THE ROLE OF INFLAMMATION MODULATION IN DENTAL PULP REGENERATION

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Abstract

A vital and healthy dental pulp (DP) is required for teeth to remain functional throughout a lifespan. Appreciating its value for the tooth, the regeneration of the DP is a highly researched goal. While inflammation of the DP marks the beginning of an eventual necrosis, it is also the prerequisite for the regenerative events of neovascularisation, stem cell mobilisation and reparative dentine deposition. In the light of a pro-regenerative inflammatory process, the present review discusses the role of the macrophage population shift from pro- to anti-inflammatory in reversible *versus* irreversible pulpitis, while also analysing the overlooked contribution of pulp innervation and locally derived neuropeptides to the process. Then, the currently practiced (pulp capping and revascularisation) and researched (cell transplantation and cell homing) approaches for DP regeneration are discussed. Focusing on the role of cell homing in modulating inflammation, some potential strategies are highlighted to harness the inflammatory process for DP regeneration, mainly by reversing inflammation through macrophage induction. Next, some potential clinical applications are discussed – especially with capping materials – that could boost macrophage polarisation and complement system activation. Finally, current challenges facing the regeneration of the DP are presented, while underlining the importance of promoting an anti-inflammatory environment conducive to a regenerative process.

Keywords: Dental pulp, inflammation modulation, regeneration, pro-inflammtory cytokines.

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List of Abbreviations

BMP-2	bone morphogenetic protein-2
CCL2	C-C motif chemokine ligand 2
CD	cluster of differentiation
CGRP	calcitonin gene-related peptide
DP	dental pulp
DPSCs	DP stromal cells
ECM	extracellular matrix
IFN	interferon
IL	interleukin
NGF	nerve growth factor
PLA	polylactic acid
RCT	root canal treatment

Introduction

The DP occupies the core of the tooth and is the reason for its vitality. Although the DP might not be considered to contribute directly to tooth function – mastication, speech, aesthetic – a vital and healthy pulp is the reason why teeth remain functional throughout a lifetime. In fact, RCT – a tooth devitalising procedure and ironically also known as "tooth-saving" – is a radical treatment that results in the eventual loss or extraction of the tooth due to reinfection, secondary decay and leakage around crown margins and periodontal involvement (Friedman and Mor, 2004; Salehrabi and Rotstein, 2004; Siqueira, 2001).

In 1951, Markley sensibly defined and approached dental decay [the most prevalent human disease (Cooper *et al.*, 2010; Heng, 2016)]: "The loss of even a part of a human tooth should be considered a serious injury and that dentistry's goal should be to preserve healthy, natural tooth structure" (Markley, 1951). This, together with the understanding of the tooth-core's invaluable role in maintaining lifelong tooth function, made DP regeneration a highly researched goal.

Root canal treatment is an appealing alternative to tooth extraction, however, not without limitations. After RCT, the physico-mechanical properties of the tooth are altered and it becomes more prone to fracture (Vongsavan *et al.*, 2000). Even with a protective covering restoration, micro leakage is highly prevalent, resulting in periodontal involvement and undetected decay due to its hidden location under the covering crown together with the lack of the alarming pain reaction (Lucarotti, 2003; Ray and Trope, 1995). Yet, more importantly, tooth devitalisation eliminates the innate immune and repair responses of a vital pulp (Farges *et al.*, 2015; Hahn and Liewehr, 2007). This inflammation-modulated immune response, orchestrated by pulp fibroblasts, odontoblasts, endothelial cells and even nerve fibres (Zhan *et al.*, 2020), plays a key role in fighting against caries invasion as well as secretion of tertiary and reparative dentine (Farges *et al.*, 2015).

The present review highlights the role of inflammation in DP repair and regeneration, while underlining the potential clinical approaches that could reverse DP inflammation, mainly by macrophage induction, towards pulp regeneration.

The role of inflammation in pulpal injury and repair

Within the dynamic DP tissue, homeostasis is disrupted by several pathologies, such as bacterial toxins or actual bacterial invasion, thermal or electrical insults (galvanism) (Certosimo and O'Connor, 1996). With tooth decay progression and before bacteria reach the DP, the diffusion of bacterial toxins into dentine tubules to the pulp-dentine interface triggers inflammatory and immune events in the underlying DP. Namely, antigen-presenting dendritic cells strategically migrate to the odontoblast layer facing the invasion, followed by accumulation of T-lymphocytes, macrophages, neutrophils and B-lymphocytes. Reversible pulpitis develops while bacterial invasion and toxins concentration are limited and inflammation is mild (Goldberg *et al.*, 2008). In fact, the dentine-pulp complex responds to insults in a dose-effect manner, with low insult levels reversed to promote regeneration, while intense immune reaction irreversibly initiates tissue necrosis (Cooper *et al.*, 2010). With fast, severe or long-lasting (chronic) insults, odontoblasts are too slow to secrete reactionary dentine and, with further toxin accumulation and invasion of the DP, irreversible pulpitis is initiated followed by necrosis, root canal infection and periapical disease.

Before tissue necrosis, prostaglandin-mediated pain impulses are manifested clinically as a pulp reaction through the transmission carried by sensory nerve endings that, along with the sympathetic nerve endings, also play a role in regulating the DP inflammatory process by neuropeptide secretion targeting membrane bound receptors located on different pulp structures (Zhan *et al.*, 2020). Neuropeptides, such as substance P, stimulate the production of pro-inflammatory cytokines from human DP fibroblasts; while CGRP is associated with immunosuppression and increased expression of BMP-2, promoting dentineogenesis within the DP (Calland *et al.*, 1997). CGRP was, in fact, reported to promote an osteodentine bridge following pulpotomy (Kline and Yu, 2009). Membrane-bound receptors for functional molecules from dental nerves are expressed on odontoblasts, fibroblasts, inflammatory cells and endothelial cells of capillaries, indicating a bidirectional cross-talks between these structures and nerve endings. Besides supporting the odontoblastic receptor theory of dentine sensitivity (Zhan *et al.*, 2020), such intimate anatomical and functional interaction between pulpal nerve endings and other pulpal structures strongly suggests their intertwining functional relationship in all DP events, including inflammation and regeneration.

A mindset shift by a cell population shift: reversible *versus* irreversible pulpitis

For years, inflammation was a term describing a vicious event of pain, redness and hotness prior to tissue necrosis and was viewed as antagonistic to regeneration. Currently, within an ascending learning curve, it is known that the inflammatory reaction is a prerequisite to initiate the reparative function of progenitor cells implicated in tissue restoration (Goldberg *et al.*, 2008). Basically, a (pro-) inflammatory phase, when tissues are lost, timely shifts to an anti-inflammatory phase, characterised by proliferation and differentiation of stem cells for healing and regeneration, indicating the significant interrelationship between the inflammatory and regeneration processes (Cooper *et al.*, 2010).

During inflammation followed by wound healing and tissue remodelling, the cellular response – especially that of macrophages – is finely tuned (Roy, 2016). Following injuries – such as bacterial endotoxin invasion through dentineal tubules, deep cavity or crown preparation – a controlled and regulated inflammatory response cascades for pulp remodelling. However, if not controlled, for example in the case of bacterial invasion of the DP, a severe blow or long-standing insult, irreversible tissue damage takes place (Kawashima *et al.*, 2005; Mutoh *et al.*, 2009).

The correlation between macrophage population shift, known as polarisation, and tissue remodelling

is under extensive investigation (Mahmoudi *et al.*, 2017; Yonehiro *et al.*, 2012). During the inflammatory process, monocytes are recruited by neutrophils and, then, migrate to the injury site where they differentiate into macrophages (Swirski *et al.*, 2009). Once recruited and differentiated, the macrophages generally attain M1 polarisation, a pro-inflammatory phenotype triggered by exposure to recruiting pro-inflammatory cytokines such as IFN-γ (St Pierre and Tidball 1994; Tidball, 2005). In about 48 h, M1 macrophages reach their maximum number. Synthesis of IL-10 and IL-4 recruits and shifts the population to the M2 immuno-regulatory and anti-inflammatory phenotype (Collins and Grounds 2001; Villalta *et al.*, 2009).

As reversible pulpitis is initiated and progresses, classically activated M1 macrophages mediate phagocytosis of bacteria, removal of dead cells and the initial exposure to the ECM-degradation products (Sicari *et al.*, 2014). Once polarised to the M2 phenotype, macrophages release anti-inflammatory cytokines that signal tissue remodelling and repair (Gordon, 2003; Gordon and Martinez, 2010) (Fig. 1). As insult intensifies, interruption of the pro-inflammatory events take place: neutrophil and macrophage infiltration, polarisation or transition are halted and, hence, remodelling or regeneration processes (Tidball and Wehling-Henricks, 2007). The interruption of the regeneration process, in the case of a DP injury, is manifested as activated osteo/odontoclastogenesis, modified vascular endothelial permeability with concomitant bone and root resorption (Lara *et al.*, 2003; Zaky *et al.*, 2020). Targeting the sustainability of a reversible
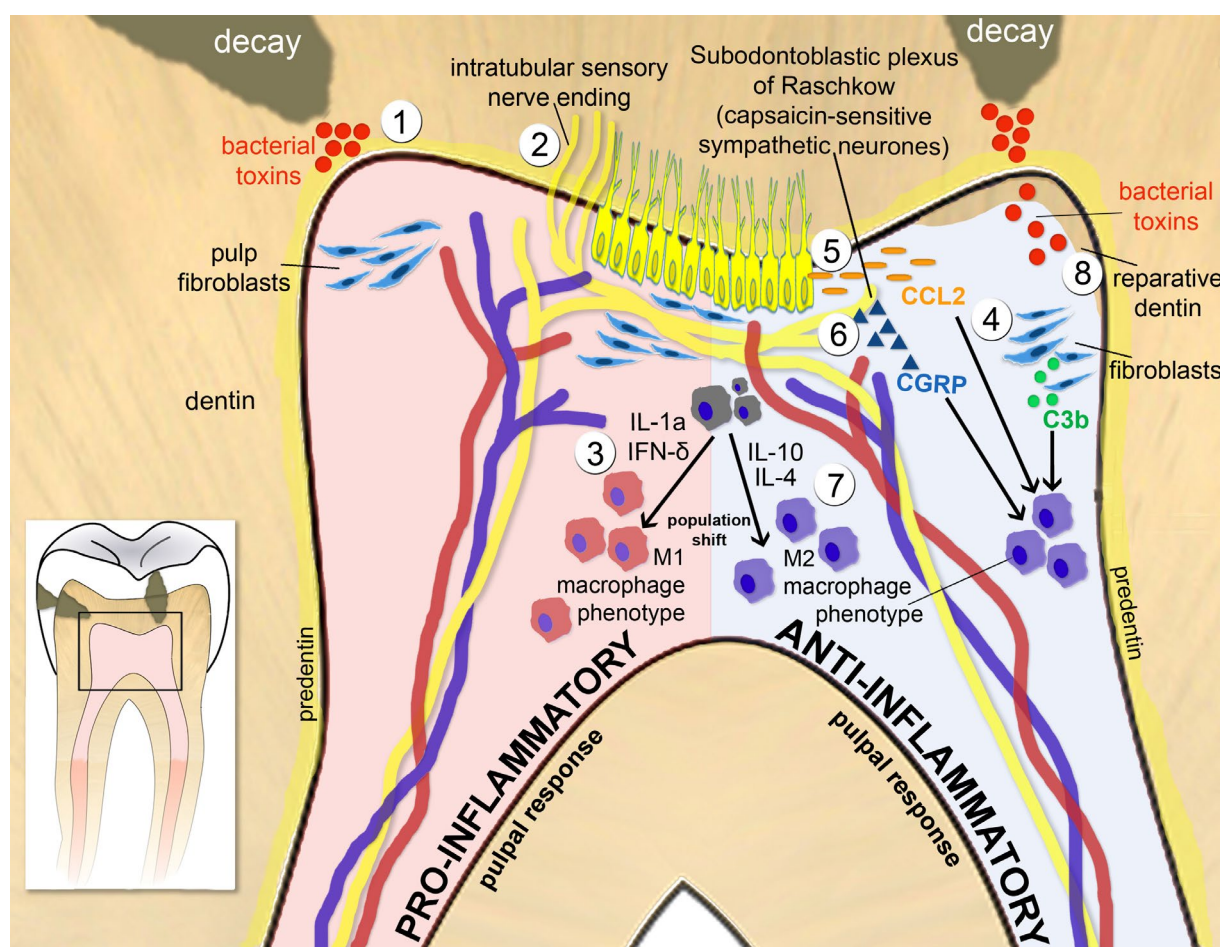
Fig. 1. Schematic drawing of pulpal events taking place in pro-inflammatory (left in red) and anti-inflammatory (right in blue) responses and the potentially tailorable approaches to modulate inflammation to achieve regeneration. Numbers indicate separate events rather than an accurate chronological order. 1: bacterial toxins (noxious stimulus) are introduced in dentineal tubules. 2: intratubular sensory nerve endings are the first to receive the noxious stimulus. 3: extravasating macrophages are signalled to an M1 phenotype by IL-1a and IFN-δ. 4: pulp fibroblasts activate C3b complement to mediate phagocytosis of bacteria toxins by M1 macrophages while contributing to their population shifts to an M2 phenotypes. As toxins advance within the dentineal tubules to the dental pulp, they stimulate a cascade of events that signal the anti-inflammatory shift to tissue remodelling and repair. 5: while still within the dentineal tubules, toxins stimulate odontoblasts to secrete CCL2. 6: reaching the dental pulp, toxins stimulate CGRP release from sympathetic nerve endings. 7: as the noxious stimulus subsides (decay removed), the macrophage population shifts to M2 phenotype by IL-10 and IL-4 action. 8: areas of reparative dentine are an indication of an anti-inflammatory, pro-regenerative homeostasis.

pulpitis from a clinical standpoint would be crucial to interrupt the pro-inflammatory events by induction of the anti-inflammatory macrophages as early as possible to prevent the progression and accumulation of a reversible damage.

The role of nerve endings and neuropeptides in anti-inflammatory macrophages induction

Neurogenic inflammation describes the direct participation of dental innervation in the innate immune response (Cooper *et al.*, 2010). From the sub-odontoblastic plexus of Rashkow in the coronal pulp, the sensory axons enter dentineal tubules to penetrate the dentine, whereas the sympathetic axons end in close proximity to odontoblast cell bodies (Ibuki *et al.*, 1996; Shimeno *et al.*, 2008). From within the tubules and receiving the bacterial toxin first-hand, the sensory group acts mainly in a pro-inflammatory manner initiating the pulpitis reaction. Progressing within the pulp, the sympathetic group, associated with the pulpal aspect of the odontoblastic layer, counteracts by an anti-inflammatory action. Such action includes the induction of anti-inflammatory macrophages by inhibition of cytokine IL-1a production, a bone-resorbing and a pro-inflammatory factor (Bletsa *et al.*, 2004; Zhan *et al.*, 2020).

NGF, a neuropeptide involved in the regulation of growth, maintenance, proliferation and survival of pulpal neurons, is highly expressed at the caries invasion site (Mitsiadis *et al.*, 2017). This takes place either directly by upregulation and even redistribution of its receptors during pulpitis (Woodnutt *et al.*, 2000) or indirectly by nerve endings outgrowth in response to human pulp fibroblast through complement system activation (Chmilewsky *et al.*, 2016). As bacterial toxins progress towards the pulp, the upregulation of NGF signals a pulpal reparative process to reverse inflammation towards regeneration.

Another neuropeptide involved in neurogenic inflammation, CGRP, was found to promote odontoblast differentiation and maintain their function (Chiego *et al.*, 1987). Together with pulpal Schwann cells, CGRP promotes macrophage population shift to the anti-inflammatory macrophage M2 phenotype to regulate the immune response (Caviedes-Bucheli *et al.*, 2008). In fact, abundant colocalisation of Schwann cells and M2 macrophages is detected in the DP under deep active decay, with faint sensitivity and without episodes of spontaneous pain (Yoshiba *et al.*, 2020).

Regeneration in endodontics

The history of root canal therapy date back to 1728 when extirpation of the DP, using a small pin, was described (Cruse and Bellizzi, 1980). Since then, manipulating inside the tooth, *i.e.* endodontics, was

coined as a branch of practicing dentistry; while the evolving instrumentation, techniques and obturation materials have been successful to make RCT an efficient treatment with more than a 95 % success rate (Naito, 2005). However, RCT is intrinsically a tooth devitalisation process whose success is judged solely by the asymptomatic retention of the tooth in place regardless of the ability of the pulp-dentine complex to react to its environment – a characteristic of a vital structure only. With that in mind, RCT is the resort when signs of irreversible pulpitis are confirmed; while approaches for vital pulp therapy are attempted to maintain the pulp vitality even with wavering reversibility. It is important to note that for RCT, vital pulp therapy or any regenerative therapy, it is of global consensus that clearing off bacterial infection, within the pulp canals or from tooth decay, is the key for treatment success.

**Clinically practiced regenerative approaches**
It is out of awareness and appreciation of the DP tissue that resorting to RCT is not a decision to be taken lightly. While considering the younger pulp favoured response (Bansal and Bansal, 2011), several approaches have been researched, developed and clinically applied to attempt regenerating the DP at different extents of its ailment: from direct pulp capping covering a pinpoint exposure to vascularisation and re-innervation of a fully lost root canal system.

Direct pulp capping is a regenerative procedure and a clinical example of vital pulp therapy. When successful (93-100 %) (Akhlaghi and Khademi, 2015), direct pulp capping confirms that pulpitis is in a reversible phase. The inflammatory process, compartmentalised within the exposed coronal pulp, can still be reversed. Stem precursors and pericytes can be mobilised and differentiate into odontoblasts to construct a reparative dentine bridge (Wells *et al.*, 2019).

Revascularisation is another regenerative approach, also referred to as a regenerative endodontic procedure, aiming at revitalising a necrotic immature permanent tooth. The necrotic tooth is debrided and disinfected with sodium hypochlorite and calcium hydroxide paste while the open apex allows stimulation of apical bleeding and invasion of a blood clot with all its healing properties, including stem cell colonisation from the apical papilla and neo-vascularisation. Some challenges exist, including patient compliance, over-manipulation and controlling infection. The success rate of this procedure ranges from bone healing, apex maturation (though unpredictable) and ultimately tooth positive response to vitality tests – indicating innervation (Ray *et al.*, 2016; Staffoli *et al.*, 2019)

DP regeneration strategies in mature teeth
In the case of adult mature teeth, pulp regeneration is primarily challenged by narrowing of the canal anatomy through dentineal sclerosis and the minimal
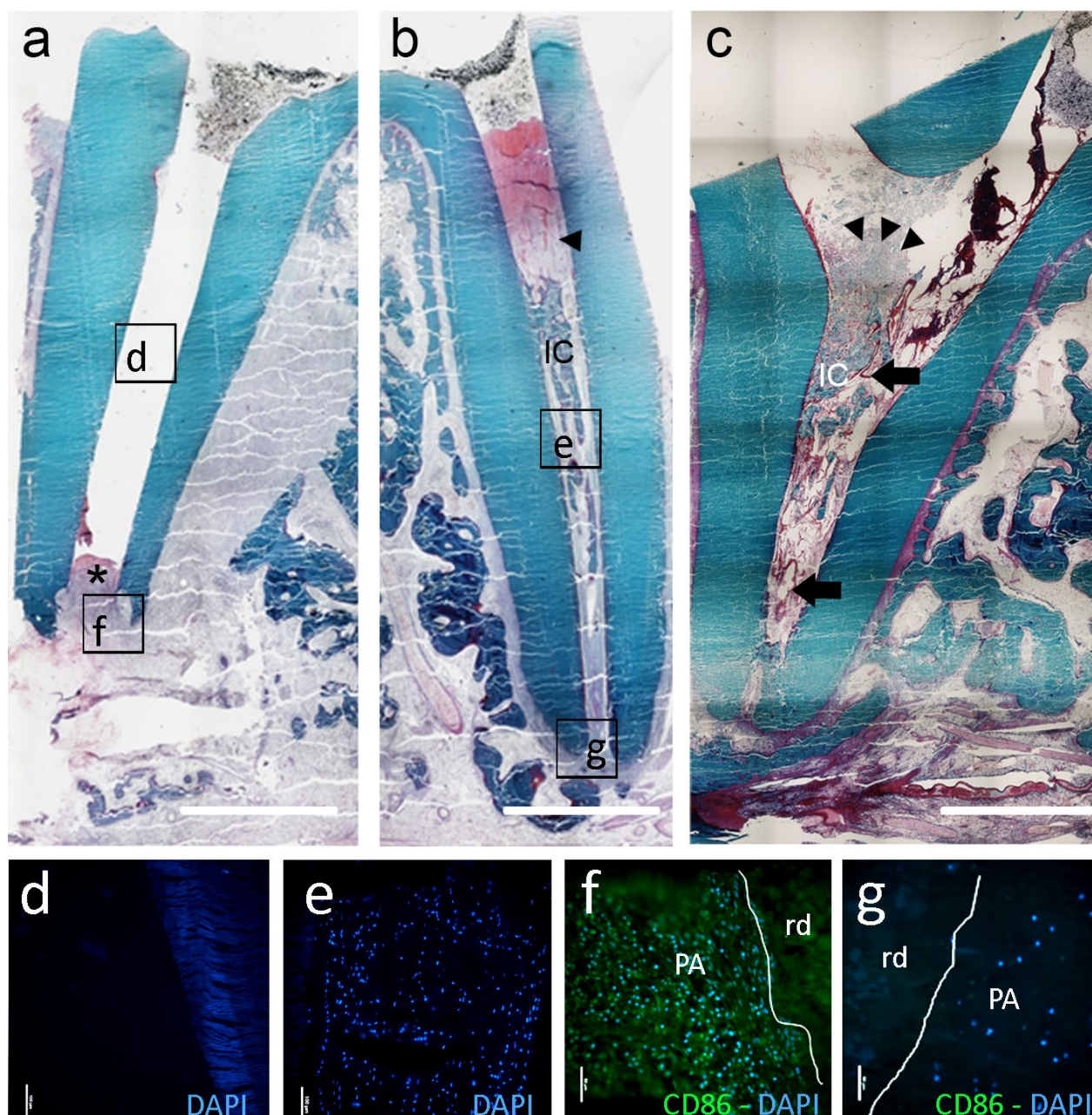
Fig. 2. Different regenerative outcomes in canine (beagles) root canal model according to the absence or presence of an inflammatory process. (**a**) With inflammation: root canal of beagle lower premolar manipulated without introduction of obturation material (only blood clot formation) while inflammation was induced periodically (*: inflammatory plug). (**f**) Immunofluorescence of CD86, an anti-M1 macrophages marker (Zaky *et al.*, 2020). (**d**) No regeneration or cell infiltration is observed while signs of destruction are seen as bone and root dentine (rd) resorption. (**b**) Without inflammation: blood-clot-filled root canal without periapical inflammation (**g**: CD86 negative) showing regenerative process and cell infiltration (**e** and arrowheads) are observed without bone or root resorption. (**c**) Inflammation modulation: Swine ECM-filled root canal shows a regenerative process with cell infiltration (arrowheads) and more vascularisation (arrows) (Alqahtani *et al.*, 2018). Although ectopic, bone/cementum-like intracanal calcifications (IC) within a remodelling pulp are a common observation in pulp regeneration literature and are considered signs of an active regenerative process (Alqahtani *et al.*, 2018; Skoglund and Tronstad, 1981; Zaky *et al.*, 2020). Hence, it is suggested to include cementogenesis inhibitor molecules while attempting pulp regeneration procedures by periapical cells homing (Zizka and Sedy, 2017). (**a-c**) Scale bars: 250 μm; (**d,e**) scale bars: 100 μm; (**f,g**) scale bars: 50 μm. PA: periapical tissues.

opening of the anatomic apical foramen – challenging the development of an anastomosing blood supply with the alveolar vessels (Huang *et al.*, 2020). Pulp regeneration in mature teeth is attempted by two approaches: cell transplantation and cell homing, both equally challenging.

Cell transplantation involves delivering of donor cells. Several animal studies with transplanted cells have reported pulp regeneration with formation of an odontoblastic layer, blood vessels and evidence of neuronal regeneration employing DPSCs; either as a total population (Souron *et al.*, 2014) or as an enriched subset (Iohara *et al.*, 2009) in pulpotomy or full pulpectomy models (Iohara *et al.*, 2020). To date, few human clinical trials report such promising results (Nakashima *et al.*, 2017). Yet, controversy still exists as to whether these protocols are ready to replace conventional endodontic treatment procedures.

Cell homing is defined as the active recruitment of stem/progenitor endogenous cells into an anatomical compartment (Laird *et al.*, 2008). Studies attempting DP regeneration by cell homing also report promising results in terms of development of pulp-like tissue that develops from recruited periapical/periodontal cells through a manipulated and widened apical foramen to colonise the root canals up to the pulp chamber (Alqahtani *et al.*, 2018; Eramo *et al.*, 2018; Huang *et al.*, 2020). Interestingly, a blood clot formation within the debrided root canal and chamber is enough to support the regeneration process that occurs regardless of the different types of inserted scaffolds (Zaky *et al.*, 2020) (Fig. 2).

While both approaches seem promising, the cell homing approach clearly circumvents, from a clinical standpoint, many of the challenges associated with cell transplantation. Undoubtedly, an acellular chair-side ready approach bypasses the hurdle of immunogenicity and the complexity of a substantial *ex vivo* cell manipulation and culture, hence is safer, more time and cost effective and more clinically applicable. Besides and more importantly, tissue regeneration by cell trafficking warrants the recruitment of all subsets of host cell populations and native growth factors to repopulate the defect and re-establish self-renewing stem-cell niches in a process mirroring physiological tissue remodelling and lifetime tissue turnover (Zaky and Cancedda, 2009).

Cell homing and inflammation modulation

Regeneration by cell homing, also including revascularisation techniques in endodontic regenerative therapy, occurs primarily by the periapical cell populations trafficking trough the root apex. These populations, later engulfed in a clot of red blood cells and platelets, include lymphocytes, endothelial cells, neuronal precursors, fibroblasts, monocytes (originating from bone marrow and differentiating into macrophages) and progenitor/

stem cells mobilised as pericytes from the blood vessel walls. All together, along with cytokines and growth factors in the specific proportions, offer the favourable cocktail for wound healing in general terms and specifically for pulp-like tissue development when within dentineal walls.

When it comes to regeneration, the role of macrophages in the process is key. Depending on the intensity of the bacterial infection or the extent of periapical instrumentation, cytokines trigger macrophagespolarisation from M1 pro-inflammatory to M2 anti-inflammatory phenotype to phagocytise bacteria and dead cells and signal a regeneration process (Sicari *et al.*, 2014). In fact, with extensive periapical inflammation and M1 macrophage colonisation, dentine and root resorption are observed with no signs of regeneration within debrided root canals. In contrast, tissues show signs of remodelling and neovascularisation within the debrided pulp space when the periapex shows no signs of inflammation (Zaky *et al.*, 2020).

Interestingly, the current literature suggests that DP fibroblasts play an important role in inflammation modulation by local activation of the complement system. Bacterial endotoxins stimulate DP fibroblasts to signal the secretion of the antimicrobial C3b complement not only to fight bacterial invasion (Le Fournis *et al.*, 2019) but also to participate in the macrophage population shift from M1 to M2 macrophages (Bohlson *et al.*, 2014) (Fig. 1).

Harnessing the inflammatory process and macrophage induction for DP regeneration

Taming the inflammatory process towards regeneration primarily involves the modulation of the process to sustain its anti-inflammatory phase over its pro-inflammatory phase. As macrophages are the main key players in this phase shift, the creation of a long-standing reversible pulpitis would be accomplished by induction of the M2 macrophages. Clinically, among several suggested approaches to modulate the inflammatory process – and the macrophages' shift to enhance regeneration – are the employment of decellularised ECM and the introduction of modulation molecules (Agrawal *et al.*, 2010; Badylak *et al.*, 2011; Frantz *et al.*, 2010).

Although a blood clot formed by the bleeding within the wounded tissues contains sufficient recruited elements for regeneration (Lehwald *et al.*, 2020; Radke *et al.*, 2018), Alqahtani *et al.* (2018) suggested an approach to modulate the inflammation process within the blood clot. The approach employs decellularised DP ECM to be introduced into a debrided root canal while the blood clot forms after canal manipulation. Such an approach has a twofold benefit: i) the introduction of the ECM with its constitutional trophic factors into the root canal space triggers periapical progenitor/stem cell trafficking for the regeneration process; ii) the ECM also plays

a role in inflammation modulation by triggering an anti-inflammatory host response that favours pulp regeneration (Agrawal *et al.*, 2010; Badylak *et al.*, 2011). The ECM would promote inflammatory modulation and create an antibacterial environment that supports DP regeneration (Fig. 2**c**) to reach homeostasis in the root canal and periapical tissues. The remodelling by ECM scaffolds is also attributed to the macrophage by-products released upon ECM digestion (Gordon and Taylor, 2005; Mantovani *et al.*, 2004; Valentin *et al.*, 2009).

Since macrophage modulation and polarisation play a crucial role in pulpal and periapical inflammation, it is intuitive to attempt DP regeneration by using macrophage-mobilising molecules to harness the inflammatory process for pulp regeneration. CGRP, a neuropeptide secreted by capsaicin-sensitive neurons in the DP, is known to promote proliferation of endothelial cells and pulpal fibroblasts and to increase DP vascular permeability (Bongenhielm *et al.*, 1995; Zhan *et al.*, 2020). CGRP is also a regulator of the immune response by acting as a signalling molecule for the M2 anti-inflammatory macrophage phenotype (Caviedes-Bucheli *et al.*, 2008). CGRP has been reported to have a protective role in the initial phase of apical periodontitis (Austah *et al.*, 2016).

Another molecule is CCL2, secreted by odontoblasts and diffused into the sub-odontoblast pulp area where they activate and mobilise various populations of immune cells (Farges *et al.*, 2015). CCL2 has been shown to favour M2 macrophage polarisation (Zhuang *et al.*, 2019) and to play a key role in the recruitment of circulating blood dendritic cells and their migration through the endothelial barrier (Goldberg *et al.*, 2008) (Fig.1).

Potential clinical application

Clinically, it is crucial to narrow down to the inflammation modulation molecule that, upon delivery in contact or within the DP, can modulate and reverse the inflammation while signalling cell homing and differentiation. Such a molecule or combinations of molecules could be case-specific, depending on the treatment modalities needed to address a certain underlying pathosis, while considering the extent of irreversible damage to DP tissues.

The polarisation of the macrophage population from M1 pro-inflammatory to M2 anti-inflammatory phenotypes would be the primary target of cytokines' application to create a pro-regenerative environment (Colombo *et al.*, 2014). CCL2 and CGRP molecules, among others, such as hypoxia-inducing factor (Colombo *et al.*, 2020) and NGF neuropeptide (Mitsiadis *et al.*, 2017), have the potential to leverage established regenerative applications such as direct pulp capping and revascularisation. These approaches rely on delivering a biocompatible,

antibacterial, Ca^{2+}-releasing material that mobilises bioactive molecules from the dentine (Cooper *et al.*, 2010). While some pulp-capping materials have been reported to have inflammation-modulating effects (Giraud *et al.*, 2019), their functionalisation with modulating molecules could boost their regenerative properties in terms of macrophage polarisation and complement system activation. Arguably, adding inflammation-modulating molecules to the capping materials could even open new horizons for mature teeth pulpotomy, currently approached as a provisional rather than a full alternative for RCT (Li *et al.*, 2019; Sadaf, 2020).

Practically, the clinical delivery of these molecules would depend on their application modality, either as a single or cocktail combination. The relevant carrying vehicles currently researched both *in vitro* and in animal models are hydrogels (Colombo *et al.*, 2020), polymeric scaffolds (Soares *et al.*, 2018), PLA microparticles (Zhuang *et al.*, 2019), nano-structured biomaterial (Keller *et al.*, 2015) and collagen, among many others (Colombo *et al.*, 2014). ECM application could also represent the delivery of a naturally occurring combination of inflammation-mediating cytokines (Alqahtani *et al.*, 2018).

Challenges and closing remarks

With the different approaches and strategies for DP regeneration, it is clear that clinicians and researchers are still at the experimentation phase and far from offering a feasible clinical alternative to the current devitalising RCT. A considerable part of the lag could be attributed to the limited understanding of pulp biology, together with the absence of precise diagnostic tools that can indicate the progression or rather the reversibility of the pulp condition early enough (Duncan and Cooper, 2020).

With all the relatively advanced understanding of tooth development and its intricate biology, all current pulp regeneration approaches are attempting dentine and pulp regeneration in ways that do not mimic their original development. Throughout the epithelium-mesenchymal interaction, the pulp tissue develops simultaneously with and within the dentine while the dentine-secreting odontoblasts and their accompanying nerve endings recede along the maturing and mineralising dentine tissue pulpal surface from the dentineoenamel junction towards the pulp core (Farges *et al.*, 2015; Zhan *et al.*, 2020). As the fully developed tooth body has lost its soft tissue core, there is an attempt to re-generate the missing pulp tissue independently and in the absence of the timely orchestrated tooth development signalling molecules. Despite the observed potential of many approaches, among which inflammation modulation, this could be not the right way to proceed.

Compared to connective tissue maturation and neovascularisation, the regeneration of neural tissue remains the most challenging part. A fully restored

and functional DP would need to acquire nerve regeneration in neural hierarchy and plexuses, not only to regain the alarming sensation of pain but primarily to regain its function in regulating inflammation and repair (Zhan *et al.*, 2020).

As for the current different strategies for pulp regeneration, a key is, after dismissing the infection, promoting an anti-inflammatory environment that is conducive to tissue regeneration at the dentine-pulp interface. This is primarily achieved by introducing molecules and cytokines that can modulate the initial pro-inflammatory reaction and mobilise its cell population into anti-inflammatory reaction for a pro-regeneration process. Undoubtedly, unfolding research will enlighten the understanding of the molecular and cellular interactions that orchestrate this intricate inflammation-regeneration coupling to develop novel therapies for DP regeneration and regeneration of the complex tooth-organ as a whole.

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Editor's note: All questions from reviewers were answered in the text, therefore there is no Discussion with Reviewers section for this manuscript. The Scientific Editor responsible for this paper was Thimios Mitsiadis.