European Cells and Materials Vol. 41 2021 (pages 233-244) DOI: 10.22203/eCM.v041a17 ISSN 1473-2262

THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IN REGENERATIVE ENDODONTICS

A. Ivica[1], M. Zehnder[1] and F.E. Weber[2,*]

[1] Preventive Dentistry, Periodontology and Cariology, Centre for Dental Medicine, University of Zurich, Zurich, Switzerland
[2] Oral Biotechnology and Bioengineering, Centre for Dental Medicine, University of Zurich, Zurich, Switzerland

Abstract

Regenerative endodontic procedures are an alternative to conventional root-canal treatment and apexification. There are two different tissue engineering approaches that are currently followed, both aiming at the colonisation of the cleaned pulp space by pluripotent cells and subsequent pulp regeneration. Firstly, the transplantation of mesenchymal stem cells (MSCs), and secondly a cell-free strategy that relies on bioactive molecules to trigger the recruitment of the patient's own cells. The first approach is hampered by costs and regulatory issues. Despite great initial enthusiasm with a clinically used cell-free approach that relies on induced bleeding into the pulp space, results have been revealed to be rather unpredictable, and mere repair rather than regeneration of the pulp-dentin complex is what is typically achieved. Moreover, the extent of further root development is variable, and the concept is limited to immature teeth. This article discusses a third possible way of regenerative endodontics that involves the application of MSC-derived exosomes. These are extracellular vesicles that contain proteins, lipids, and nucleic acids, reflecting the secretome of MSCs. Based on the first *in vitro* and *in vivo* studies, exosomes appear to be a potent tool to improve pulp regeneration. This narrative review aims to investigate the therapeutic use of human MSCs or dental pulp-derived exosomes in regenerative endodontics. Furthermore, the focus of this review is on targeting important questions that should be investigated in future *in-vivo* and clinical studies, such as the choice of scaffold material for exosome delivery into the pulp space.

Keywords: Regenerative endodontics, dental pulp, exosomes, extracellular vesicles, regeneration.

***Address for correspondence**: Franz E. Weber, Schönleinstrasse 2, 8032 Zurich, Switzerland.
Telephone number: +41 44 6343140 Email: franz.weber@zzm.uzh.ch

Introduction

Regenerative medicine is a fast-growing field. Its concepts aim to replace or restore diseased cells, tissues, or organs, and to restore their functionality. Because oral diseases pose a significant public health problem, there is a continued search for better and more efficient treatments, including attempts to implement regenerative approaches. It was estimated that at least 3.58 billion people suffer from oral diseases worldwide (Kassebaum *et al.*, 2017). The health of the tooth may be compromised due to infection, trauma, or some developmental abnormalities, which can lead to irreversible pulpitis or necrosis. Traditional endodontic treatments merely seek to clean and then seal the canal space with synthetic materials, and thus result in the repair of the non-infected tissues. However, these procedures fail to promote continued root development, leaving immature teeth susceptible to fractures and without internal vitality (Diogenes *et al.*, 2016). Because an endodontically treated tooth does not contain a vital pulp, it cannot fight against caries and other attacks. Regenerative endodontics with the goal to regenerate lost pulp tissue was thus proposed as an alternative to conventional endodontic treatment for immature teeth (Hargreaves *et al.*, 2008; Mao *et al.*, 2012).

As a common concept, tissue engineering treatments utilise a combination of stem cells, growth factors, and scaffolds to induce functional tissue regeneration (Langer and Vacanti, 1993). One of the best-studied stem cell types in this context are mesenchymal stem cells (MSCs). They can be isolated from dental tissues, are widely available throughout

all life stages and represent an important tool, not only for craniofacial regeneration, but also to treat a growing number of other conditions (Sharpe, 2016). Since dental tissues represent a particularly attractive source for stem cells because of their availability, dental MSCs have been extensively investigated. Moreover, there are stem cell banks worldwide, offering MSCs to be transplanted and used in clinical and commercial applications (Codispoti *et al.*, 2018). The formation of pulp-like tissue after the transplantation of dental pulp cells into an empty tooth was first shown in dogs and later in humans (Iohara *et al.*, 2011; Nakashima *et al.*, 2017). Despite these positive results, however, there are many technical problems with the direct application of MSCs. At the current stage of the technology, the cost of such a therapy would be forbiddingly high to save one tooth and, even more importantly, there may be a risk of contamination and immunorejection (Mao *et al.*, 2012). Therefore, clinical protocols for regenerative endodontics are not and will probably never be based on cell transplantation, but rely on cell homing (Galler *et al.*, 2016). The latter is defined as the recruitment of a patient's own stem cells to a damaged part of a tissue induced by chemotaxis (Kim *et al.*, 2010). The main limitation of this strategy is however the predictability of the number of the recruited cells. Moreover, as discussed below, the current concept does not yield predictable results and can lead to blood- and material-induced tooth discoloration (Chrepa *et al.*, 2020; Kahler *et al.*, 2014). The use of recombinant growth factors may induce non-desirable side effects, because of their pleiotropic effects on metabolism.

The paradigm shift from cell-based approaches to possibly the concepts discussed in this article was spurred by growing evidence that, while current cell-free tissue engineering approaches in endodontics do not yield the expected results, there may be an intermediate route in between cell-based and cell-free concepts. This idea is based on the historic evidence that conditioned medium of MSCs could have therapeutic effects on ischemic heart disease (Gnecchi *et al.*, 2006). That phenomenon started the recent research boom in exosomes that are found in the conditioned medium together with other paracrine mediators. Since then, there has been a tremendous interest in use of exosomes instead of MSCs for the purpose of regenerative medicine (Phinney and Pittenger, 2017). In dentistry, however, where such concepts may find earlier clinical application than in other fields dealing with more systemically important organs, research is still limited to laboratory and animal investigations. This review therefore focuses on the therapeutic potential of MSC-derived exosomes in regenerative endodontics and aims at giving an overview of the recent developments in this field.

MSCs and their therapeutical effects

Stem cells are defined as immature, primitive, undifferentiated cells that have the ability to differentiate into multiple lineages (Miyajima *et al.*, 2014). Eventually, they can give rise to a progenitor cell that differs slightly from the initial cell and that will differentiate into a more mature cell of the respective tissue. This is a process important for tissue homeostasis. The older cells are removed, and the tissue is constantly being regenerated from stem cells (Clevers *et al.*, 2014). Likewise, a stem cell may also divide into another stem cell or self-renew, which is a tool to protect the stem cell pool from exhaustion.

The term MSCs refers to cells isolated from stroma, the connective tissue that surrounds other tissues and organs. Because these cells did not show any teratogenic potential in animal models and can easily be cultured from different human tissues, such as bone marrow, dental pulp and fat, they have been frequently used and investigated (Pittenger *et al.*, 1999). MSCs may support regeneration in several ways. They release cytokines and growth factors to orchestrate cell migration, proliferation, angiogenesis, immune system recognition and the expansion of B and T cells (Dexter *et al.*, 1990). The first time the allogeneic MSCs were used was to treat patients with osteogenesis imperfecta. The improvements in bone structure and function obtained were reported (Horwitz *et al.*, 1999). Since then, MSCs have been used in a large number of different animal models and randomised clinical trials, including treatment of acute myocardial infarction, ischaemic stroke, acute kidney failure and Crohn´s disease as well as steroid-refractory acute graft-*versus*-host disease (acute GvHD), which arises as a side effect of allogeneic hematopoietic stem cell transplantation therapies (Giebel *et al.*, 2017).

In many aspects, MSC therapy showed numerous advantages over conventional treatments. Adding MSCs into bone tissue engineering seems to be a key improvement, since they promote rapid tissue remodelling and trigger vascular differentiation, which is especially important in a tissue with a limited vascular capacity (Khojasteh *et al.*, 2017). Moreover, MSC-based therapy significantly improves wound healing and angiogenesis in skin defects (Pelizzo *et al.*, 2018). Because of their immunomodulatory capacity, MSCs are also used for liver regeneration in acute liver failure (Shi *et al.*, 2017). However, this MSC-based therapy may also have serious side effects. Upon studying the distribution of intravenously injected MSCs it was reported that, because of their large size, MSCs are rapidly trapped within the pulmonary capillaries, causing pulmonary and haemodynamic problems (Schrepfer *et al.*, 2007). In the literature there are several studies suggesting that the application of MSCs may lead to tumourigenesis,

post transplantation infection, secondary injury, and congenital abnormalities (Dlouhy *et al.*, 2014; Goldstein *et al.*, 2010; Rebelatto *et al.*, 2008; Hovatta, 2011).

Extracellular vesicles

Cells secrete different types of membrane vesicles, known as extracellular vesicles. Various names have been used to refer to these vesicles (van Niel *et al.*, 2018). Depending on their origin, they can be broadly divided into exosomes and microvesicles or ectosomes. Exosomes are of endocytic origin and secreted by fusion of these vesicles with the cell membrane. Microvesicles form from the surface of the plasma membrane by outward budding (Abels and Breakefield, 2016). Despite the difference in their origin, the mechanism responsible for biogenesis of both types of vesicles is the same (van Niel *et al.*, 2018). Further, extracellular vesicles can be subdivided according to their size into apoptotic bodies, microparticles and exosomes. The latter range from 40 nm to 150 nm in diameter. Some scientists prefer the term exosome as a generic alternative instead of extracellular vesicle. To solve this nomenclature disagreement, the International Society for Extracellular Vesicles suggested that the term extracellular vesicles be used (Witwer and Théry, 2019).

Extracellular vesicles are encapsulated small portions of the parent cell, which are secreted into extracellular fluid or culture media (Koritzinsky *et al.*, 2017). For a long time, it was believed that these vesicles were just cellular waste and they were considered insignificant. However, their lipid bilayer contains a complex cargo of proteins, lipids, and nucleic acids, which mirrors their cell source and exert similar effects to the cells they derived from (Gurunathan *et al.*, 2019). Extensive research has been carried out to characterise the cargo of extracellular vesicles. The content of vesicles is highly heterogenous and depends on the cell type and the physiologic conditions (Abels and Breakefield, 2016). Proteomics, a large-scale study of proteins and their functions (Kupcova Skalnikova, 2013) of exosomes revealed common proteins in extracellular vesicles like tetraspanins (CD9, CD63, CD81, and CD82), Rab GTPases, flotillin, Alix, TSG101, heat shock proteins (Hsc70, Hsp90), antigen-presentation proteins (MHC-I and MCH-II) and receptors (tumour necrosis factor receptor 1) (Qing *et al.*, 2018; Deng *et al.*, 2018). Proteins that are associated with basic cellular functions, such as membrane trafficking, cell junction, the cytoskeleton, and structure in general, are present in all extracellular vesicles. In addition, the proteome of MSC-derived extracellular vesicles often includes enzymes and signalling molecules like cytokines, interleukins, chemokines, and growth factors (Deng *et al.*, 2018). Therapeutic efficacy could be mediated by exchanging cell signalling proteins such as TNF-α, TGF-β, Wnt5, β-catenin and *etc.* from the extracellular vesicles to their surroundings. Thus, MSC-derived exosomes could potentially help tissue regeneration by providing catalytically active enzymes or growth factors (Anderson *et al.*, 2016). Extracellular vesicles are rich in miRNA, small single-stranded non-coding RNA molecules. Transfer of miRNA through exosomes has been widely studied as a possible mechanism of intercellular communication. The concept that miRNA from the exosomes can be delivered to other cells, resulting in direct modulation of their mRNA targets, has become one of the most attractive hypotheses in the field (Tkach and Théry, 2016). It was demonstrated *in vivo* that exosomes from Epstein-Barr virus-infected cells transferred the virus to noninfected cells (Pegtel *et al.*, 2010).

Extracellular vesicles act in both physiological and pathological processes. They are important for cell communication and the transmission of information to further locations (He *et al.*, 2018). They may enter into a cell *via* more than one route. All extracellular vesicles carry surface molecules to be recognised by recipient cells (Tkach and Théry, 2016). They are usually taken up by endocytosis/phagocytosis (Montecalvo *et al.*, 2012). The recipient cell may also fuse directly with the membrane of a vesicle, or the uptake occurs by receptor-ligand interactions (Mulcahy *et al.*, 2014). Thus, extracellular vesicles represent a key concept in mediating changes in cellular behaviour by affecting cells in a paracrine or an endocrine manner, which makes them useful therapeutically (Zhang *et al.*, 2019). Because of their rapid uptake, it is more likely that their primary function is to exchange information with neighbouring cells and not to interfere with distant cellular targets (Smyth *et al.*, 2015). Unlike MSCs, extracellular vesicles cannot renew themselves and thus lack endogenous potential for tumourigenesis (Giebel *et al.*, 2017).

Exosomes and their potential therapeutic effects

It was demonstrated that MSCs have immune-modulating properties (Bartholomew *et al.*, 2002). Since this does not require cell-cell interaction, it has been suggested that the anti-proliferation activity of MSCs on T-cells could be due to the secretion of modulating factors by these cells (Di Nicola *et al.*, 2002). This led to the hypothesis that tissue repair may be orchestrated through paracrine factors of MSCs and stimulation of host cells, and not necessarily by cell replacement (Caplan and Dennis, 2006). Moreover, experiments on cardioprotective effects of conditioned media suggested that vesicular structures, identified as exosomes, have cardioprotective properties (Lai *et al.*, 2010). These active components in the conditioned medium of

human MSCs were within the 100 to 200 nm range (Timmers *et al.*, 2007). This gave a new perspective into intercellular mediation of tissue repair.

A major advantage of exosomes is that they represent a cell-free therapy. Moreover, unlike other nanoparticles, exosomes show the rapid clearance and the absence of unwanted accumulation in liver, which could explain their favourable toxicity profile (Fu *et al.*, 2019; Smyth *et al.*, 2015). Most importantly, because they do not express HLA (human leukocyte antigen) class II, exosomes are hypoimmunogenic (Phinney and Pittenger, 2017). Most experimental evidence suggests that exosomes harvested from MSCs show a very similar biological activity to the MSCs proper (Nakamura *et al.*, 2015; Timmers *et al.*, 2007; Zhang *et al.*, 2015). Due to their great biocompatibility exosomes have attracted wide attention in the medical community (Kalimuthu *et al.*, 2018).

There has been a fundamental change of focus from using MSCs in a therapeutic manner to the mere application of exposomes, which is reflected in the number of more than 180 clinical trials using exosomes as reported in www.clinicaltrials.gov in August 2020 (Web ref. 1). Some clinical trials using exosomes from human specimens are completed and results showed that exosomes were well-tolerated. Depending on the cell source, exosomes can promote or suppress inflammatory activity or boost anti-tumour response (Chen *et al.*, 2019).

The harvesting of exosomes is usually performed using centrifugation steps on a size-based isolation strategy. This is the most widely adopted method, but it is time consuming and works only with large sample volumes (He *et al.*, 2018). There are also other methods for exosome isolation, such as ultrafiltration, chromatography, polymer-based precipitation, immunoaffinity capture or antibody-coupled magnetic beads (Gurunathan *et al.*, 2019). Exosomes may be isolated from human plasma, saline, urine, blood, semen or culture medium.

Extracellular vesicles are less complicated to handle than cells. To attain routine clinical usage, however, a crucial point is to identify optimal isolation and storage conditions, as the use of fresh extracellular vesicles is not practical. Recently, Ibsen *et al.* established an alternating current electro kinetic microarray chip device for rapid isolation and recovery of exosomes from human plasma within 15 min (Ibsen *et al.*, 2017). Interestingly, there is also a novel approach named "NANOBIOME" that offers the biobanking of exosomes (Codispoti *et al.*, 2018). MSCs have the capacity to mass produce exosomes. Following this route, high-quality exosomes may be collected, processed, and preserved for clinical application. For their preservation and storage, there are options like freezing or lyophilisation. Interestingly, the freezing cycles do not negatively affect the stability of exosomes, neither in terms of structure nor composition (Codispoti *et al.*, 2018). Freezing at – 80 °C in trehalose with or without protease inhibitors seems to be a convenient way of storage (Le Saux *et al.*, 2020). Moreover, freeze-drying allows satisfactory shipping conditions and long-term storage, avoiding any risk of thawing or degradation (Frank *et al.*, 2018; Le Saux *et al.*, 2020). Such techniques are gradually advancing the routine manufacture of exosomes and their clinical distribution. Because of their small size, extracellular vesicles may be sterilised through filtration (Giebel *et al.*, 2017).

Extracellular vesicles derived from oral cavity MSCs

Dental tissues represent a particularly attractive source of MSCs, wherefrom exosomes may be extracted. Because teeth are often discarded in the clinic as medical waste, their examination does not necessarily require ethical approval (Egusa *et al.*, 2012). These tissues consist of heterogeneous cells, as they, beside MSCs, include progenitor cells, fibroblasts, neural cells, endothelial cells and immune system cells. Besides dental pulp stem cells (DPSC) and stem cells from exfoliated deciduous tooth (SHED), MSCs are also present in the periodontal ligament, apical papilla, gingiva, tooth germ and bone marrow. Further work is required to effectively classify the properties of exosomes derived from those specific MSCs to better understand which source may be used for regenerative purposes. Because exosomes could be relatively easily harvested from a patient's wisdom teeth or from deciduous teeth, these cell sources have a great potential to be tested in clinical trials.

Exosomes derived from gingival MSCs combined with poly(lactide) scaffold promoted bone regeneration in an animal model for the study of calvarial bone repair (Diomede *et al.*, 2018b). Moreover, an extensive vascular network was observed, suggesting an osseointegration process (Trubiani *et al.*, 2019). Extracellular vesicles released from periodontal ligament stem cells showed similar results (Diomede *et al.*, 2018a). It may be assumed that exosomes boost the osteoinductive activity through the activation of endogenous bone marrow MSCs in the bone defect.

Regenerative endodontics now

Regenerative endodontics has its roots in the work of Nygaard-Østby in the 1960s (Nygaard-Østby, 1961). In 2001, Iwaya and Ikawa reopened the field by showing a case report on the continued root growth of an immature permanent tooth with apical periodontitis and a sinus tract (Iwaya *et al.*, 2001). The tooth, however, had a *dens evaginatus*, and its endodontic condition was hardly that of a tooth affected by caries or trauma. Despite a great deal of enthusiasm at the beginning, which was partly based on mis-interpretation of such individual case reports

and the disregard of pulp tissue possible conditions in necrotic teeth with caries *versus* trauma, there are many challenges that must be overcome in order to achieve better clinical results. At the core of these problems is the fact that in the absence of a vital pulp stump, there is little pulpal regeneration (Edanami *et al.*, 2020), and the pulp space repairs with periodontal tissues (Lui *et al.*, 2020; Nygaard-Ostby and Hjortdal, 1971).

One clinical pilot study and several animal studies supported the potential applicability of cell transplantation for pulp regeneration therapy (Iohara *et al.*, 2011; Nakashima *et al.*, 2017; Xuan *et al.*, 2018). However, there are many regulatory barriers, such as a risk of contamination and immunorejection (Mao *et al.*, 2012). It is noteworthy that cell transplantation requires sophisticated materials and techniques. To circumvent all these negative aspects, other possibilities should be further explored and optimised. Mao and his group were the first to demonstrate that, in an animal model with human teeth, the regeneration of dental pulp was achievable by the attraction of host endogenous cells and without cell transplantation, *i.e.* cell homing (Kim *et al.*, 2010). However, they used a mix of morphogens that was applied into the cleaned pulp space to investigate their research question. Clinically, the cell-free concept that has been accepted both by the American Association of Endodontists (AAE) and the European Society of Endodontology (ESE) for the treatment of immature teeth with pulp necrosis (Galler *et al.*, 2016), however, merely relies on controlled bleeding into the formerly cleaned pulp space and blot clot formation (Nygaard Ostby, 1961; Trope, 2010). It may be surmised that application of tissue factors has not been followed by any dental company because of regulatory impasses. The ESE suggested the protocol shown in Fig. 1 that

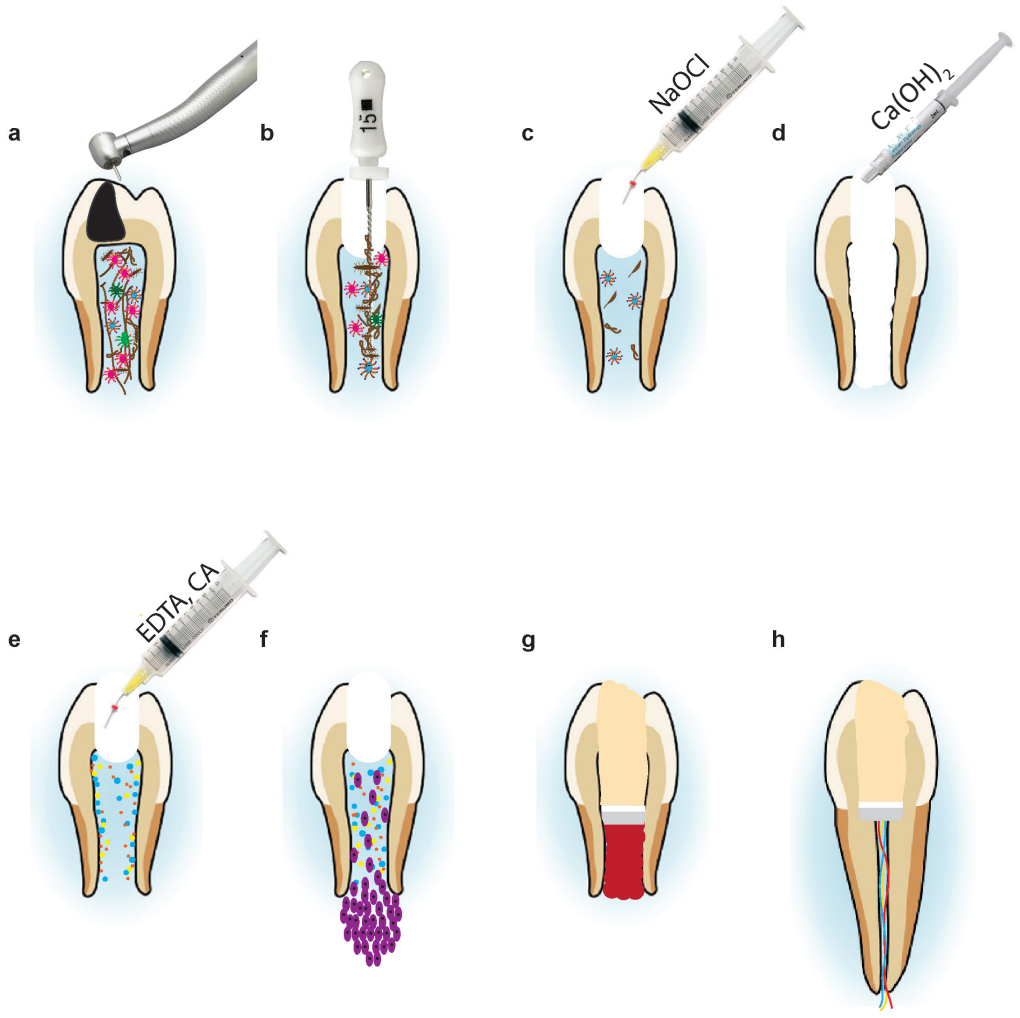

Fig. 1. Current protocol recommended by ESE based on cell homing strategy. (**a**) The first step is to open a pulp chamber. (**b**) This is followed by pulp extirpation and (**c**) disinfection. (**d**) Calcium hydroxide dressing is used as intracanal medicament between two appointments. (**e**) Next step is dentine conditioning to release growth factors that are (**f**) embedded in dentine to recruit stem cells. Afterwards bleeding is induced so that blood clot may serve as a scaffold. MTA plug ensuring a thickness of 2-3 mm should be placed on the top of the blood clot underneath cemento-enamel junction. A flowable, light-curable glass-ionomer is applied on top of it. (**g**) The tooth is sealed with adhesive restoration. (**h**) Pulp regeneration with re-continued root development.

includes access cavity preparation and removal of necrotic pulp tissue followed by disinfection. Sodium hypochlorite (NaOCl) is the first choice for disinfection (Martin *et al*., 2014). Although there is no consensus on the application of calcium hydroxide as a medicament in-between two appointments, several studies have shown a positive effect of this medicament on stem cell survival (Ruparel *et al*., 2012). Dentin conditioning with some chelating agent such as ethylenediaminetetraacetic acid (EDTA) or citric acid liberates growth factors from dentin (Galler *et al*., 2015). This helps to recruit stem cells and to differentiate them into cells that resemble those found in normal pulps (Ivica *et al*., 2019). Bleeding induced by over-instrumenting is used to form a blood clot. However, blood clot formation is not always achievable and it may lead to tooth discoloration by the diffusion of haemoglobin into dentinal tubules (Saoud *et al*., 2014). A hydraulic calcium silicate cement (*e.g*. MTA or tricalcium silicate cement) is placed coronally to this clot, which is then followed by the final restoration. Parameters that define success of such a treatment are the healing of a pre-existing periapical lesion, increase of root thickness and length, apical closure, and positive response to sensitivity testing (Kahler *et al*., 2017).

The histological assessment of human teeth, treated by the regenerative approaches that are currently allowed, mostly showed tissue repair or a combination of repair and regeneration. In some cases, replacement of the dentin-pulp complex by periodontal supporting tissues, including bone and cementum, was observed (Digka *et al*., 2020; Lui *et al*., 2020). Between researchers, there is no consensus on the types of tissues that are generated in the pulp space following regenerative therapy. However, for clinicians it seems to be unimportant if there is repair or regeneration as long as the patient is asymptomatic, a tooth responds positively to vitality testing, and there is lengthening and thickening of the dentine walls of immature teeth (Galler *et al*., 2016). From a research perspective, however, the final goal of the regeneration treatment is to re-establish the original form and function of the damaged structures, in our case, the pulp-dentin complex. This would result in a deposition of tissues that are at the histological level similar to non-damaged dentine and with well-known architecture of the pulp. In addition, there are some patient-related outcomes that need to be added into the equation, such as crown discoloration, tooth functionality, pain, the number of dental appointments and patient satisfaction (Diogenes *et al*., 2016). As an example, a meta-analysis showed that tooth discoloration was reported in 40 % of the cases treated by current regenerative techniques, which is a severe problem since front teeth are the most common teeth in need of such treatment (Torabinejad *et al*., 2017).

In summary, it may be stated that while there is a will to use tissue engineering concepts in clinical endodontics, the results obtained with what is currently available to practitioners must be improved upon. The predictability of these treatment outcomes and other unwanted consequences are due to a shortage of recruited autologous stem cells in necrotic root canals and the lack of a suitable scaffold to replace the blood clot (Song *et al*., 2017).

Potential therapeutic effects of MSC-derived extracellular vesicles in regenerative endodontics and beyond

Thanks to its accessibility, the dental pulp is of particular interest and displays great advantages compared to other sources of MSCs. Therefore, exosomes derived from dental pulp cells have also been studied extensively in the past few years. It would appear that they can promote healing in neurological and vascular diseases, osteoarthritic disorders, liver and lung regeneration and many other conditions (Kichenbrand *et al*., 2019). The use of dental pulp-derived exosomes for applications other than regenerative endodontics was not the focus of the current review and should be discussed in future communications. In terms of pulp regeneration, most of the studies investigating the role of exosomes on regenerative properties used DPSC as a source of exosomes (Table 1). Since fibroblasts and endothelial cells contribute to dentine repair and pulp healing, it may be useful to harvest exosomes from the whole dental pulp, *e.g*. the mixture of cells and not only DPSC (Ivica *et al*., 2020; Xian *et al*., 2018). Odontoblasts secrete exosomes with anti-apoptotic features, which may be particularly important during inflammation (Wang *et al*., 2019). However, whether exosomes from different cell types contribute to the better microenvironment for triggering pulp regeneration or not awaits further clarification. It may be so that since exosomes carry the functions of their maternal cells, different exosomes may be necessary for specific applications within dentistry. A further question is whether there are differences between the exosomes secreted from young and adult patients (Iezzi *et al*., 2019).

Huang *et al*. demonstrated the beneficial effect of exosomes isolated from dental pulp stem DPSC on odontoblastic differentiation of naïve cells (Huang *et al*., 2016). It was suggested that exosomes promoted the odontogenic differentiation by the transfer of microRNAs. Moreover, when the cells were cultured with odontogenic medium and afterwards the exosomes were extracted, the effect was even stronger compared to the normal culturing conditions.

To clarify the underlying mechanism, micro-RNA sequencing was conducted (Hu *et al*., 2019). The analysis showed that microRNA levels in exosomes extracted from the cells cultured in odontogenic medium were significantly changed when compared to those in regular medium. Furthermore, these authors demonstrated that exosomes promote odontogenic differentiation by

Table 1. Dental pulp derived exosomes in pulp regeneration and repair.

Origin of exosomes	Type of study	Observation	Reference
DPSC	*in vivo* – induced acute inflammation in mice	Exosomes show strong anti-inflammatory effects	Pivoraitė *et al*., 2015
DPSC	*in vivo* – the root slices implanted into the back of mice	Exosomes promote differentiation of stem cells; exosomes isolated under odontogenic conditions have stronger effect	Huang *et al*., 2016
Epithelium and mesenchyme cells of developing tooth	*in vivo* in rats	Exosomes travel between epithelium and mesenchyme during tooth development leading to reciprocal cell differentiation and matrix synthesis	Jiang *et al*., 2017
Dental pulp	*in vitro*	Exosomes promoted endothelial cell proliferation, paracrine angiogenic factor expression and tube formation	Xian *et al*., 2018
DPSC	*in vitro*	MicroRNAs significantly changed in odontogenic exosomes compared to normal conditions; exosomes trigger odontogenic differentiation *via* TGFβ1/Smads signalling pathway	Hu *et al*., 2019
Odontoblasts and odontoblast-like cells	*in vitro*	Exosomes reduce apoptosis in neighbouring cells; increased levels of CD63 (exosome marker) in the region affected by caries	Wang *et al*., 2019
Dental pulp	*in vitro*	Exosomes trigger migration and proliferation of BMMSCs; fibrin gel and exosomes have synergistic effect on stem cell attraction	Ivica *et al*., 2020
DPSC	*in vivo* – rat pulpotomy model	Exosomes loaded into biodegradable polymeric scaffold and used as pulp capping material enhance tertiary dentineogenesis and dentine bridge formation	Swanson *et al*., 2020
SCAP	*in vivo* – the root fragments implanted subcutaneously into immunodeficient mice	Exosomes are endocytosed by BMMSCs and orchestrate their specific dentineogenesis	Zhang *et al*., 2020
DPSC	*in vitro*	Fibrin gel loaded with exosomes supports neovascularisation and collagen I, III, IV deposition; fibrin preserved the activity of exosomes for at least 7 d	Zhang *et al*., 2020

the transforming growth factor-β (TGF-β) signalling pathway by downregulating the inhibitory molecule LTBP1. TGF-β signalling is well known to play important roles in odontogenic differentiation and tooth morphogenesis (He *et al*., 2014). Moreover, exosomes derived from human DPSC have strong anti-inflammatory effects (Pivoraitė *et al*., 2015).

It is well known that tooth development requires interaction of cells in different tissues. In mice it was shown that during tooth formation, exosomes are secreted by both the epithelium and mesenchyme (Jiang *et al*., 2017). Those exosomes reciprocally trigger cell differentiation and matrix secretion. Epithelium exosomes are taken in by mesenchymal cells that afterwards produce dentine sialoprotein and start mineralisation. At the same time, mesenchymal exosomes induced epithelium cells to produce ameloblastin and amelogenenin.

Wang *et al*. established an *in vitro* model to study the possible role of exosomes in dental caries (Wang *et al*., 2019). In caries progression, odontoblasts show different levels of inflammation. The odontoblasts that are closer to the site of injury exhibited more severe signs of inflammation. At the same time, those odontoblasts secreted an increased number of exosomes, comparing to the mildly injured counterparts along the periphery of the caries lesion. Interestingly, exosomes helped to block apoptosis.

As discussed above, in regenerative endodontic procedures that are based on a cell homing strategy, the main problem is to recruit the patient's endogenous mesenchymal stem cells (Kling *et al*., 1986). Human bone marrow-derived mesenchymal stem cells are mobilised into the circulation and represent an important source of stem cells for pulp regeneration. It was shown that exosomes from human pulp attract bone marrow-derived mesenchymal stem cells *in vitro* (Ivica *et al*., 2020). Moreover, in the same study, it was reported that stem cell proliferation was strongly affected by exosomes. Differentiation towards odontoblasts was also detected in BMMSCs after they were endocytosed (Zhuang *et al*., 2020).

Delivery and matrix systems for pulpal regeneration in conjunction with exosomes

In order to use exosomes as a therapeutic tool, an appropriate delivery system which allows the direct application to the injury site is required. Drug delivery strategies are a tremendous challenge in tissue engineering (Jain *et al*., 1998). To investigate how the exosomes may be applied clinically in regenerative endodontics, they were combined with a collagen membrane and used to fill the roots of human teeth before being subcutaneously implanted in rats (Huang *et al*., 2016). Results showed that exosomes could enhance the regeneration of pulp-like tissue. An increase in the expression levels of growth factors TGF-β1, BMP-2, RUNX2 and PDGF, markers for odontogenic differentiations, was observed in newly formed tissue in the groups where exosomes were used. Moreover, the results suggest that exosomes from dental pulp cells trigger angiogenesis,

which is a critical step for the regeneration of the dentine-pulp complex (Xian *et al.*, 2018). The level of vascular endothelial cell growth factor A (VEGF-A), the key growth factor that regulates angiogenesis, were increased when exosomes were used. Moreover, p38 MAPK signalling inhibition driven by exosomes enhance tube formation and stimulate endothelial cell angiogenesis. Similar results were seen when conditioned medium from human dental pulp stem cell was applied (Bronckaers *et al.*, 2013). Fibrin-based scaffolds showed some advantage over other scaffolds used in the field of regenerative endodontics (Galler *et al.*, 2018). When tested for use as delivery system for exosomes, results showed a synergistic effect of fibrin and the dental pulp-derived exosomes on stem cell attraction (Ivica *et al.*, 2020). In another study, fibrin gel loaded with exosomes provoked vascular-like structure formation in less than 7 d (Zhang *et al.*, 2020). Importantly, fibrin gels are commercially available clinically approved biomaterials and appear to be suitable delivery vehicles for exosomes in pulp regenerative procedures. However, additional preclinical and clinical studies are needed, especially related to their thrombin/fibrinogen composition and other physicochemical features relating to a controllable application in the root canal system (Hertig *et al.*, 2017).

A recent study showed that controlled release of human DPSC exosomes from poly lactic-*co*-glycolic acid (PLGA)-based biodegradable scaffolds may be useful in pulp capping therapy (Swanson *et al.*, 2020). Exosomes stimulated tertiary dentineogenesis and dentin bridge formation by promoting stem cell migration, odontogenic differentiation and mineralisation.

Conclusions and future perspectives

Because there are currently many challenges in regenerative endodontic treatment, a search for alternative clinically applicable strategies is indicated. MSCs have been used in different studies. Evidence has been provided that their therapeutic activities is mediated by their extracellular vesicles. In this context, exosomes are considered as potential therapeutic tools to avoid problems that are inherent to the use or application of whole stem cells. Moreover, along with tissue-engineering techniques, exosomal modification strategies are promising for the development of improved clinical therapies. Exosomes could be used as biomimetic tools to induce migration, proliferation and odontoblast-specific differentiation of stem cells in regenerative endodontic treatments (Table 1). Most of the current evidence for the use of exosomes in pulp regeneration come from *in vitro* or animal *in vivo* studies. Based on these studies, it is possible that the exosomes may have a positive effect on differentiation of naïve cells, their migration, proliferation and angiogenesis (Fig. 2). It seems like the exosomes derived from DPSC may be as good, if not better than using MSCs to regenerate a pulp. It was shown that transplantation of MSCs into injured tooth could regenerate dental pulp with odontoblasts at the periphery, histologically very similar to normal pulp tissue of a human tooth (Xuan *et al.*, 2018). Because exosomes mimic the effects of MSCs, it is to be expected that they will trigger the regeneration and new tissue formation in the same way that MSCs do and, at the same time, by using exosomes technical problems that MSCs bring can be

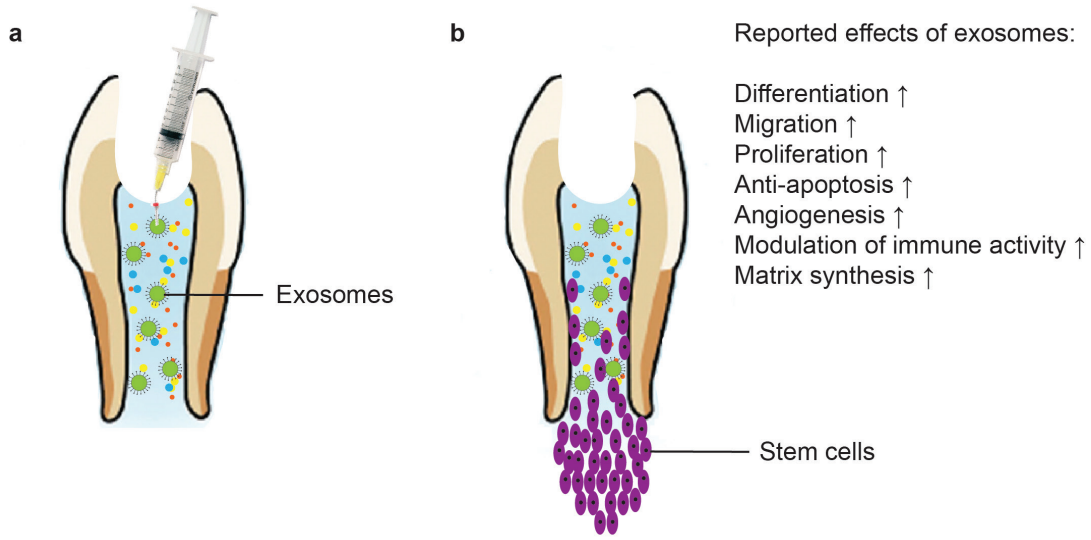

Fig. 2. Schematic representation of using exosomes in pulp tissue regeneration. Firstly, a tooth is prepared as was explained in Fig. 1**a**-**e**. (**a**) Once a pulp chamber is cleaned and conditioned, MSC-derived exosomes are injected. (**b**) Exosomes could be used to promote the recruitment of patient's cells, differentiation and proliferation of naïve cells as well as anti-apoptosis and angiogenesis.

avoided. Further clinical studies are needed to make a step forward in the application of this modifications in regenerative endodontics. To approach clinical use, this promising therapeutic strategy for regenerative endodontics has to prove its efficacy in clinical trials, and its effectiveness in everyday clinical use (Haynes, 1999). Moreover, an ideal vehicle for exosome delivery and scaffold formation in the pulp space needs to be identified. In that context, fibrin gels hold the greatest promise.

References

Abels ER, Breakefield XO (2016) Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol **36**: 301-312.

Anderson JD, Johansson HJ, Graham CS, Vesterlund M, Pham MT, Bramlett CS, Montgomery EN, Mellema MS, Bardini RL, Contreras Z, Hoon M, Bauer G, Fink KD, Fury B, Hendrix KJ, Chedin F, El-Andaloussi S, Hwang B, Mulligan MS, Lehtiö J, Nolta JA (2016) Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis *via* nuclear factor-kappaB signaling. Stem Cells **34**: 601-613.

Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Exp Hematol **30**: 42-48.

Bronckaers A, Hilkens P, Fanton Y, Struys T, Gervois P, Politis C, Martens W, Lambrichts I (2013) Angiogenic properties of human dental pulp stem cells. PLoS One **8**: e71104. DOI: 10.1371/journal.pone.0071104.

Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem **98**: 1076-1084.

Chen YS, Lin EY, Chiou TW, Harn HJ (2019) Exosomes in clinical trial and their production in compliance with good manufacturing practice. Ci Ji Yi Xue Za Zhi **32**: 113-120.

Chrepa V, Joon R, Austah O, Diogenes A, Hargreaves KM, Ezeldeen M, Ruparel NB (2020) Clinical outcomes of immature teeth treated with regenerative endodontic procedures – a San Antonio study. J Endod **46**: 1074-1084.

Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science **346**: 1248012. DOI: 10.1126/science.1248012.

Codispoti B, Marrelli M, Paduano F, Tatullo M (2018) NANOmetric BIO-banked MSC-derived exosome (NANOBIOME) as a novel approach to regenerative medicine. J Clin Med **7**: 357. DOI: 10.3390/jcm7100357.

Deng H, Sun C, Sun Y, Li H, Yang L, Wu D, Gao Q, Jiang X (2018) Lipid, protein, and microRNA composition within mesenchymal stem cell-derived exosomes. Cell Reprogram **20**: 178-186.

Dexter TM, Heyworth CM, Spooncer E, Ponting IL (1990) The role of growth factors in self-renewal and differentiation of haemopoietic stem cells. Philos Trans R Soc Lond B Biol Sci **327**: 85-98.

Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood **99**: 3838-3843.

Digka A, Sakka D, Lyroudia K (2020) Histological assessment of human regenerative endodontic procedures (REP) of immature permanent teeth with necrotic pulp/apical periodontitis: a systematic review. Aust Endod J **46**: 140-153.

Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM (2016) Regenerative endodontics: a way forward. J Am Dent Assoc **147**: 372-380.

Diomede F, D'Aurora M, Gugliandolo A, Merciaro I, Ettorre V, Bramanti A, Piattelli A, Gatta V, Mazzon E, Fontana A, Trubiani O (2018a) A novel role in skeletal segment regeneration of extracellular vesicles released from periodontal-ligament stem cells. Int J Nanomedicine **13**: 3805-3825.

Diomede F, Gugliandolo A, Cardelli P, Merciaro I, Ettorre V, Traini T, Bedini R, Scionti D, Bramanti A, Nanci A, Caputi S, Fontana A, Mazzon E, Trubiani O (2018b) Three-dimensional printed PLA scaffold and human gingival stem cell-derived extracellular vesicles: a new tool for bone defect repair. Stem Cell Res Ther **9**: 104. DOI: 10.1186/s13287-018-0850-0.

Dlouhy BJ, Awe O, Rao RC, Kirby PA, Hitchon PW (2014) Autograft-derived spinal cord mass following olfactory mucosal cell transplantation in a spinal cord injury patient: case report. J Neurosurg Spine **21**: 618-622.

Edanami N, Yoshiba K, Shirakashi M, Ibn Belal RS, Yoshiba N, Ohkura N, Tohma A, Takeuchi R, Okiji T, Noiri Y (2020) Impact of remnant healthy pulp and apical tissue on outcomes after simulated regenerative endodontic procedure in rat molars. Sci Rep **10**: 20967. DOI: 10.1038/s41598-020-78022-w.

Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K (2012) Stem cells in dentistry – part I: stem cell sources. J Prosthodont Res **56**: 151-165.

Frank J, Richter M, de Rossi C, Lehr CM, Fuhrmann K, Fuhrmann G (2018) Extracellular vesicles protect glucuronidase model enzymes during freeze-drying. Sci Rep **8**: 12377. DOI: 10.1038/s41598-018-30786-y.

Fu W, Lei C, Liu S, Cui Y, Wang C, Qian K, Li T, Shen Y, Fan X, Lin F, Ding M, Pan M, Ye X, Yang Y, Hu S (2019) CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity. Nat Commun **10**: 4355. DOI: 10.1038/s41467-019-12321-3.

Galler KM, Brandl FP, Kirchhof S, Widbiller M, Eidt A, Buchalla W, Göpferich A, Schmalz G (2018) Suitability of different natural and synthetic biomaterials for dental pulp tissue engineering. Tissue Eng Part A **24**: 234-244.

Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G (2015) Influence of root canal disinfectants on growth factor release from dentin. J Endod **41**: 363-368.

Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, Lambrechts P (2016) European Society of Endodontology position statement: revitalization procedures. Int Endod J **49**: 717-723.

Giebel B, Kordelas L, Börger V (2017) Clinical potential of mesenchymal stem/stromal cell-derived extracellular vesicles. Stem Cell Investig **4**: 84. DOI: 10.21037/sci.2017.09.06.

Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J **20**: 661-669.

Goldstein RH, Reagan MR, Anderson K, Kaplan DL, Rosenblatt M (2010) Human bone marrow-derived MSCs can home to orthotopic breast cancer tumors and promote bone metastasis. Cancer Res **70**: 10044-10050.

Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH (2019) Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells **8**: 307. DOI: 10.3390/cells8040307.

Hargreaves KM, Giesler T, Henry M, Wang Y (2008) Regeneration potential of the young permanent tooth: what does the future hold. J Endod **34 Suppl 7**: S51-S56.

Haynes B (1999) Can it work? Does it work? Is it worth it? The testing of healthcareinterventions is evolving. BMJ **319**: 652-653.

He C, Zheng S, Luo Y, Wang B (2018) Exosome theranostics: biology and translational medicine. Theranostics **8**: 237-255.

He W, Zhang J, Niu Z, Yu Q, Wang Z, Zhang R, Su L, Fu L, Smith AJ, Cooper PR (2014) Regulatory interplay between NFIC and TGF-β1 in apical papilla-derived stem cells. J Dent Res **93**: 496-501.

Hertig G, Zehnder M, Woloszyk A, Mitsiadis TA, Ivica A, Weber FE (2017) Iodixanol as a contrast agent in a fibrin hydrogel for endodontic applications. Front Physiol **8**: 152. DOI: 10.3389/fphys.2017.00152.

Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med **5**: 309-313.

Hovatta O (2011) Infectious problems associated with transplantation of cells differentiated from pluripotent stem cells. Semin Immunopathol **33**: 627-630.

Hu X, Zhong Y, Kong Y, Chen Y, Feng J, Zheng J (2019) Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through TGFβ1/smads signaling pathway *via* transfer of microRNAs. Stem Cell Res Ther **10**: 170. DOI: 10.1186/s13287-019-1278-x.

Huang CC, Narayanan R, Alapati S, Ravindran S (2016) Exosomes as biomimetic tools for stem cell differentiation: applications in dental pulp tissue regeneration. Biomaterials **111**: 103-115.

Ibsen SD, Wright J, Lewis JM, Kim S, Ko S-Y, Ong J, Manouchehri S, Vyas A, Akers J, Chen CC (2017) Rapid isolation and detection of exosomes and associated biomarkers from plasma. ACS nano **11**: 6641-6651.

Iezzi I, Pagella P, Mattioli-Belmonte M, Mitsiadis TA (2019) The effects of ageing on dental pulp stem cells, the tooth longevity elixir. Eur Cell Mater **37**: 175-185.

Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng Part A **17**: 1911-1920.

Ivica A, Ghayor C, Zehnder M, Valdec S, Weber FE (2020) Pulp-derived exosomes in a fibrin-based regenerative root filling material. J Clin Med **9**: 491. DOI: 10.3390/jcm9020491.

Ivica A, Zehnder M, Mateos JM, Ghayor C, Weber FE (2019) Biomimetic conditioning of human dentin using citric acid. J Endod **45**: 45-50.

Iwaya SI, Ikawa M, Kubota M (2001) Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol **17**: 185-187.

Jain R, Shah NH, Malick AW, Rhodes CT (1998) Controlled drug delivery by biodegradable poly(ester) devices: different preparative approaches. Drug Dev Ind Pharm **24**: 703-727.

Jiang N, Xiang L, He L, Yang G, Zheng J, Wang C, Zhang Y, Wang S, Zhou Y, Sheu TJ, Wu J, Chen K, Coelho PG, Tovar NM, Kim SH, Chen M, Zhou YH, Mao JJ (2017) Exosomes mediate epithelium-mesenchyme crosstalk in organ development. ACS Nano **11**: 7736-7746.

Kahler B, Mistry S, Moule A, Ringsmuth AK, Case P, Thomson A, Holcombe T (2014) Revascularization outcomes: a prospective analysis of 16 consecutive cases. J Endod **40**: 333-338.

Kahler B, Rossi-Fedele G, Chugal N, Lin LM (2017) An evidence-based review of the efficacy of treatment approaches for immature permanent teeth with pulp necrosis. J Endod **43**: 1052-1057.

Kalimuthu S, Gangadaran P, Rajendran RL, Zhu L, Oh JM, Lee HW, Gopal A, Baek SH, Jeong SY, Lee SW, Lee J, Ahn BC (2018) A new approach for loading anticancer drugs into mesenchymal stem cell-derived exosome mimetics for cancer therapy. Front Pharmacol **9**: 1116. DOI: 10.3389/fphar.2018.01116.

Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W, GBD OHC (2017) Global, regional, and national prevalence, incidence, and disability-adjusted life

years for oral conditions for 195 countries, 1990-2015: a systematic analysis for the global burden of diseases, injuries, and risk factors. J Dent Res **96**: 380-387.

Khojasteh A, Fahimipour F, Jafarian M, Sharifi D, Jahangir S, Khayyatan F, Baghaban Eslaminejad M (2017) Bone engineering in dog mandible: coculturing mesenchymal stem cells with endothelial progenitor cells in a composite scaffold containing vascular endothelial growth factor. J Biomed Mater Res B Appl Biomater **105**: 1767-1777.

Kichenbrand C, Velot E, Menu P, Moby V (2019) Dental pulp stem cell-derived conditioned medium: an attractive alternative for regenerative therapy. Tissue Eng Part B Rev **25**: 78-88.

Kim JY, Xin X, Moioli EK, Chung J, Lee CH, Chen M, Fu SY, Koch PD, Mao JJ (2010) Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng Part A **16**: 3023-3031.

Kling M, Cvek M, Mejare I (1986) Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endod Dent Traumatol **2**: 83-89.

Koritzinsky EH, Street JM, Star RA, Yuen PS (2017) Quantification of exosomes. J Cell Physiol **232**: 1587-1590.

Kupcova Skalnikova H (2013) Proteomic techniques for characterisation of mesenchymal stem cell secretome. Biochimie **95**: 2196-2211.

Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK (2010) Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res **4**: 214-222.

Langer R, Vacanti JP (1993) Tissue engineering. Science **260**: 920-926.

Le Saux S, Aarrass H, Lai-Kee-Him J, Bron P, Armengaud J, Miotello G, Bertrand-Michel J, Dubois E, George S, Faklaris O, Devoisselle JM, Legrand P, Chopineau J, Morille M (2020) Post-production modifications of murine mesenchymal stem cell (mMSC) derived extracellular vesicles (EVs) and impact on their cellular interaction. Biomaterials **231**: 119675. DOI: 10.1016/j.biomaterials.2019.119675.

Lui JN, Lim WY, Ricucci D (2020) An immunofluorescence study to analyze wound healing outcomes of regenerative endodontics in an immature premolar with chronic apical abscess. J Endod **46**: 627-640.

Mao JJ, Kim SG, Zhou J, Ye L, Cho S, Suzuki T, Fu SY, Yang R, Zhou X (2012) Regenerative endodontics: barriers and strategies for clinical translation. Dent Clin North Am **56**: 639-649.

Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, Diogenes A (2014) Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod **40**: 51-55.

Miyajima A, Tanaka M, Itoh T (2014) Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. Cell Stem Cell **14**: 561-574.

Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, Baty CJ, Gibson GA, Erdos G, Wang Z, Milosevic J, Tkacheva OA, Divito SJ, Jordan R, Lyons-Weiler J, Watkins SC, Morelli AE (2012) Mechanism of transfer of functional microRNAs between mouse dendritic cells *via* exosomes. Blood **119**: 756-766.

Mulcahy LA, Pink RC, Carter DR (2014) Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles **3**: . DOI: 10.3402/jev.v3.24641.

Nakamura Y, Miyaki S, Ishitobi H, Matsuyama S, Nakasa T, Kamei N, Akimoto T, Higashi Y, Ochi M (2015) Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. FEBS Lett **589**: 1257-1265.

Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Ariji Y, Matsushita K (2017) Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. Stem Cell Res Ther **8**: 61. DOI: 10.1186/s13287-017-0506-5.

Nygaard-Ostby B, Hjortdal O (1971) Tissue formation in the root canal following pulp removal. Scand J Dent Res **79**: 333-349.

Nygaard-Østby BN (1961) The role of the blood clot in endodontic therapy. an experimental histologic study. Acta Odontol Scand **19**: 324-353.

Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Würdinger T, Middeldorp JM (2010) Functional delivery of viral miRNAs *via* exosomes. Proc Natl Acad Sci U S A **107**: 6328-6333.

Pelizzo G, Avanzini MA, Mantelli M, Croce S, Maltese A, Vestri E, De Silvestri A, Percivalle E, Calcaterra V (2018) Granulation tissue-derived mesenchymal stromal cells: a potential application for burn wound healing in pediatric patients. J Stem Cells Regen Med **14**: 53-58.

Phinney DG, Pittenger MF (2017) Concise review: MSC-derived exosomes for cell-free therapy. Stem Cells **35**: 851-858.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science **284**: 143-147.

Pivoraitė U, Jarmalavičiūtė A, Tunaitis V, Ramanauskaitė G, Vaitkuvienė A, Kašėta V, Biziulevičienė G, Venalis A, Pivoriūnas A (2015) Exosomes from human dental pulp stem cells suppress carrageenan-induced acute inflammation in mice. Inflammation **38**: 1933-1941.

Qing L, Chen H, Tang J, Jia X (2018) Exosomes and their microRNA cargo: new players in peripheral nerve regeneration. Neurorehabil Neural Repair **32**: 765-776.

Rebelatto CK, Aguiar AM, Moretão MP, Senegaglia AC, Hansen P, Barchiki F, Oliveira J, Martins J, Kuligovski C, Mansur F, Christofis A, Amaral VF,

Brofman PS, Goldenberg S, Nakao LS, Correa A (2008) Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med (Maywood) **233**: 901-913.

Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A (2012) Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod **38**: 1372-1375.

Saoud TM, Zaazou A, Nabil A, Moussa S, Lin LM, Gibbs JL (2014) Clinical and radiographic outcomes of traumatized immature permanent necrotic teeth after revascularization/revitalization therapy. J Endod **40**: 1946-1952.

Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP (2007) Stem cell transplantation: the lung barrier. Transplant Proc **39**: 573-576.

Sharpe PT (2016) Dental mesenchymal stem cells. Development **143**: 2273-2280.

Shi D, Zhang J, Zhou Q, Xin J, Jiang J, Jiang L, Wu T, Li J, Ding W, Li J, Sun S, Li J, Zhou N, Zhang L, Jin L, Hao S, Chen P, Cao H, Li M, Li L, Chen X, Li J (2017) Quantitative evaluation of human bone mesenchymal stem cells rescuing fulminant hepatic failure in pigs. Gut **66**: 955-964.

Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ (2015) Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. J Control Release **199**: 145-155.

Song JS, Takimoto K, Jeon M, Vadakekalam J, Ruparel NB, Diogenes A (2017) Decellularized human dental pulp as a scaffold for regenerative endodontics. J Dent Res **96**: 640-646.

Swanson WB, Gong T, Zhang Z, Eberle M, Niemann D, Dong R, Rambhia KJ, Ma PX (2020) Controlled release of odontogenic exosomes from a biodegradable vehicle mediates dentinogenesis as a novel biomimetic pulp capping therapy. J Control Release **324**: 679-694.

Timmers L, Lim SK, Arslan F, Armstrong JS, Hoefer IE, Doevendans PA, Piek JJ, El Oakley RM, Choo A, Lee CN, Pasterkamp G, de Kleijn DP (2007) Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res **1**: 129-137.

Tkach M, Théry C (2016) Communication by extracellular vesicles: where we are and where we need to go. Cell **164**: 1226-1232.

Torabinejad M, Nosrat A, Verma P, Udochukwu O (2017) Regenerative endodontic treatment or mineral trioxide aggregate apical plug in teeth with necrotic pulps and open apices: a systematic review and meta-analysis. J Endod **43**: 1806-1820.

Trope M (2010) Treatment of the immature tooth with a non-vital pulp and apical periodontitis. Dent Clin North Am **54**: 313-324.

Trubiani O, Marconi GD, Pierdomenico SD, Piattelli A, Diomede F, Pizzicannella J (2019) Human oral stem cells, biomaterials and extracellular vesicles: a promising tool in bone tissue repair. Int J Mol Sci **20**: 4987. DOI: 10.3390/ijms20204987.

van Niel G, D'Angelo G, Raposo G (2018) Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol **19**: 213-228.

Wang HS, Yang FH, Wang YJ, Pei F, Chen Z, Zhang L (2019) Odontoblastic exosomes attenuate apoptosis in neighboring cells. J Dent Res **98**: 1271-1278.

Witwer KW, Théry C (2019) Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. J Extracell Vesicles **8**: 1648167. DOI: 10.1080/20013078.2019.1648167.

Xian X, Gong Q, Li C, Guo B, Jiang H (2018) Exosomes with highly angiogenic potential for possible use in pulp regeneration. J Endod **44**: 751-758.

Xuan K, Li B, Guo H, Sun W, Kou X, He X, Zhang Y, Sun J, Liu A, Liao L, Liu S, Liu W, Hu C, Shi S, Jin Y (2018) Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. Sci Transl Med **10**: eaaf3227. DOI: 10.1126/scitranslmed.aaf3227.

Zhang B, Wang M, Gong A, Zhang X, Wu X, Zhu Y, Shi H, Wu L, Zhu W, Qian H, Xu W (2015) HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. Stem Cells **33**: 2158-2168.

Zhang S, Thiebes AL, Kreimendahl F, Ruetten S, Buhl EM, Wolf M, Jockenhoevel S, Apel C (2020) Extracellular vesicles-loaded fibrin gel supports rapid neovascularization for dental pulp regeneration. Int J Mol Sci **21**: 4226. DOI: 10.3390/ijms21124226.

Zhang Y, Liu Y, Liu H, Tang WH (2019) Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci **9**: 19. DOI: 10.1186/s13578-019-0282-2.

Zhuang X, Ji L, Jiang H, Liu Y, Liu X, Bi J, Zhao W, Ding Z, Chen X (2020) Exosomes derived from stem cells from the apical papilla promote dentine-pulp complex regeneration by inducing specific dentinogenesis. Stem Cells Int **2020**: 5816723. DOI: 10.1155/2020/5816723.

Web Reference

1. https://clinicaltrials.gov/ct2/results?cond=&term=exosomes&cntry=&state=&city=&dist= [20-08-2020]

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