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ABSTRACT

Dentistry is entering an exciting era in which many of the advances in biotechnology offer opportunities for exploitation in novel and more effective therapies. Pulp healing is complex and dependent on the extent of injury, among many other factors. Many of the molecular and cellular processes involved in these healing events recapitulate developmental processes. The regulation of odontoblast activity is clearly central to pulp healing, and an understanding of the mechanisms involved in these processes is necessary to enable laboratory studies to be translated to clinic application. Transcriptome analysis has identified changes in many odontoblast genes during the life-cycle of this cell and its responses to injurious challenge. The p38 MAPKinase pathway appears to be central to the transcriptional control of odontoblasts and may provide a key target for therapeutic intervention. The many recent advances in knowledge of pulpal stem cells and molecular signaling molecules within the tooth, now provide exciting opportunities for clinical translation to novel therapies. Such translation will require the partnership of researchers and skilled clinicians who can effectively apply advances in knowledge to appropriate clinical cases and develop novel therapies which can be realistically introduced into the clinic.

Dentistry is entering an exciting era in which the many advances in biotechnology offer opportunities for exploitation in novel and more effective therapies. Regenerative medicine provides many advantages for restorative dentistry in terms of restoration survival rates and long-term treatment prognosis. In fact, dentistry has long been a pioneer of regenerative medicine, with the introduction of pulp capping some 60 to 70 years ago and is well-placed to continue to provide a lead in this area (Zander, 1939; Zander and Glass, 1949).

Clinically, there are two situations commonly encountered in pulp disease. First, when the dental pulp is still vital and potentially inflamed, the focus is very much on the maintenance of vitality. The treatment strategy will be to locally regenerate new dentin and promote reorganization of the underlying connective tissue. In the second situation, there is complete loss of the pulp, due to cell and tissue death in response to infection and uncontrolled inflammation, resulting in the root canal system becoming empty of any vital tissue and, frequently, highly infected. In this situation, the strategy is to endeavor to regenerate a new vital connective tissue, ideally mimicking the dental pulp.

Significant progress in the field of caries management has led to a much improved understanding of the mineralization of teeth and the biological behavior of the dentin-pulp complex. It is apparent that the dentin-pulp complex is able to adapt to a variety of stimuli-invoking defense responses to maintain its vitality, and the main role of the dentin-pulp complex is to secrete dentin. When tooth development is complete, the pulp sustains the dentin through homeostatic and self-protective mechanisms. The dental pulp is also able to re-initiate dentinogenesis to protect itself from external injury and insult. The pulp-healing processes are complex and dependent on the extent of injury, among many other factors. With mild injury, healing involves a simple up-regulation of dentinogenic events by existing primary odontoblasts (reactionary dentinogenesis). However, with greater tissue injury, more complex defense and healing responses occur, with recruitment of stem/progenitor cells, their differentiation to odontoblast-like cells, and subsequent up-regulation of secretory activity (reparative dentinogenesis) (Lesot *et al*., 1993; Smith *et al*., 1995). Many of the molecular and cellular processes involved in these healing events are hypothesized to recapitulate developmental processes, although the absence of odontogenic epithelium and adapted regulatory processes highlight differences from development (Tziafas *et al*., 2000; Smith and Lesot, 2001). The regulation of odontoblast activity is clearly central to pulp healing, and an understanding of the mechanisms involved in these processes is necessary to enable laboratory studies to be translated

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Key Words

regeneration, dentin-pulp complex, bioengineering, tissue engineering, pulp capping, regenerative medicine.

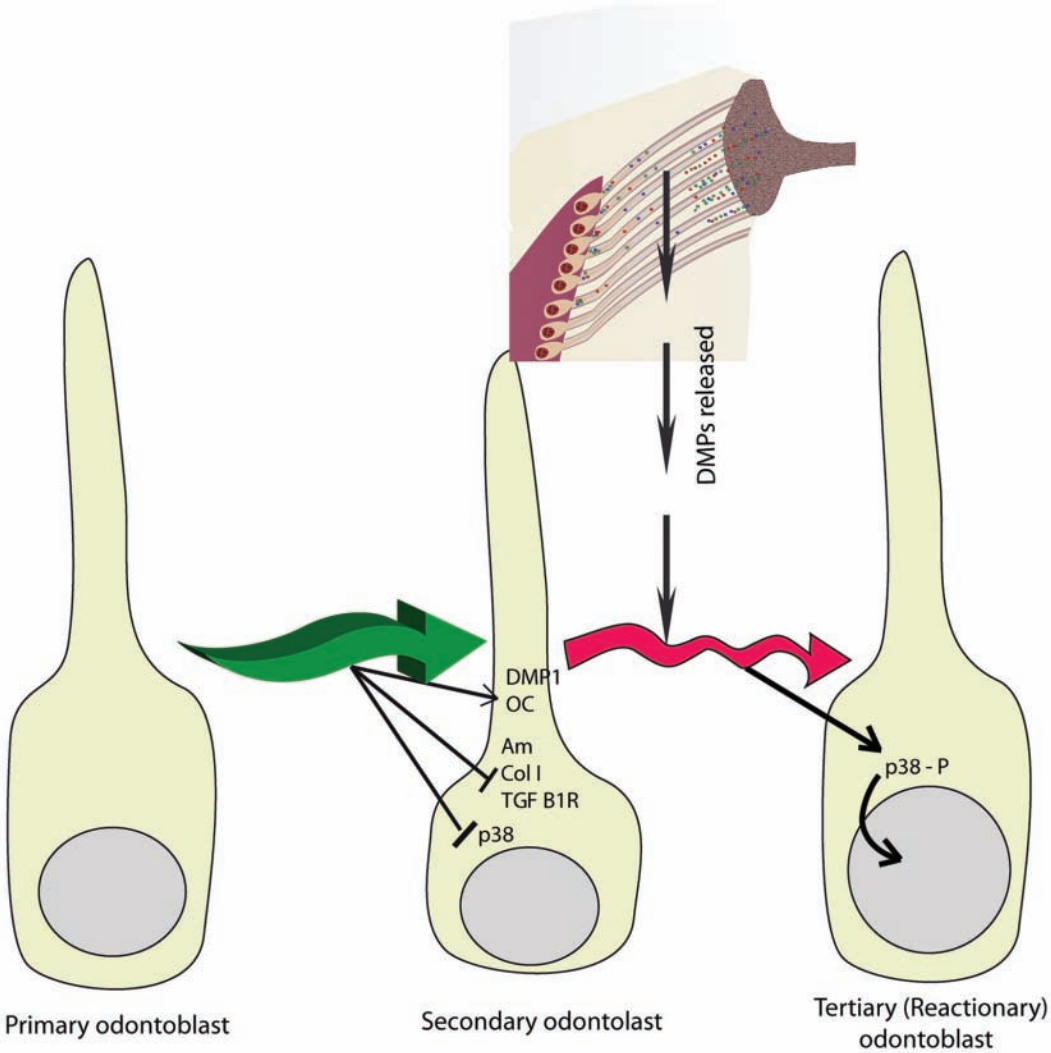

Figure. Dentin secretion and odontoblast activity are related to gene and cascade pathway regulation. Whereas p38 gene expression is down-regulated in secondary odontoblasts compared with primary ones, as for many other genes (Simon *et al.*, 2009), its expression and phosphorylation are up-regulated when odontoblasts are stimulated by dentin matrix proteins, and phosphorylated proteins are translocated to the nucleus (Simon *et al.*, 2010). *In vivo*, embedded dentin matrix proteins are released by carious disease, and reach the odontoblasts *via* the dentinal tubules. Am, amelogenin; Col I, collagen I; TGF β1-R, tissue growth factor β1 receptor; DMPs, dentin matrix proteins; p38-P, phosphorylated p38 protein.

to clinical application. Investigation of genes and/or signaling pathways which might represent targets for the switch from secondary to tertiary dentinogenesis may provide a valid approach to try to address the clinical question of how we regulate dentin secretion. Comparison of the transcriptomes of odontoblasts at different stages of maturity has allowed us to highlight differential regulation of several genes (Simon *et al*., 2009). Among these genes, 6 were involved in regulatory control of the p38 MAPKinase pathway, and this signaling cascade appears to be central to the control of odontoblast secretory activity (Simon *et al*., 2010) (Fig.). Notably, the MAPK/ERK pathway is a central signal transduction pathway that couples intracellular responses to the binding of growth factors at cell-surface receptors. This pathway is complex and includes many signaling mediators (Herlaar and Brown, 1999; Kaminska, 2005; Fernandes *et al*., 2007). It has also been well-established that the MAPK signaling pathway is involved in cellular differentiation, and while the role of TGF-β1 is well-established in odontoblast differentiation during primary development and in tertiary dentinogenesis (D'Souza *et al*., 1992, 1998; Smith and Lesot, 2001; Unterbrink *et al*., 2002; Botero *et al*., 2010), recent publications now indicate that TGF-β1 may also regulate MAPK pathway activity (Ning *et al*., 2002; Zhao *et al*., 2004). The regulatory role of the MAPK pathway therefore warrants further investigation so that we may gain a better understanding of its potential for targeting clinically.

Much of the research in pulp biology has focused on the unique environment of the pulp and the specific nature of the cells and extracellular matrix molecules therein. However, a growing body of evidence indicates similarities between pulp and bone cells (Simon *et al*., 2009), and greater comparison of data derived from bone and dental studies may help in the

development of new regenerative therapies. While much of the stimulus to date has been to regenerate tissues which specifically mimic dentin and pulp, there may be merit in regenerating more bone-like matrices in their place to provide a less-permeable barrier within the dental tissue.

Despite the potential offered by our increased understanding of the basic biology of the dental tissues, these advances have not yet been fully translated into improved treatments in day-to-day dental practice beyond perhaps treatment of early caries. When caries has progressed through much of the depth of the dentin, it is often assumed clinically to be too late for intervention with regenerative techniques. Once caries reaches the pulp, a pulpectomy is usually the first choice of treatment to prevent further painful, infectious complications. However, to date, no guidelines clearly define the indications for pulpectomy *vs*. conservation of pulp vitality. Future progress in the field of regenerative dentistry faces many challenges if there is to be significant change to clinical practice. Translation of the many biotechnological advances will begin to emerge only as a result of pioneer clinicians attempting to test their efficacy within everyday practice.

INFLAMMATION – CHALLENGE OR OPPORTUNITY?

Inflammation is central to defense throughout the body's tissues, and in the dental pulp it has long been considered as a strong clinical contraindication for pulp capping. However, inflammation might also be considered as an early integral stage of reparative or regenerative processes. As soon as caries reaches the dentin (even during superficial disease), dental pulp cells respond *via* a localized and reversible inflammatory process.

Recent publications on inflammation and associated biological markers stress that, depending on the depth of the carious disease, the markers of inflammation are differentially expressed within the pulp tissue (McLachlan *et al*., 2003; Cooper *et al*., 2010). It now appears clear that the initial inflammatory response might be at the heart of the regenerative process, as well as also being involved latterly in the degeneration of the tissue. The balance existing between infection/inflammation and regeneration appears to be fundamental to treatment prognosis.

PULP STEM CELLS – POTENTIALITY AND SPECIFICITY?

The recent reports of several populations of stem cells in and around the tooth [Dental Pulp Stem Cells (DPSC) (Gronthos *et al*., 2000; Miura *et al*., 2003), Stem Cells of Apical Papilla (SCAP) (Sonoyama *et al*., 2008), Periodontal Dental Ligament Stem Cells (PDLSC) (Seo *et al*., 2004)] and our ever-increasing knowledge about mesenchymal stem cell (MSC) processes have been key areas of progress in regenerative medicine over the past decade. Nevertheless, we still have much to learn about the specificity and potentiality of stem or progenitor cell populations in the pulp, as well as their localization and factors responsible for the maintenance of their niches. A central question still to be fully resolved is whether the stem cell populations in pulp are fundamentally distinct from MSCs and to what extent other MSC sources are recruited to injury sites within the pulp *via* the vasculature in health and disease. Cell recruitment, whether from within the pulp or from other sites in the body, will likely be a key event in regeneration, and the concept of cell homing offers exciting opportunities (JY Kim *et al*., 2010; K Kim *et al*., 2010). Such recruitment of stem cells from within the body might be obviated by direct transplantation of autologous stem cells at sites of injury in the tooth. However, this will require availability of autologous stem cells with dentinogenic potentiality in sufficient numbers for transplantation. This approach, however, may be hampered, since *in vitro* cell expansion can lead to phenotypic changes in isolated cell populations (Patel *et al*., 2009). Furthermore, considerable hurdles have to be overcome before autologous stem cell isolation and transplantation can be realized within the environment of routine dental practice. It is important, however, that basic sciences research on stem cell biology in the pulp continue to advance to enable new dental tissue-engineering approaches to be developed.

IS DENTAL TISSUE ENGINEERING A DREAM?

Proof-of-principle for whole-tooth tissue engineering (Modino and Sharpe, 2005; Hu *et al*., 2006; Duailibi *et al*., 2008; Ikeda *et al*., 2009) represents one of the most exciting recent advances, although it may be some years before this can be realistically translated clinically. However, engineering of component tissues of the tooth, *e.g.*, pulp and dentin, may be more easily achieved, and a recent report has demonstrated the feasibility of engineering pulp (Cordeiro *et al*., 2008). It is tempting to speculate that such approaches might allow for the development of injectable hydrogel-based engineered pulp for use in bacteria-free root canals in place of a traditional root canal filling.

Clearly, such tissue-engineering approaches have significant potential for future clinical translation; however, what will be needed to allow for their successful introduction into general practice? First, these approaches must be demonstrated to be more efficacious than more traditional restorative approaches. In view of the relatively poor long-term survival of traditional restorations (Lucarotti *et al*., 2005), this may not be an issue, but since general dental practice is strongly driven by treatment costs, then a cost-benefit analysis will be critical. Technique sensitivity must also be considered, since this is already perceived to be an issue for traditional adhesive restoration systems. Thus, other key requirements for any new tissue-engineering-based therapy might be ease of use, reproducibility, and maintenance of tooth vitality. There would also be a requirement for harvesting of autologous cells for the tissue construct, use of general practitioners' facilities, and ethical acceptance of the procedure. All of these requirements provide several hurdles which need to be overcome; however, even if dental tissue engineering does not become a routine procedure in dental practice, it is likely that we will gain a much deeper understanding of pulp behavior, which can valuably be applied to clinical management of the diseased pulp and may facilitate the development of novel dental materials which promote natural regenerative mechanisms.

ROOT CANAL CONNECTIVE TISSUE REGENERATION

Following infection of the dental pulp, the connective tissue remaining in the root canal initiates various defense responses because of the presence of bacteria. Thus far, the endodontic community has mainly focused its research on root canal disinfection and subsequent filling with an inert material.

Several cross-sectional clinical studies have shown a large percentage of failures associated with periapical disease on badly treated teeth. Since root canal treatments are technically difficult to complete, especially by non-specialist clinicians, endodontics would benefit from alternative approaches (Tavares *et al*., 2009). Early studies attempted to regenerate a vascularized tissue in an empty canal; however, the absence of infection in these studies limited their relevance to the clinical situation (Ostby, 1961; Nygaard-Ostby and Hjortdal, 1971; Horsted and Nygaard-Ostby, 1978; Skoglund *et al*., 1978). Subsequently, the endodontic community has largely focused on more mechanical aspects of root fillings, including the use of disinfection methods, files and instrumentation, and alternative filling materials and techniques. For more than 30 years, little progress has been made on the design of new approaches in endodontics, other than shaping, disinfecting, and novel filling methods.

Over the past decade, revascularization of the root canal has been re-proposed with a two-visit therapeutic approach (Iwaya *et al*., 2001; Trope, 2008). In this procedure, the root canal system is disinfected with a mix of antibiotics, and a blood clot is subsequently induced in the canal itself by irritation of the periapical apex area with an endodontic file. This clot is then protected by a mineral trioxide aggregate plug, and the coronal cavity is sealed and restored by conventional treatment. Although case reports have been published based around this approach (Jung *et al*., 2008), few have attempted to describe the regenerative processes taking place. Although this was initially presented as a regenerative technique, indicating that the regenerated connective tissue was a dental-pulp-like tissue, many authors have described it as a revascularization approach rather than a regenerative one, meaning that there is no histological proof that the new tissue forming in the root canal is comparable with the pulp. Recently, Wang *et al*. described a combination of dentin, cementum, and pulp regeneration in a dog tooth following the use of a revascularization approach (Wang *et al*., 2010). So far, our limited knowledge regarding the healing process has limited the development of new techniques. The hypothetical involvement of SCAP cells remaining viable even in very aggressive infection conditions implies a limitation to this therapy in immature necrotic teeth (Huang *et al*., 2008); however, further work on SCAP cell use in this area is required.

Many other scientific/clinical questions still remain in this area. For example, is the histological nature of regenerated tissue important? While regeneration of the whole dental pulp would be ideal clinically, regenerating a connective tissue, mineralized or not, might provide an acceptable compromise. Indeed, the aim of our current research is to prevent any further infection of the tooth and protect the periapical tissues. The inert materials used in endodontics have limited sealing ability and, ultimately, a limited clinical longevity. Currently, very few materials used in this process are truly biocompatible and, consequently, promote beneficial biological responses. Clearly, filling of the canal with a biological tissue might provide an optimal clinical solution for restoration longevity.

Bioengineered connective tissue requires neo-vascularization and, ideally, new innervation. Indeed, innervation is necessary to maintain the homeostasis of the regenerated tissue. Our current diagnostic tools are used only to monitor tissue innervation (thermal tests or electric pulp testing), while none of these tests enables the blood circulation to be monitored, which is considered as the only true proof of tissue vitality.

DENTAL MATERIALS FOR ENDODONTIC THERAPY

For a long time, clinicians have focused on two main properties of the materials used: (1) their sealing ability and (2) their biocompatibility or lack of cytotoxicity. Calcium hydroxide has been proposed for many years as the 'gold standard' for pulp capping. In the past 15 years, mineral trioxide aggregate (MTA) has received much attention and has been proposed as a new endodontic dental material for pulp capping based on its excellent sealing abilities. The action of both of these materials has never been completely understood, although their release of bioactive molecules from the dentin matrix may offer some explanation (Graham *et al*., 2006).

The sealing ability alone of a material is not a sufficient property to induce pulp regeneration, although it may create the conductive environment required for natural regeneration. Direct pulp-capping with bonded resins has shown promising clinical results in terms of maintenance of the pulp vitality (Heitmann and Unterbrink, 1995); however, no biological activity of this material has been demonstrated, including stimulation of dentin bridge formation (Koliniotou-Koumpia and Tziafas, 2005). It could be postulated, however, that maintenance of pulp vitality is sufficient for the technique to be considered reliable and justifiable, but the deterioration of the sealing ability over time due to the degradation of the bonding agent with resins is a concern for long-term prognosis. Clearly, a biological closure of the wound probably provides a better and more reliable approach for the treatment of disease. Based on these observations, calcium hydroxide and MTA might be considered as bioactive materials, since they induce the formation of a dentin bridge. Few histological differences are noticeable, with osteodentin being commonly observed beneath calcium hydroxide restorations, while orthodentin is generally observed under MTA restorations, with odontoblast-like cells that express dentin sialoprotein also present (Simon *et al*., 2008).

A possible activity of local dentin dissolution and bioactive dentin matrix protein release by these materials has been proposed (Tomson *et al*., 2007), and this process may explain the biological effects of these products. The differential concentrations of the released products between calcium hydroxide and grey and white MTA have been suggested as a potential explanation for the histological differences in the generation of a dentin bridge (Nair *et al*., 2008). Based on the hypothesis of this controlled dentin dissolution by these materials, other authors have proposed the use of other products, such as orthophosphoric acid or other cavity etchants, to induce the release of growth

factors initially entombed in the dentin matrix (Ferracane *et al*., 2010). Dentin conditioners and bonding agents provide a viable target for regenerative dentistry, since they might be an ideal support for the delivery and release of biological active agents. Indeed, several companies have already exploited this approach by adding antimicrobial agents in their bonding products, allowing for a prolonged and controlled effect on the underlying dental pulp (Protect Bond®, Kuraray, Frankfurt, Germany).

An alternative approach has also been explored by other groups, proposing the role of specific pulp extracellular matrix proteins (Goldberg *et al*., 2006), such as amelogenin (Goldberg *et al*., 2008), DMP1 (Chaussain *et al*., 2009), or MEPE fragment (Dentonin) (Six *et al*., 2007) to stimulate regeneration. A hypothesis presented by these authors is that these molecules could be used to induce mineralization of the pulp, thereby sealing the root canal. If complete mineralization of the root canal system is to be a reliable technique, the inducing factors will have to be incorporated during the restorative approach. Nanotechnological approaches might ultimately provide a realistic solution for such a controlled delivery process, but we should not underestimate the challenges in developing reliable delivery systems for biological agents.

CONCLUSIONS

A main focus of current scientific research is to improve our knowledge regarding the biological processes involved in dental tissue regeneration to gain a better understanding of the disease and repair processes. Clearly, this knowledge has the potential to be translated clinically, providing new therapies for patient benefit. Indeed, the many recent advances in our knowledge of pulp stem cells and molecular signaling molecules within the tooth now provide exciting opportunities for clinical translation to novel therapies in the future. The regenerative endodontics field must be focused not only on disinfection and pulp tissue regeneration, but also on partial regeneration of dentin-pulp-like tissues, allowing for the maintenance of root pulp vitality.

A more biological approach to endodontics will likely increase the success rate of dental treatments globally within both developed and developing countries and has potential for strong impact on long-term public health. However, such translation will require the partnership of researchers and skilled clinicians who can effectively apply advances in knowledge to appropriate clinical cases and develop novel therapies which can be realistically tested and subsequently used within the clinic.

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