dentine surface are detected by the expression of TRPM8 (TRP melastatin subfamily member 8) and TRPA1 (TRP ankyrin subfamily 1) (Tsumura et al., 2013). TRPA1 is also activated in alkaline environments (Kimura et al., 2016) while TRPV1 (TRP cation channel subfamily V1), TRPV2 and TRPV4 are activated to mediate reactionary dentinogenesis (Tsumura et al., 2013).

Currently there are no reproducible chairside methods to assess dental pulp inflammation, however with the knowledge of the various biomarkers and pathways involved, reproducible methods could be developed that will help clinicians assess the stage of the inflammatory process, accurately diagnose dental pulp health and administer appropriate bioactive treatments in regenerative dental medicine that modulate inflammation to promote healing and repair.

2.4. An overview of regenerative dental medicine

The bioactive materials used successfully for vital pulpotomy in both primary and permanent teeth with 'reversible pulpitis' via direct or indirect pulp capping materials are calcium hydroxide ($\text{Ca}(\text{OH})_2$), calcium silicate blend mineral trioxide aggregate (MTA) and MTA-derived materials (hydraulic calcium silicate cements) (Prati & Gandolfi, 2015). Examples of hydraulic calcium silicate cements include Biodentine™ (Septodont, Saint-Maur-des-Fossés, France), Harvard MTA Caps® (Harvard Dental International GmbH, Hoppegarten, Germany), Ledermix® MTA (Riemser, Riems, Germany), MM-MTA™ (Micromega, Besancon, France), MTA Angelus (Angelus Dental Solutions, Londrina, PR, Brazil), MTA Plus™ (Prevest Detpro Limited, Jamnu, India), Pro-Root® MTA (Dentsply Tulsa, Johnson City, TN, USA), Tech Biosealer capping (Isasan Sr, Rovello Porro, Co, Italy), TheraCal® (Bisco Inc, Schaumburg, IL, USA). Application of calcium hydroxide-based biomaterials induces tertiary dentinogenesis through a series of events (Sangwan, Sangwan, Duhana, & Rohilla, 2013). Initial application of $\text{Ca}(\text{OH})_2$ that has a high pH and anti-bacterial properties, causes an area of necrosis when in contact with pulp tissues (Schroder & Granath, 1971). Interestingly, more recent studies have shown that the antibacterial activity is limited to the superficial layers and effective with minimal bacterial infection (Goldberg et al., 2008). The anti-inflammatory action of $\text{Ca}(\text{OH})_2$ is caused by denaturation of pro-inflammatory cytokines that gives the biomaterial an immunomodulatory effect that reduces the intensity of the inflammation (Khan, Sun, & Hargreaves,

2008). Resolution of the initial inflammation caused by the material is anticipated within the first week (Sangwan et al., 2013). The high pH of $\text{Ca}(\text{OH})_2$ denatures local dentinal tubules releasing solubilised bioactive molecules trapped within the dentinal tubules (Smith et al., 2012). In addition, the high pH is able to neutralise the acidic environment created by the bacterial by-products, corresponding inflammation (Heithersay, 1975) and denature pro-inflammatory mediators (Khan et al., 2008) that assists with immunomodulatory effects and tips the balance from dentinoclast activity to favourable odontogenic mechanisms (Segura et al., 1997). The optimal physiological pH limit of odontoblasts is around the pH 10 range (Heithersay, 1975), higher than this, cell death may occur and odontoblasts are replaced with odontoblast-like cells.

The bioactive molecules released, stimulate the repair and healing observed through activation of the innate immunity produces tertiary dentine (Farges et al., 2015). Formation of tertiary dentine occurs in four weeks (Yoshida, Yoshida, Nakamura, Iwaku, & Ozawa, 1996). The repair process is uncontrolled with unpredictable outcomes, perhaps due to the state of the pulp in regard to the degree of inflammation and presence of microorganisms and metabolic products, viable undifferentiated pluripotent cells, the presence of biomolecules, growth factors, genes and the local environment to sustain and support healing. Calcium silicate-based bioactive materials like MTA (Giraud et al., 2018) and hydraulic calcium silicate cements (Prati & Gandolfi, 2015) act in a similar manner. The calcific bridge (reparative dentine) formation is the product of odontoblast-like cells.

A greater understanding of the roles and relationships between odontoblasts and dental pulp cells allows researchers to use the innate healing and regenerative capabilities of these cells to produce reproducible and predictable outcomes. The greater challenge is to develop a successful treatment for teeth with irreversible pulpitis and necrotic pulps. The current management of these non-vital teeth is either root canal treatment or extraction. Interestingly, success has been reported in the management of teeth with irreversible pulpitis in immature and mature permanent teeth by partial or full pulpotomy using MTA and Biodentine™ (Taha & Abdulkhader, 2018; Taha & Khazali, 2017).

Newer experimental methods include bioactive molecules, tissue engineering and epigenetic modifications. Bioactive molecules may be acquired naturally from the within the dentinal walls by means of demineralising agents such as ethylenediaminetetraacetic acid (EDTA) (Cassidy, Fahey, Prime, & Smith, 1997), lactic acid (Smith, Patel, Graham, Sloan, & Cooper, 2005), cavity etching agents (Smith & Smith, 1998) and alkaline bioactive materials (Giraud et al., 2018; Prati & Gandolfi, 2015). Direct application of recombinant growth factors (Hu, Zhang, Qian, & Tatum, 1998; Nakashima, 1994; Rutherford, Wahle, Tucker, Rueger, & Charette, 1993; Tziafas, Alvanou, Papadimitriou, Gasic, & Komnenou, 1998) or growth factor hydrogel (Dobie, Smith, Sloan, & Smith, 2002) onto the amputated dental pulp has been attempted. However, due to the short half-life of the growth factors, its effectiveness has been limited. The ideal method would require a controlled gradual release that will maintain signalling to promote healing and repair (Smith, Lumley, Tomson, & Cooper, 2008). Experimental methods involve allogenic bioactive molecules incorporated into control release kinetics in multiple layers, encapsulated PLGA (poly-lactico-glycolic acid) microspheres or into bioactive materials (da Rosa et al., 2018). Further research in this area is warranted.

In instances of dental pulp death, tissue engineering appears promising (Kim, Malek, Sigurdsson, Lin, & Kahler, 2018). Regenerative endodontic therapy aims to regenerate the pulp dentine complex (Kim et al., 2018) by disinfection of root canal with irrigation (sodium hypochlorite) and intracanal dressing (calcium hydroxide paste or triple antibiotic paste). Once the root canal is sufficiently disinfected, EDTA (Ethylenediaminetetraacetic acid) rinse to release growth factors is done prior to the injection of suitable scaffold (blood or platelet-rich plasma or platelet-rich fibrin) which is seeded with stem cells, growth

factors, signalling and anti-inflammatory molecules to regenerate a functional dental pulp with odontoblast-like cells that produce calcified dentine-like tissue (Diogenes & Ruparel, 2017; Kim et al., 2018). Active research is ongoing investigating potential scaffold/delivery systems for the stem cells and bioactive molecules. These may be natural, synthetic or hybrid biocompatible materials that mimic the dental pulp to allow stem cells to attach, differentiate and migrate (Bakhtiar et al., 2018; da Rosa et al., 2018). While there are advances in this area, there are no long-term high-quality studies with favourable predictable clinical outcomes (Tong et al., 2017).

Similarly, epigenetic modification occurs when an alteration to gene expression and cellular function is brought about without changing the original DNA sequence (Allis & Jenuwein, 2016; Stefanska & MacEwan, 2015). The mechanism involves DNA methylation, histone modification and noncoding RNAs (ncRNAs). Kearney and colleagues have provided an in-depth review of the topic, the potential development of accurate diagnostic and targeted dental biomaterial applications (Kearney et al., 2018). However, with any genetic manipulation, potential concerns exist on the possible side-effects and clinical approval needed to use these methods safely. Currently, there is no approval for any epigenetic-based drug in the United States (FDA) for medical and dental use, although in the UK there are epigenetic-based drugs available, especially for oncology patients (Kearney et al., 2018).

3. Conclusion

Regenerative dental medicine has provided many new possibilities for the future management of dental caries and its sequelae. Understanding the molecular and genetic interactions within the dental pulp complex in health and disease will assist researchers to fine-tune the available methods in bioactive materials, molecules, epigenetic modifications and bioengineering. It is our hope that in the near future we may be able to heal or regenerate the dental pulp to help preserve structurally sound teeth.

Author contribution

SR, AL, DJM, AEB and MM conceived and developed the idea as part of the literature review for SR's PhD thesis. All authors reviewed and approved the final version of the manuscript.

Declaration of Competing Interest

None.

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