Tissue Engineering in Regenerative Endodontics

Introduction

Regenerative Endodontics

Regenerative endodontics encompasses a range of strategies that aim to preserve tooth vitality, maintain homeostatic functions, and promote tissue repair and regeneration following pulpal disease, to avoid the need for pulpectomy or tooth extraction (Simon, 2014). The strategies employed range from creation of clinical protocols to harness the natural wound healing capacity of pulp, to procedures promoting revascularisation of an empty root canal, to the use of stem cells in tissue engineering (Simon S., 2013;216:(E13)).

Stem Cells and Differentiation

Differentiation is the process by which cells become more specialised to carry out a specific function.

Stem cells are undifferentiated cells capable of self-renewal and giving rise to an indefinite number of daughter cells, capable of differentiation; i.e. becoming adapted for their required function. There are 2 main categories of stem cells: embryonic and non-embryonic, also known as adult stem cells. Embryonic stem cells (ESCs) are described as pluripotent, as they have the ability to differentiate into a wide range of cell type lineages. In contrast to this, adult stem cells (ASCs) are termed 'multipotent', as their range of potential cell lines to differentiate into is more limited (Tuch, 2006;35(9)). ASCs undergo a process called asymmetric cell division, meaning that when an ASC divides, one daughter cell remains a stem cell (thus achieving self-renewal the stem-cell population), and the other daughter cell will continue to divide and differentiate, subsequently producing cells with specific characteristics for certain tasks or environment, such as how red blood cells are adapted to maximise oxygen-carrying capacity, or how nerve cells are adapted to optimise action potential transmission (Ouellet J., 2012;196).

Induced pluripotent stem cells are a form of stem cell reverse engineered from a differentiated cell via a process of dedifferentiation and re-differentiation, under the influence of a limited number of transcription factors (proteins that cause specific gene expression), effectively shifting their phenotype, or role, from one type to another. The application of induced pluripotent stem cells may provide some highly interesting breakthroughs in all areas of regenerative medicine and dentistry in the future (Goldberg M. , 2014).

Formation and Biology of Dentine Pulp Complex

Dentine is a mineralised hard tissue layer beneath dental enamel, made up largely of inorganic materials, collagens, water and, interestingly, a wide variety of bioactive molecules, with a series of fluid filled tubules running from the enamel-dentine junction to the pulp. These are cytokines and growth factors embedded within the dentine matrix, including vascular endothelial growth factor (VEGF) fibroblast growth factor 2 (FGF2) and bone morphogenic proteins (BMPs) (Smith A.J., 2012;57). Primary dentine is formed by the action of odontoblasts during tooth development, secondary dentine due to odontoblast action post-developmentally, throughout the lifespan of the tooth, and tertiary dentine is laid down in response to injury (see below). The pulp is a soft connective tissue at the core of a tooth containing blood vessels, nerves, a variety of cell types and the extracellular matrix. Whilst appearing anatomically distinct, the dentine and pulp act synergistically as a dentine-pulp complex. The tubules at the dentine-pulp interface contain

extensions of some pulpal cells, such as odontoblast extensions, afferent nerve terminals and processes of some immunocompetent cells; which are supplied with nutrients and structural materials via the pulp's extensive vasculature. Trauma or disease, such as caries, can cause an inflammatory response in the pulp. Depending on the extent, this can be mild and reversible or may result in the death of the pulp (Orchardson, 2001; 28).

The Odontoblast

Odontoblasts form a lining layer at the dentine-pulp junction, and are described as fully differentiated, post-mitotic cells. This means that once odontoblasts form, they are unable to divide and produce daughter cells. This dearth of cell turnover leads to the odontoblast's continued presence for the entire lifespan of the tooth, in the absence of injury (Simon S., 2013;216:(E13)).

Pulpal Response to Operative Procedures

Any operative procedure provokes a pulp reaction, the extent of which determines the degree of injury and subsequent required treatment. The responses may involve neural, vascular, dentinogenic and immune components in any combination. Cavities or decay deep into dentine can result in disruption or death of odontoblasts in the odontoblast layer (Orchardson, 2001; 28).

Pulp Wound Healing

Wound healing is a pathological process, thus it would follow that the regulatory control of the local environment would differ from physiological processes such as homeostasis and tissue development (Simon S., 2013;216:(E13)).

It is important to ascertain the extent of injury sustained by the pulp, either by traumatic or carious origin, when considering the pulpal response, as this has a significant effect on the subsequent cellular events (Simon S., 2013;216:(E13)). If the injury is due to caries, pulpal inflammation arises due to cariogenic bacteria and their byproducts diffusing through dentinal tubules. With disease progression towards the pulp, both innate and adaptive immunities are activated (Zhong, 2012;38(6)), and the cellular events within the pulp shift significantly, resulting in the death of odontoblasts [as these are the first cells encountered in the pulp by cariogenic bacteria (Love, 2002;13(2))] local to the site of injury. This will further exacerbate inflammatory responses within the pulp (Smith A J, 1995).

Reactionary vs Reparative Dentinogenesis

A low grade or initial injury, not sufficient enough to kill off local odontoblasts, causes upregulation of odontoblast secretory activity at the site of injury. This results in deposition of reactionary dentine, a form of tertiary dentine, at the dentine-pulp interface in an attempt to 'wall-off' the pulp from the injury (Smith A J, 1995). This is known as 'reactionary dentinogenesis', and as it is due to action of existing odontoblasts, the new dentine layer formed expresses a regular tubular structure, in continuity with, and acting identically to the remaining primary or secondary dentine present (Simon S., 2013;216:(E13)).

Reparative dentinogenesis occurs when the insult to the pulp is so great that local primary odontoblasts are killed off. As a consequence of the post-mitotic nature of these cells, they can only be replaced by the formation of new odontoblast-like cells. These cells are formed by recruitment of stem/progenitor cells, accompanied by sufficient cellular signalling to cause their differentiation into odontoblast-like cells (Simon S., 2013;216:(E13)). In During dentine-pulp regeneration, the driving force of reparative dentinogenesis is the interplay between pulp stem cells and signalling molecules

derived from the dentine matrix (Goldberg M. e., 2004:15(1)). The new generation of odontoblast-like cells secrete a 'reparative' variant of tertiary dentine at the site of injury (Smith A J, 1995). This process has been harnessed in conventional endodontic therapy when pulp capping is indicated after a pulpal exposure. Formation of a dentine bridge between the site of injury and pulp after sufficient time has passed is well documented. (Simon S., 2013;216:(E13)). Unlike reactionary dentine, the structure of reparative dentine can be highly variable, ranging from a regular tubular structure to a highly disorganised atubular structure, reflecting the pathological nature of reparative dentinogenesis (Simon S., 2013;216:(E13)).

Angiogenesis

Angiogenesis, or the formation of new blood vessels from existing ones, is paramount in wound healing and tissue development, and is tightly regulated in the dental pulp. It is well documented that at sites of pulpal injury, blood micro-vessel density can be rapidly adapted to account for the increased demand of nutrients and oxygen required by cells in the regeneration process (de Peralta, 2014). It is also well known that certain bacterial by-products; lipoteichoic acid (LTA) in gram-positive and lipopolysaccharide (LPS) from gram-negative cariogenic species induce vascular endothelial growth factor (VEGF) secretion by pulp cells. VEGF is widely recognised as causing vascular permeability and oedema, both of which are required in an inflammatory and angiogenic response. (Nagy, 2008;11(2)).

Role of Stem Cells

1. Stem/Progenitor Recruitment

   There have been 3 populations of dental stem cells that have been identified as having capacity to give rise to odontoblast like cells: dental pulps stem cells (DPSCs), stem cells from the apical papilla (SCAPs) and stem cells from human exfoliated deciduous teeth (SHEDs). Whilst these cells have distinguishing cell surface markers (i.e. characteristic proteins on each subpopulation to allow each to be identified), and varied localisations; from perivascular niches to the apical papillae of the tooth, they all arise from mesenchymal stem cells (MSCs). This ease of access to MSCs in the pulp, especially in the case of exfoliated deciduous teeth, offers a much less invasive approach to obtain an abundant supply of MSCs, currently isolated form bone marrow, for use in regenerative medicine (Simon S., 2013;216:(E13)).

   Carious dissolution of dentine hard tissue results in the release of dentine matrix proteins 'fossilised' within the inorganic material. One function of these molecules is to promote stem cell recruitment (Smith JG., 2012:318), via cell homing through the vasculature formed upon natural wound healing (Kim JY., 2010;16).

   When odontoblast-like cell formation is required, once stem/progenitor cells have migrated to the injury site, they are exposed to the pulpal environment, resulting in specific phenotypic expression. One advantage of additional stem cell recruitment from outside the pulp (as well as those residing within the pulp), would be the significant increase in number of cells available post-injury (Simon S., 2013;216:(E13)).

2. Odontoblast-Like Cell Differentiation

   During tooth development, odontoblast differentiation is regulated by enamel epithelium. However, as this is not present in mature teeth, an alternative driving factor is required for odontoblast-like cell differentiation in dentine-pulp regeneration. The cytokines and growth factors throughout the dentine matrix (released on dissolution of dentinal hard tissue) are

recognised to be involved in signalling of odontoblast differentiation (Simon S., 2013;216:(E13)). This has been shown in experiments whereby dentine bridge formation was induced using dentine chips placed in the base of a prepared cavity (Anneroth G., 1972;23). Sequestration of these bioactive molecules in a fossilised state in dentine grants a limited self-repair mechanism for the tooth (Simon S., 2013;216:(E13)).

3. Odontoblast-like Cell Secretory Activity

Whilst achieving successful tertiary dentinogenesis is key in pulpal healing, the process must be regulated to avoid pulp obliteration and maintenance of pulp vitality (McCabe, 2012:45). Unfortunately, the majority of research on this topic has focussed on odontoblast differentiation and activation of secretory activity, whilst the subsequent regulation of this activity and the required eventual downregulation of odontoblast activity have been neglected.

DPSCs have been shown to readily differentiate into functional odontoblast-like cells, expressing dentine sialophosphoprotein (DSPP) and dentine matrix protein 1 (DMP1), capable of synthesising new tubular dentine. (Sakai VT., 2010;89).

P38-MAPK signalling has been proposed as playing a major role in the regulation of odontoblast activity, as it has been found to be active on odontoblast (Simon S, The MAPK pathway is involved in odontoblast stimulation via p38 phosphorylation, 2010;36) and pulp cell (Botero TM, 2010;89) activation on exposure to bacterial products and growth factors and cytokines in the dentine matrix, during tertiary dentinogenesis and regenerative events within the pulp (Simon S., 2013;216:(E13)). Targeting this pathway with inhibitory agents may allow pharmaceutical control of regulation of dentinogenesis.

Reaction of the Pulp to Dental Materials in Conventional Endodontic Therapy

Various irrigants and etchants used in conventional endodontic therapy can cause release of the bioactive molecules sequestered in dentine, similar to carious demineralisation (Smith AJ, 1998;77), causing dentinogenic events (Murray PE, 2008;41). This effect can also be stimulated by pulp capping agents on pulpal exposure, such as calcium hydroxide and mineral trioxide aggregate (MTA) (Graham L, 2006;27). The local release of these cytokines can result in recruitment/homing or endogenous stem cells to the site of injury, inducing area specific pulpal regeneration or repair (Smith JG., 2012:318).

Sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) are widely used in canal preparation. However, the order of their application has a marked effect on tissue damage, thus careful attention to canal irrigation and disinfection protocols will be paramount to successful treatment outcomes in regenerative endodontics and considerable scope remains for optimisation of these protocols (Simon S., 2013;216:(E13)).

Regenerative Endodontic Procedures

DPSCs function to be quiescent until required to differentiate into the required cells e.g. odontoblasts, for response to injury, such as deep caries. Their discovery provided an explanation for the regenerative potential of the pulp, previously widely documented (Fitzgerald, 1990;35). DPSCs are multipotent cells, consistent with MSCs, and can differentiate into one of three distinct cell lineages: (1) osteo/odontogenic, (2) adipogenic and (3) neurogenic (Gronthos S., 2000;97). If accompanied with VEGF, DPSCs have also been shown to differentiate into vascular endothelial cells,

capable of anastomosing with the existing vasculature, showing their role in the perfusion of the newly regenerated dental pulp tissue (Sakai VT., 2010;89).

DPSCs have been shown to be isolated from clinically compromised dental pulps with irreversible pulpitis. The stem cells isolated from such pulps were demonstrated to have great proliferative potential, including *ex vivo* odonto/osteogenic differentiation capacity for subsequent *in vivo* tissue regeneration. This experiment shows how even these compromised teeth may act as a reservoir of stem cells for regenerative procedures, despite the high demands of replacing odontoblasts (Alongi DJ., 2010;5(4)).

Creation of culture conditions to store DPSCs that simultaneously maintain their stem cell-like functions, whilst also enabling their proliferation in the absence of animal derived reagents will be of utmost important for the clinical use of DPSCs in cell-based tissue-engineering. This highlights the need for more research into the cell markers present on the surface of DPSCs, as these are the targets of cellular signalling that drives cellular events and subsequent differentiation (de Peralta, 2014).

Case Selection

Most case reports focus on the use of regenerative endodontics in immature permanent teeth, with the aim of completing root growth. Regenerative endodontic approaches have similar primary determinants of treatment outcome to conventional endodontics, namely control of inflammation and infection (Simon S., 2013;216:(E13)), thus with regards to case selection, one must consider if the tooth could be treated with conventional endodontic strategies with respect to operative techniques, such as obtaining optimal moisture control. If the answer is yes, the tooth in question may be a good candidate for regenerative endodontics.

However, whilst control of infection and inflammation is important in regenerative endodontics, there is a greater emphasis of carrying this out in a gentler, more controlled fashion, so as avoid unnecessary damage to tissues from which we aim to regenerate pulpal cells. This control could, in theory, increase the risk of procedure failure in terms of treatment efficacy, thus case selection is highly important (Simon S., 2013;216:(E13)).

Therefore, in the future, regenerative endodontics will likely be the first line treatment option in cases where infection and inflammation are judged clinically, to be minimal. However, this emphasises the need for further research into diagnostic techniques to ascertain pulp status, as current conventional methods leave a lot to be desired (Mejare IA, 2012:45).

Materials

1. Stem Cells

   A. Adult Post-natal Stem Cells

      I. Stem Cells Permanently Present in Adult Teeth

         Dental Pulp Stem Cells (DPSCs) can be derived from the pulp of permanent third molars. They are derived from neural crest cells, a group of multipotent stem cells responsible for formation of the majority of bone and cartilage that forms the face and head (Achilleos A, 2012;22). How readily available DPSCs and stem cells from human exfoliated deciduous teeth (SHEDs) are as a source of autologous stem cells provides an attractive option for use in regenerative dentistry (Demarco, 2011:22). In an experiment where DPSCs were transplanted into immunocompromised mice, a dentine-like structure, lined with human odontoblast-like cells, surrounding a pulp-like structure was

produced (Gronthos S., 2000;97). Stem cells from the apical papilla (SCAPs) could provide an alternative reservoir of odontoblasts. These cells have already been elucidated as pivotal in continuous formation of root dentine (Huang GT-J, 2008;34).

II. Stem Cells Originating from Periodontal and Other Tissues

Other groups of mesenchymal stem cells from the surrounding dental tissues may also be viable sources for tooth tissue regeneration: stem cells of the apical part of the papilla (SCAP), stem cells from the dental follicle (DFSC), periodontal ligament stem cells (PDLSC) and bone marrow derived mesenchymal stem cells (BMSC) are all potential sources of stem cells (Peng L., 2009;1). Non-dental stem cells are currently being looked into as they are known to share similar properties to non-dental stem cells, including: a high proliferation rate, the ability to differentiate into multiple tissue and cell types, and ease of induction (Gronthos S., 2000;97).

B. Niches of Stem Cells

The microenvironments inhabited by stem cells are known as a cell niches. A niche comprises all elements in the stem cells immediate vicinity, including non-stem cells, which are in direct contact with the stem cell, as well as soluble molecules in the surrounding extra-cellular matrix (Kolf, 2007;9). The local microenvironment, i.e. niche, regulates stem cell behaviour and is characterised by three properties: (1) a niche is an anatomic space, creating a place for stem cells to be regulated; (2) within this anatomic space, stem cells are instructed to control maintenance, quiescence, self-renewal, and recruitment towards differentiation, fate determination and long-term regenerative capacity; (3) the niche guides cell motility. Specific niches have been characterised in the dental pulp (Ema, 2012;120). The role of the extracellular matrix is to provide a medium for diffusion of chemical and mechanical signals, whilst providing structural support, thus is functional in processes such as in inflammatory and circulating cell recruitment to the cell niche (Goldberg M. , 2014).

2. Bioactive Molecules

The dentine matrix is a reservoir of bioactive molecules, shown to be vital in the process of pulp regeneration, such as growth factors and cytokines. These molecules are released from the dentine hard tissue due to acid demineralisation from bacterial attack (Roberts-Clark, 2000;45(11)). The role of these dentine-derived morphogenic and angiogenic signals in pulp tissue regeneration is their ability to induce stem cell differentiation (de Peralta, 2014).

I. Vascular Endothelial Growth Factor (VEGF)

VEGF is one such growth factor. It has been hypothesised to contribute to the enhanced vascular permeability and vasodilation seen in pulp tissue during pulpitis (Soden, 2009;88(9)), causing a subsequent increase in intrapulpal pressure (Heyeraas, 1992;88(1)). Therefore, whilst VEGF appears a necessary factor to induce angiogenesis, a necessary requirement for new tissue growth, too high a concentration may result in an increased interstitial pressure within the root canal, with the risk of causing tissue damage, hindering any expected new tissue growth. More research will be required to determine a suitable protocol with respect to finding an appropriate amount of exogenous angiogenic factors, such as VEGF, to introduce, to maximise revascularisation and new tissue growth, without causing damage to the remaining tissues (de Peralta, 2014).

3. Scaffold

A. Introduction

The third component of scaffold-based tissue engineering concepts is the scaffold itself; a structural matrix which functions to support cell adhesion, proliferation, differentiation and tissue formation (Galler K. , 2014). In the past, the importance of a scaffold has been overlooked, using bio-inert materials simply as carriers for stem cells to deliver them to the appropriate site. Any subsequent tissue regeneration would rely solely on the intrinsic capability of the stem cells (Galler KM, 2010;132(9)). As experiments using this method failed to induce sufficient regeneration, there was a need for construction of novel biometric scaffolds that play an active role in promoting various cell-matrix interactions via mechanical and biochemical cues (Galler K. , 2014). The subsequent transition of scaffolds from inert, passive cell carriers to inductive and instructive matrices means the required cellular behaviour can be stimulated. Functions of these more sophisticated biomaterials that can be adapted to improve their efficacy include stiffness, degradation rate and pattern, controlled release of growth factors and cytokines for differentiation, and bioactive motif incorporation (Galler K. , 2014).

A scaffold for dentine-pulp-complex engineering should enable and support vascularisation, cell-matrix interactions, biodegradation, growth factor incorporation, biomineralisation, and contamination control. Control of these features is adapted through customising the scaffold to incorporate specific features (Galler K. , 2014), thus a range of scaffolds could be constructed and implemented based on the clinical scenario indicated.

B. Optimal Scaffold Properties:
    1. Optimal flow on application to avoid bubbles and reduce contraction after hard tissue formation
    2. Biocompatible with a high bioactivity to be impregnated with growth factors and cytokines secreted by transplanted stem cells for homing, engraftment, anti-apoptosis and proliferation of endogenous stem/progenitor cells.
    3. Biodegradability
    4. Close resemblance of cell's physiological environment.
    5. No stimulation of differentiation of odontoblasts and mineral deposition inside the root canal except along the dentinal wall and in the dentine defect (Yuan Z, 2011:17(5)).

C. Materials

    Self-assembling peptides are a promising development in scaffold engineering, and are a good example of a material that can be modified and customised, tackling issues surrounding the specific requirements of pulpal regeneration. They consist of a protein matrix, with each protein 15-20 amino acids in length, which self-assemble, constructing a nanofibrous network which traps water, thus forming a hydrogel. As these proteins are constructed from naturally occurring amino-acids, the scaffold is fully biocompatible and biodegradable. The advantages of using hydrogel-like materials include viscoelastic properties akin to soft connective tissues and rapid metabolite diffusion. The amino acid and subsequent peptide sequence of a protein determines the properties of the resulting material, thus due to the customisable nature of the amino acid sequence and thus incorporation of multiple protein domains, a further advantage of self-assembling peptides is the increased control over material properties and the potential of tailoring their uses to a wide range of clinical applications (Galler K. e., 2011;23(3))

D. Biomineralisation

    During dentine and enamel synthesis, an organic matrix is secreted prior to mineralisation by ameloblasts and odontoblasts. Negatively charged surface proteins and

phosphorylated serine amino acids on the scaffold attract calcium ions, initiating crystal growth. Non-collagenous proteins in the secreted matrix act to control orientation and elongation of the nascent hydroxyapatite crystals (Galler K. e., 2011;23(3)). Mineral nucleation along the nanofibers of self-assembling peptides in the presence of dental stem cells has been previously demonstrated by Galler et al (Galler K. e., 2008;(14)), thus using self-assembling peptides with a similar amino acid sequence and motifs, may allow formation of the mineralised dental component of the dentine pulp complex (Galler K. e., 2011;23(3)).

E. Conclusion

The functions and qualities of an ideal delivery system include: (1) delivery of, or assist in homing of resident stem cells, (2) binding and prolonging the bioavailability of several growth factors and cytokines, (3) allowing connection to the established pulpal vasculature, (4) can be inserted into small defects whilst (5) allowing suitable contamination control (Galler K. , 2014), which at present does not exist. Optimisation of a scaffold that can fulfil these criteria would greatly contribute to future regenerative endodontic strategies.

Experimental Methods and Models of Pulpal Regeneration

1. Intro

Despite the success of initial conventional pulp therapy, incomplete disinfection, coronal leakage etc. can lead to reinfection, subsequent periapical disease and if unamenable to re-root treatment, in the worst case, extraction of the offending tooth (Nakashima M A. A., 2005; 31 (10)). The success rate of re-treatment of an endodontically failed tooth with a periapical lesion and/or other clinical symptoms is 50-70% (Matchou P, 2003). Whilst material science has progressed rapidly in recent decades, we are yet to create a synthetic restorative material superior to natural pulp and dentine. Therefore, regeneration of the dental pulp would be optimal to restore tooth function and morphology (Nakashima M A. A., 2005; 31 (10)).

2. Ectopic Approach for Pulp Regeneration

This is a distinct approach from revascularisation/regeneration whilst still employing the tissue engineering triad, discussed above, namely, stem/progenitor cells, morpho-genetic signalling molecules and an extracellular scaffold (Nakashima M A. A., 2005; 31 (10)). It involves full length human roots injected with a scaffold that contains SHEDs, transplanted subcutaneously with the subsequent aim of replantation in the appropriate site. This resulted in pulp-like tissue regeneration with odontoblasts found to be able to generate tubular dentine (Rosa V, 2013;92 (11)).

3. Orthotopic Models for Pulp Regeneration in Mature Teeth

A. Pulp Stem/Progenitor Cells

For pulp regeneration to stimulate stem cell migration/homing, proliferation and extracellular matrix formation, re-innervation and ingress of blood vessels from the remaining pulpal vasculature (angiogenesis) is paramount (Nakashima M A. A., 2005; 31 (10)). Thus a further requirement of stem cells, besides multi-differentiation potential and self-renewal is influencing induction of angiogenesis and neurogenesis. Sub-fractions of DPSCs with increased angiogenic/neurogenic potential have been isolated from human adult teeth, however the extracellular matrix surrounding this sub-fraction of DPSCs contained a high concentration of angiogenic and neurotrophic factors (Nakashima M I. K., 2009;20 (5-6)), therefore it may be

hard to elucidate the extent of the role this sub-fraction plays in induction of new growth of blood vessels and nerves.

B. Partial Pulp Regeneration

This approach would be applicable in cases of coronal pulpotomies once caries have been removed. Two methods have been researched:

1. Transplantation of Engineered Pulp/Dentine Tissue or DPSC Sub-fractions with Scaffold

   This approach yielded regenerated pulp tissue with well-developed vascularisation and innervation with tubular dentine formation along the dentinal wall (Iohara K, 2009;4(3)).
2. Application of Homing/Migration Factors e.g. Fibroblast Growth Factor 2 (FGF2) with Scaffold –

   This technique is advantageous over the other as the matrix applied is acellular. This simultaneously tackles issues with immune rejection of allogenic cells, cost with respect cell isolation, or lack thereof, handling, storage etc. (Kim JY, 2010; 16(10)). There are multiple studies showing promising results using this method: (1) The results from Kim et al., showed endogenous progenitor cell recruitment with subsequent partial pulp regeneration (Kim JY, 2010; 16(10)); (2) Vascularisation and re-cellularisation was observed in human teeth endodontically treated with FGF2 followed by ectopic transplantation (Suzuki T, 2011;90(8)), (3) Osteodentine formation has been observed on the surface of regenerated pulps when exposed to a controlled release of FGF2 from a gelatin hydrogel (scaffold) (Kitamura C, 2012).

   Matrix metalloproteinase 3 (MMP3) is also a migration/homing factor implicated in proliferative, migratory and anti-apoptotic effects on endothelial cells *in vitro*. This had the effect of promoting pulp wound healing and angiogenesis on amputated rat incisors (Zheng L, 2009;175(5)).

C. Models of Complete Pulp Regeneration with DPSC Sub-fractions and Homing/Migration Factors

The discovery of SCAPs has posed the theory of whole pulp regeneration after pulpectomy in immature (i.e. developing) teeth with incomplete apical closure (Sonoyama W, 2008;34(2)). Once a pulpectomy has been carried out on a tooth, it raises the issue of the nutrient and oxygen requirement of transplanted cells if regeneration is to be attempted. Therefore, a DPSC sub-fraction with high migration, angiogenic and re-innervation properties would be most beneficial to optimise ingrowth of pulp-like tissue in now empty root canals (Nakashima M I. K., 2009;20 (5-6))

Experimental Models for Complete Pulp Regeneration

Model 1 – The tooth is extracted and the apical 1mm of the root is resected and the apical foramen is enlarged to 0.8mm after the whole pulp is removed. Autologous DPSCs impregnated on a scaffold is injected into the apical part of the root canals, with the coronal aspect filled with homing/migration factors e.gs. FGF2, stromal cell derived factor 1 (SDF-1), through a conventionally prepared access cavity. The tooth is then re-implanted into the alveolar bone. Subsequent sectioning of these teeth showed pulp-like tissue formation, and odontoblast-like cells extending their processes into dentinal tubules was seen at 14, and 35 days respectively (Iohara K., 2014).

Model 2 – Similar to the first model, except the tooth is kept *in situ*, thus the apical 1mm is kept in place, with the apex being widened to 0.6-0.7mm. This method resulted in similar pulpal regeneration seen at 14 days. This method is advantageous over

extraction method in that no external or internal resorption of the root was noted (Iohara K., 2014). The pulp tissue regenerated by transplantation of DPSC sub-fractions and SDF1 has been shown to be identical to normal, functional pulp tissue by expression of similar pulp tissue markers. This has been confirmed by two-dimensional electrophoretic analyses and microarray analyses, showing qualitative and quantitative protein and mRNA expression patterns of regenerated tissues are practically identical to normal pulp. (Iohara H, 2011;17(15-16)). Pulp regeneration based on DPSC sub-fractions and SDF-1 mechanisms are postulated as follows: Induction of migration of stem/progenitor cells into the root call and subsequent induction of differentiation into endothelial cells and odontoblasts. Angiogenesis and re-innervation are stimulated by angiogenic/neurotrophic factors secreted by transplanted stem cells (Iohara K., 2014).

4. Complete Pulp Regeneration with Periapical Disease

Model 3 – The method for this model is similar to model 2 but in a tooth with periapical disease. The results showed a marked decrease in the amount of regenerated tissue, with the tissue containing inflammatory cell inclusions. (Iohara K., 2014).

5. Complete Pulp Regeneration for the Aged

Model 4 – Due to canal sclerosis, widely accepted to correlate with age, teeth from older patients have a reduced quantity of pulpal tissue available, thus contain fewer viable DPSCs than young patients. Despite this, it has been reported that DPSCs can be obtained from a small amount of pulpal tissue from samples from older patients (Iida K, 2010;55(9)). The noticeable decline in pulp regeneration in older patients may be related to the decrease in regenerative potential of the residing stem cells. However, this is still unclear and further work is needed to elicit cellular and molecular mechanisms underpinning age-related changes of the stem cell niche which effect stem cell function

6. Other Sources of Tissue Stem Cells for Pulp Regeneration

Model 5 – The use of stem cells can carry several ethical, and other, more practical issues, such as immune rejection. Autologous MSCs from either bone marrow or adipose cover many ethical and to some-extent the immunoreactive issues. However, there are differences in protein expression to DPSCs (Noel D, 2008;314(7)). Adipose and bone marrow stem cells were found to be express lower migration activity and decreased angiogenic/neurotrophic factor expression than DPSCs. Transplantation of bone marrow stem cells resulted in a significantly decreased amount of pulp-like tissue regeneration, whilst adipose stem/progenitor cells induced a similar amount of regenerated pulp tissue, compared to pulpal stem cells (Ishizaka R, 2012;34(8)). The advantage of using adipose stem cells, however, in favour of DPSCs requires further research.

Further Problems to Overcome

An important determinant of treatment outcome to consider is the extent of surgical intervention during canal preparation with regards to preservation of tissue vitality. Pulpotomies are commonly used in the management of an infected pulp, especially in younger patients, where efforts to maintain at least partial pulp vitality are maximised, however, due to the difficulties in determining the depth of infection and disease progression, more radical, overtreatment is common (Simon S., 2013;216:(E13)). Evidence showing pulpal inflammation to a depth of two millimetres in

deep carious lesions (Mjor IA, 1972;34), led to the proposition of limiting surgical intervention to a more superficial partial pulpotomy (Cvek., 1978;4).

It is evident there is a strong correlation between canal preparation procedures and clinical outcomes for regenerative endodontic procedures. Careful attention to infection control, the extent of surgical excision of inflamed tissue and case selection in the context of preservation of tissue vitality are central features of regenerative endodontics (Simon S., 2013;216:(E13)).

Future Prospects and Areas for Research

This paper has highlighted a number of areas requiring further research before an optimal regenerative endodontic procedure protocol can be designed.

It is recognised that clinical signs and symptoms do not correlate with the histological status of the pulp (Dummer, 1980;13(1)). Since treatment success is based on symptoms reported by the patient, as well as conventional, rudimentary testing methods such as thermal, electrical, tenderness to percussion or palpation and radiographic assessment (Simon, 2014), this highlights the importance of developing more sensitive methods of detecting pulp status, both in treatment planning and in assessing clinical outcome. The advanced assessment techniques currently available to assess histological structure, cell behaviour and immunological/inflammation status involving assessing a tooth section etc are not a viable option for obvious reasons.

With regards to the manufacturing of a matrix for use in pulpal regeneration for a successful clinical outcome, a protocol for stem/progenitor cell isolation and expansion in a safe, stable, efficient and economic manner must be drawn up, before regenerative endodontics can replace conventional root canal treatment. The availability of an off-the-shelf allogenic stem/progenitor cells, however, will profound effect on the clinical practice of endodontics.

Despite propositions for the use of autologous stem cell transplantation in regenerative endodontics, there is still considerable debate as to whether these cell based approaches are necessary, when cell-free approaches may generate similar results, whilst avoiding the challenge of introducing cell transplantation protocols into general dental practice (Simon S., 2013;216:(E13)).

Some clinical aspects that require further research include: (1) focus on mechanisms that result in quiescence and/or decreased function of pulpal stem cells witnessed with patient ageing, (2) the subsequent regulation of odontoblast secretory activity and downregulation after sufficient dentinogenesis (the P38-MAPK may prove a promising pharmaceutical target).

Should a reliable method of stem cell transplantation be elucidated to give results as successful as conventional root canal therapy, there still remains the need to determine the longevity of treated teeth. As these teeth still require access cavity preparation, this poses issues of potential pulpal inflammation and reinfection through marginal leakage of the access cavity (even with the newly regenerated pulp laying down tertiary dentine) and the detrimental structural effects of removal of such a large amount of dental hard tissue for access cavity preparation. The latter of these two points poses the question of, should load-bearing teeth that have undergone this therapy require cast cuspal coverage restorations to account for their weakened state due to tooth tissue removal? This in turn raises issues due to the well ascribed notion that placing a crown on a (newly) vital tooth increases the risk of devitalisation and subsequent pulp-necrosis (Bergenholtz, 1984; 55), effectively making the process of pulp regeneration of that tooth pointless.

Conclusion

The ease of access to pulp tissue combined with the advantages of multipotent/pluripotent stem cell therapies make adult stem cells an attractive option in regenerative medicine and dentistry (Goldberg M. , 2014). Regenerative endodontics is an exciting development in the field, with applications ranging from promotion of natural wound healing to tissue engineering principles applied to treatment protocols, with the aim of restoring tooth function by preserving pulp vitality after incidences of disease or trauma. The mechanism of these therapies is through the recruitment of endogenous stem/progenitor cells or introduction of allogenic stem/progenitor cells, incorporation of the function of bioactive molecules sequestered in dentine matrix, all based around a biocompatible scaffold (Simon S., 2013;216:(E13)).

Self-assembling peptides, an example of a tubule matrix, are a promising class of biomaterials for tissue engineering as they have multiple advantageous, customisable properties, allowing construction of a versatile, tailor-made matrix (Galler K. e., 2011;23(3)).

Regeneration of pulp tissue by stem cell therapy has resulted in: (1) Well-vascularised and innervated pulp tissue, (2) The cell density and architecture of extracellular matrix that is identical to normal pulp, (3) The lining of functional and aligned odontoblasts along the dentinal wall with nascent dentine, demonstrating functional regeneration. However, there are still many more areas of research to be looked in to before regenerative endodontic therapies can enter the mainstream of clinical dental practice.

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