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THE EFFECTS OF AGEING ON DENTAL PULP STEM CELLS, THE TOOTH LONGEVITY ELIXIR

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Abstract

Stem cells are essential for tissue homeostasis and regeneration throughout the lifespan of multicellular organisms. The decline in stem cell function during advanced age is associated with a reduced regenerative potential of tissues that leads to an increased frequency of diseases. Age-related changes also occur in the dental pulp that represents a reliable model tissue, with high regenerative capability, for studying senescence mechanisms. However, little information is available concerning the effects of ageing on dental stem-cell function. In this mini-review, recent data on how the molecular and functional alterations that accumulate in stem cell populations during ageing result in modifications of dental pulp physiology are discussed. Changes that accumulate during ageing such as how reduction of pulp chamber volume, decreased vascular supply and modifications to the stem cell niches affect stem cell functions and, therefore, dental pulp regenerative potential in response to various stressful agents. Dental pulp cells from aged individuals are still metabolically active and secrete pro-inflammatory and matrix-degrading molecules. Furthermore, miRNAs and exosomes derived from dental pulp stem cells constitute an attractive source of nanovesicles for the treatment of age-related dental pathologies. Further investigation of the epigenetic alterations in dental pulp stem cells, accumulating during ageing, might reveal crucial information for potential stem cell-based therapeutic approaches in the elderly.

Keywords: Tooth, dental pulp, dental pulp stem cells (DPSCs), senescence, ageing, inflammation, exosomes, miRNAs, stem cells, regeneration.

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Introduction

The world's population is ageing. By 2050 the number of people of 65 years of age and older will reach about 1.5 billion (Web ref. 1). The occurrence of general pain in the elderly is high, with the prevalence of chronic pain ranging from 27 % to 86 % (Larsson *et al*., 2017). This might be due to longstanding persistent disease processes, such as impaired vascular function and age-specific autoimmune conditions (Ungvari *et al*., 2018). The process of altered immune capability that accompanies ageing is known as immune senescence, which leads to increased susceptibility of older individuals to infections (Preshaw *et al*., 2017). Also, in dentistry, chronic pain in the elderly is frequently attributed to several factors, in particular neuropathy and dysregulation of immune responses (Zakrzewska *et al*., 2013; Ástvaldsdóttir *et al*., 2018). Consequently, there is an increasing interest in studying ageing processes, with the aim of preventing age-related pathologies and developing cell-based therapies tailored for older people (Partridge *et al*., 2018). Indeed, several novel medical disciplines such as regenerative and personalised medicine are evolving very rapidly in the attempt to meet these contingencies. In this regard, regenerative dentistry is still underdeveloped, although dental pathologies and disorders affect virtually all the

world's population (Kassebaum *et al.*, 2015; Web ref.2), particularly the aged (Ástvaldsdóttir *et al.*, 2018).

It has long been established that the dental pulp possesses remarkable regenerative abilities (Fig. 1). Upon tooth injury, odontoblasts, the pulp-derived cells responsible for dentine production, degenerate and are replaced by undifferentiated mesenchymal cells that migrate to the affected site from the deeper regions of the pulp (Mitsiadis and Rahiotis, 2004; Mitsiadis *et al.*, 2015). These cells differentiate into new odontoblast-like cells and produce reparative dentine (Mitsiadis and Rahiotis, 2004; Mitsiadis *et al.*, 2015; Orsini *et al.*, 2018). Dental pulp stem cells (DPSCs) exhibit high proliferative activity and are able to differentiate into odontogenic, osteogenic, chondrogenic, adipogenic, vascular, myogenic and neurogenic lineages (Gronthos *et al.*, 2000; Mitsiadis

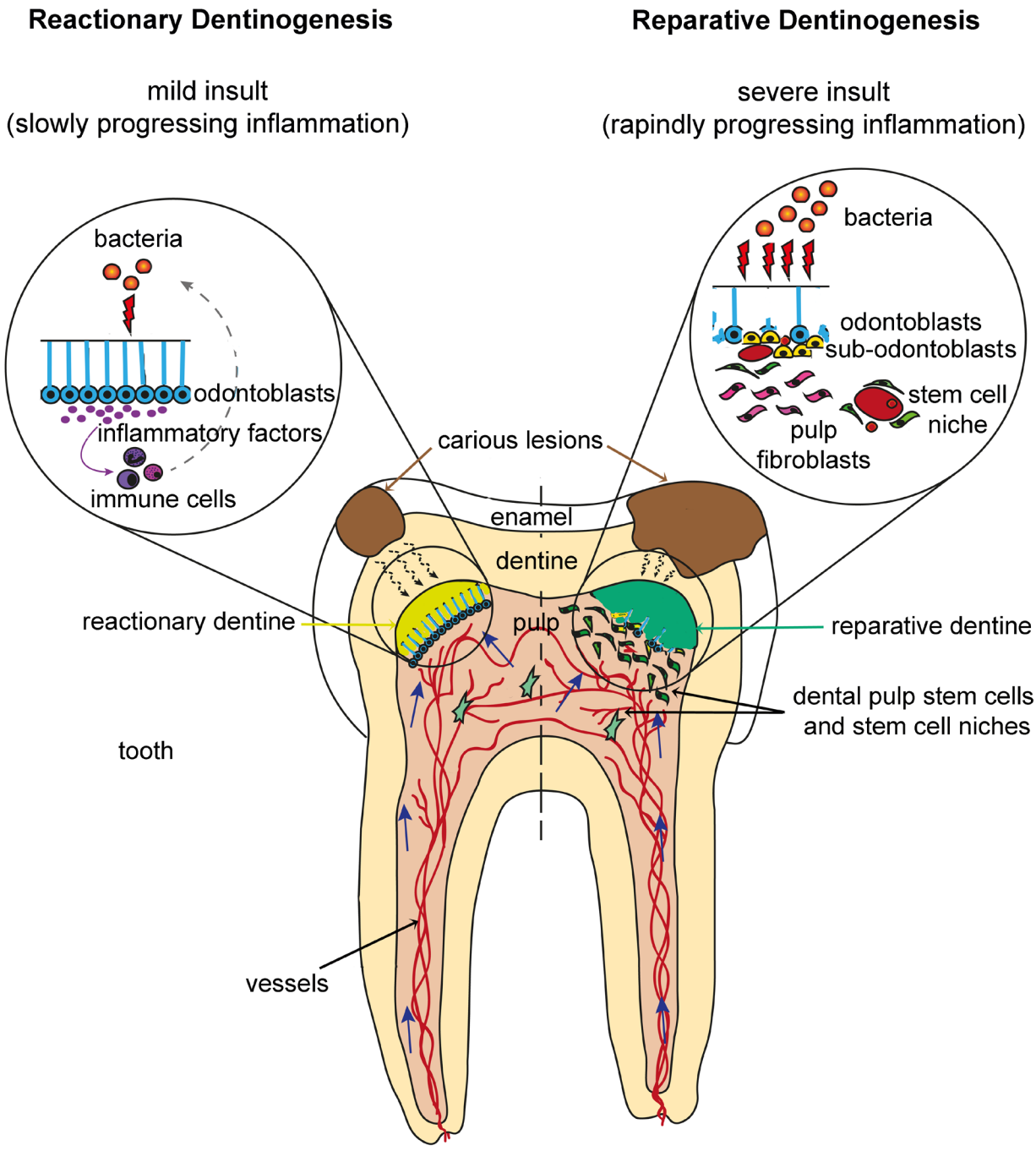

Fig. 1. Schematic representation of a tooth indicating the regenerative capacity of the dentine-pulp complex upon a carious lesion. Reactionary dentine is deposited by odontoblasts in response to a mild insult (*e.g.* dentine injury by minor caries, without pulp tissue exposure), potentially including proinflammatory mediators. These molecules are in charge to activate the immune response against the pathogens. Reparative dentinogenesis is a more complex mechanism and requires the generation of new odontoblast-like cells from stem/progenitor pulp in response to a severe insult.

et al., 2015). Due to their characteristics, DPSCs are the subject of intense investigations aiming at allowing craniofacial tissue regeneration.

In this mini-review, an overview is provided of the alterations occurring in DPSCs in response to ageing, to better understand pro and cons of potential stem-cell-based therapeutic approaches in the elderly.

Cell and tissue impairment with ageing

Ageing is manifested as an overall decline in organs' functional capacity, which normally maintains tissue homeostasis and physiological reactions (Hennrich *et al*., 2018). Age-related regressions are often gradual, mild in middle-aged patients, and become preeminent later in life, particularly under stressful conditions that require regenerative responses. The age-associated decrease in tissue cellularity and the consequent inadequate tissue reparative reactions are intimately linked to weak immune responses and impaired wound healing (Franceschi *et al*., 2018). Although senescence was first described in long-term cultures of fibroblast cells as a loss of proliferation capacity (Hayflick and Moorhead, 1961), cellular senescence also takes place in all body tissues during ageing (Fridlyanskaya *et al*., 2015; McHugh and Gil, 2018). Senescence constitutes an active mechanism that provides cellular homeostasis by blocking proliferation of aberrant cells that are under stressful conditions (Fridlyanskaya *et al*., 2015). However, senescence constitutes a stress response activated by insults associated with ageing (McHugh and Gil, 2018). Accumulation of senescent cells in aged tissues may create a favourable environment for the onset and progression of various age-related diseases (Childs *et al*., 2017; Ermolaeva *et al*., 2018; Aramillo Irizar *et al*., 2018; Gude *et al*., 2018).

The response to gradual loss of genomic, proteomic and metabolic integrity in ageing tissues (Lopez-Otin *et al.* 2013) is triggered and controlled by two main tumour suppressor pathways: p53-p21-retinoblastoma (RB) and $p16^{INK4A}$-RB proteins. Interestingly, these pathways crosstalk but can independently stop cell-cycle progression (Campisi and d'Adda di Fagagna, 2007). The activation of chronic DNA damage response, telomere shortening and upregulation of lysosomal protein levels are other typical signs of ageing cells (Hernandez-Segura *et al*., 2018). The activity of the specific lysosomal enzyme senescence-associated beta-galactosidase (SA-β-Gal) is widely used as marker for the augmented lysosomal content of aged cells (Lee *et al*., 2006). Accumulating evidence also indicates that ageing is associated with a decrease in autophagy (Lopez-Otin *et al.* 2013). Autophagy is a highly conserved pathway that removes redundant or defective organelles and protein aggregates. Its decrease in older individuals has been associated with several age-dependent pathologies, spanning from neurodegenerative diseases to metabolic disorders (Martinez-Lopez *et al.* 2015).

Aged cells significantly affect their microenvironment, as they secrete pro-inflammatory and matrix-degrading molecules, a process known as senescence-associated secretory phenotype (SASP) (Childs *et al*., 2015). SASP includes a variety of soluble signalling factors (*e.g*. interleukins, chemokines and growth factors), secreted proteases, and insoluble extracellular matrix components. These molecules affect neighbouring cells by activating different cell-surface receptors and the corresponding signal transduction pathways (Coppé *et al*., 2010). Such non-cell-autonomous processes have now been associated with many age-related conditions, including oral diseases (*e.g*. caries and periodontitis) (Preshaw *et al*., 2017).

Increasing evidence suggests a role for exosomes in the establishment of age-dependent tissue alterations (Xu and Tahara, 2013; Web ref. 3). Exosomes are nanovesicles (30-120 nm) secreted by all cell types and found in most of the body fluids (Keller *et al*., 2006). Along with trans-membrane proteins, they contain RNA material (mRNA and miRNA) and cytosolic proteins (Thery *et al*., 2002). The contents normally reflect the status of parental cells and influence the behaviour of recipient cells, both locally and systemically (Tkach and Thery, 2016). Their active release from aged cells (Xu and Tahara, 2013) could influence the cellular microenvironment and lead to tissue degeneration and age-related diseases (Web ref. 3).

MiRNAs are non-coding RNA molecules, composed of about 22 nucleotides, which function in RNA silencing and interference-mediated post-transcriptional gene regulation (Bartel, 2018). They are considered essential to normal cellular physiology and provide regulation of gene expression at the post-transcriptional level (Mendell, 2005). miRNAs can directly contribute to age-related senescence by deregulating the cell cycle and modulating cytoskeletal dynamics (Maes *et al*., 2008; Li *et al*., 2011).

Age-related changes in the dental pulp tissue

Ageing affects all tissues and organs of the human body (Lopéz-Otín *et al*., 2013; Kubben and Misteli, 2017). The dental pulp also undergoes age-related changes and several studies have focused on its ageing. The dental pulp is a highly specialised, cranial neural-crest-derived mesenchymal tissue that hosts many cell types and is responsible for the production of dentine (*i.e*. odontoblasts) and the perception of pain (*i.e*. nerve fibres) (Cobourne and Mitsiadis, 2006; Mitsiadis and Graf, 2009; d'Aquino *et al*., 2009) (Fig. 1). Upon tooth damage by external insults, odontoblasts respond by increasing their secretory activity to produce reparative dentine, and the pulp cells activate inflammatory responses in the case of bacterial invasion (Mitsiadis and

Rahiotis, 2004; Veerayutthwilai *et al.*, 2007; Farges *et al.*, 2011; Mitsiadis *et al.*, 2015). The dental pulp is also characterised by high collagen content and the presence of few scattered fibroblasts. Pulp fibroblasts are responsible for the formation and turnover of extracellular matrix and play an important role during tooth damage (Shimabukuro *et al.*, 2009). The core of the pulp region contains a vast mesoderm-derived vascular network plexus as well as nerves, which contribute to the establishment of DPSC niches (Pagella *et al.*, 2015).

All of these dental pulp cell populations undergo age-related modifications, which include the reduction of the pulp chamber caused by continuous formation of dentine (Burke and Samarawickrama, 1995), a reduced vascular supply, the formation of fibrous bundles, and the reduction of fibroblast density. Extensive calcification of the pulp is also a particular condition occurring with ageing. Calcifications in the coronal region are known as pulp stones, whereas those in the radicular pulp are diffuse and may lead to a complete calcific degeneration, a process termed pulpal obliteration (Murray *et al.*, 2002; Goldberg, 2014; Montoya *et al.*, 2015). All these events take place approximately in the same period (20-39 years of age), and they are often followed by a decrease in odontoblast cellularity (40-59 years of age). Moreover, with increasing age, pulp cells modify their morphology and acquire a flattened and spindle-like shape (Daud *et al.*, 2016). Similarly, odontoblasts from older individuals show clear signs of decrease of autophagic activity, which results in the accumulation of intracellular lipids and a subsequent loss of functionality (Couve and Schmachtenberg, 2011). Changes in pulp cell density and decreased pulp stemness in advanced age affect the regenerative capability of the pulp upon tooth injury.

Impact of ageing on dental pulp stem cells

Organs possess an astonishing capacity for extensive and continuous tissue renewal throughout the individual's lifetime, which is maintained by reservoirs of various stem cell populations (Mitsiadis *et al.*, 2007). As physiological functions of all organs decay with age, stem cells have gained increasing consideration in age-associated regenerative processes. It is indeed essential to preserve a sufficient number of stem cell populations in order to maintain organ functionality with advancing age. It has long been recognised that the function and proliferative potential of mesenchymal stem cells (MSCs) declines with age and this might influence the effect of autologous MSC transplantation in the elderly (Zhang *et al.*, 2005). Recent studies showed that age-related dysfunctions also occurred in DPSCs (Yi *et al.*, 2017). Ageing affects DPSCs, which exhibit typical senescence features such as enlarged cell shape, decreased proliferation and decreased differentiation potential. Increases of SA-β-gal activity and $p16^{INK4A}$ expression were also noticed in adult DPSCs when compared to cells from younger individuals (Feng *et al.*, 2014) (Fig. 2).

Ageing also affects the ability of DPSCs to contribute to mineralisation processes. In fact, a decreased osteogenic potential was observed in aged human DPSCs (Yi *et al.*, 2017; Iezzi *et al.*, 2019). The decline of differentiation potential towards mineralised tissues (*i.e.* bone and dentine) with age has been associated with changes in expression of bone-related genes (*e.g. BMP2*, β-catenin, *RUNX2* and *BGLAP*). Moreover, a strong decrease in the expression of *DMP1* and *DSPP*, coding for key odontogenic differentiation markers, was observed in DPSCs cultured in odontogenic medium derived from older patients (Iezzi *et al.*, 2019). During cytodifferentiation, DMP1 that regulates mineralisation processes, is translocated from the nucleus to the cytoplasm and thereafter is secreted to the extracellular space, where it regulates the nucleation of hydroxyapatite (HA) (Narayanan *et al.*, 2003; Qin *et al.*, 2003). Upon odontogenic differentiation, aged DPSCs have lower amounts of cytoplasmic DMP1 when compared to younger DPSCs (Iezzi *et al.*, 2019). This correlates with the diffusing calcific degeneration of the pulp, a pathologic condition occurring as a response to ageing (Piattelli and Trisi, 1993).

Adult DPSCs also display lower neurogenic differentiation potential. Various studies have shown that markers of neurogenic differentiation decreased with age (Feng *et al.*, 2013), and that this is associated with impaired localisation of β-tubulin III (Martens *et al.*, 2012) and β-catenin expression upon neural induction (Feng *et al.*, 2013).

Age-related features of DPSCs can possibly be reverted by providing appropriate extracellular cues and substrates. DPSCs from older individuals display similar regenerative properties to DPSCs isolated from younger patients, when cultured on nanostructured HA scaffolds and used *in vivo* to repair calvaria defects in rats (Bressan *et al.*, 2012).

Ageing and the role of secretory factors in dental pulp inflammaging

Ageing is characterised by the accumulation of senescent cells and correlates with changes in pro-inflammatory events (Campisi *et al.*, 2011; Freund *et al.*, 2011; Lopez-Otin *et al.*, 2013). "Inflammaging" refers to the chronic, low-grade inflammation that characterises ageing (Franceschi *et al.*, 2018). In this context, chronic inflammations, along with the loss of the normal immune response capability during ageing, can alter immunocompetence and promote age-related diseases (Franceschi and Campisi, 2014). Dental caries represents one of the most common health problems in humans and refers to the degenerative process causing decalcification of tooth hard tissues (Selwitz *et al.*, 2007). The extent of caries increases progressively with age and might lead to

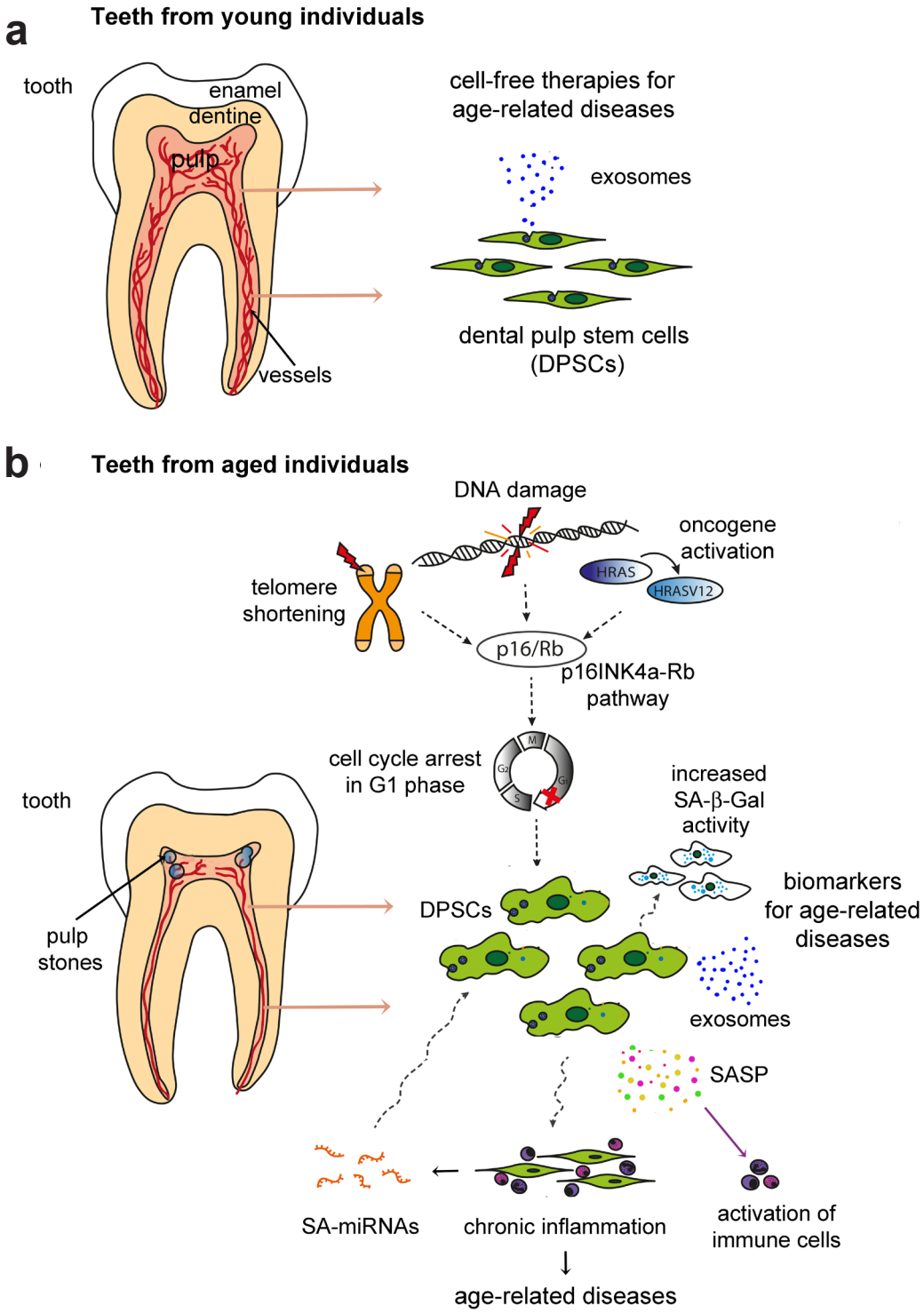

Fig. 2. Schematic representation showing the effects of ageing in the dental pulp tissue. (**a**) The dental pulp from healthy teeth from young individuals has an appropriate volume and receives a rich vascularisation. Exosomes released from dental pulp stem cells (DPSCs) represent a valid source of free-cell based therapy for age-associated diseases. (**b**) Age-related changes in dental pulp and DPSCs. Dental pulp volume and its vascularisation decrease when compared to the pulp tissue of young subjects. A diffusing calcific degeneration of the pulp also occurs during ageing. Telomere shortening, DNA damage, and oncogene activation are the primary drivers of pathology in ageing. This process activates the Rb/p16 pathways to block cell cycle and sustain growth arrest, leading to age-related cell senescence. Senescent cells (SNCs) are positive for SA-β-gal, indicating lysosomal content augmentation, and are characterised by specific senescence-associated secretory phenotype (SASP). SASP recruit immune cells (*e.g.* macrophages, neutrophils and natural killer (NK) cells) to phagocytose and remove SNCs. This can in turn drive chronic inflammation of dental pulp, that triggers multiple age-related diseases. Impairment of miRNAs may impact the chronic condition of dental pulp tissue (*e.g.* miR-181 family, miR150, miR-584, miR766 and miR-433). DPSCs-derived exosomes are involved in ageing processes and may constitute novel therapeutic tools in age-related diseases.

pulpitis, a pathological condition of the dental pulp characterised by tissue inflammation (Lee *et al.*, 2013; Bernabé and Sheiham, 2014).

Ageing affects the secretion of some senescence-associated factors, including matrix metalloproteinases (MMPs) (Coppè *et al.*, 2010). Several studies have shown that the concentration of specific MMPs increased significantly in inflamed pulp compared to the normal pulp (Hannas *et al.*, 2007). In pulp tissue from patients suffering from acute pulpitis, the levels of MMP-2 and MMP-3 were significantly higher than in pulp from healthy individuals, suggesting that MMPs may play a role in the progression of pulp inflammation and/or damage. Indeed, MMP-3 may activate the expression of other MMPs, such as MMP-1, -7 and -9, which is crucial for triggering the collagen degradation that will eventually lead to changes in the extracellular matrix. These events have been observed in tooth tissues pathologies such as acute and chronic pulpitis and periapical lesions (Shin *et al.*, 2002; Goda *et al.*, 2015).

The progression of dental caries into the dental tissues leads to the accumulation of inflammatory cells within the dental pulp. These cells release inflammatory cytokines such as tumour necrosis factor α (TNFα) that promote mineralisation (Pezelj-Ribaric *et al.*, 2002; Liu *et al.*, 2005). This could explain the generation of nucleation points, which drive pulp stone formation in teeth of aged individuals (Lee *et al.*, 2013).

These results suggest that ageing may also affect the secretome of DPSCs (Benatti *et al.*, 2009; Domon *et al.*, 2014). Indeed, using the secretome from young DPSCs could be an alternative approach to autologous DPSCs transplantation for cell-free treatments of the elderly population. For example, it has been reported that providing the secretome from DPSCs can improve diabetic polyneuropathy by improving nerve/muscle blood flow and suppressing inflammation (Martens *et al.*, 2012).

DPSCs-derived exosomes and miRNA involvement in age-related diseases

Understanding the connection between ageing and age-related diseases is of great significance, especially for undertaking prevention measures and improving modern therapeutic strategies. In this regard, exosomes are thought to play a key role in several physiological and pathological conditions, and are currently being tested as disease biomarkers (Melo *et al.*, 2015). Exosomes derived from DPSCs are an attractive source of nanovesicles for the treatment of many age-related pathologies. Their ability to suppress inflammation in mice has been shown and was comparable to that obtained with corticosteroids (Pivoraité *et al.*, 2015). It has been demonstrated that DPSCs-derived exosomes were able to trigger dental pulp-like tissue regeneration in a tooth root-slice *in vivo* model (Huang *et al.*, 2016). This regenerative potential might also be due to their pro-angiogenic properties. Recently, it has been shown that the application of DPSCs-derived exosomes leads to increased proliferation of human umbilical vein endothelial cells (HUVEC) and the formation of new vessels (Xian *et al.*, 2018). DPSCs-derived exosomes may also have a potent neuroprotective capacity. In particular, nanovesicles derived from stem cell populations of young teeth were able to suppress 6-hydroxy-dopamine (6-OHDA)-induced apoptosis in dopaminergic neurons (Jarmalavičiūtė *et al.*, 2015).

Emerging evidence suggests that miRNAs might also exert important functions in age-related oral diseases (Gay *et al.*, 2014; Li *et al.*, 2015). Differences in miRNA expression levels have been detected between DPSCs from healthy and inflamed pulp tissues, the latter being more common in the elderly. For instance, the expression of different miRNAs of the miR-181 family was downregulated in DPSCs from pathological pulp tissues. These miRNAs include: miR-181a, a modulator of *IL-6* levels (Pichiorri *et al.*, 2008), miR-181b which regulates the chemokine ligand 8 (*CCL8*) (Dave and Khalili, 2010), miR-181 which controls *IL-2* (Xue *et al.*, 2011), and miR-181d which regulates the expression of metallopeptidase 9 (*MMP9*) (Wang *et al.*, 2010). In addition, it has been shown that miR150, miR-584, and miR766 were significantly upregulated in DPSCs from inflamed dental pulps when compared to DPSCs of healthy pulps (Zhong *et al.*, 2012). A recent work demonstrated that miR-433 is an important senescence-associated miRNA in human DPSCs, regulating morphological, proliferative, apoptotic, and mineralisation events (Wang *et al.*, 2015).

Taken together, these results show that DPSC-derived exosomes are clearly involved in ageing processes and may provide novel therapeutic tools in age-related diseases. In addition, miRNAs from DPSCs may play a crucial role in age-related inflammatory process within the dental pulp.

Conclusions and future perspectives

A direct consequence of ageing is the impairment of cell physiology that affects the function of all tissues and organs. Systemic and environmental factors profoundly influence tissue ageing. Therefore, experimental therapeutic approaches that are closely related to age-dependent senescence constitute a nascent and promising area of translational research. Experiments using a heterochronic parabiosis model, in which one young and one old mouse are surgically joined in order to develop a shared blood circulation, could offer important information about treatment of age-related diseases. It has been observed that older parabiotic mice have improved stem cell function in muscles (Conboy *et al.*, 2005), brain (Villeda *et al.*, 2014), and heart (Loffredo *et al.*, 2013). Small molecules released by cells could

represent a potential therapeutic approach to trigger and strengthen immune responses in senescent cells, or event to rejuvenate tissues. Accumulating scientific knowledge on the secretome, microvesicles, nanovesicles, and exosomes of stem cells is crucial, since they could be potentially used for the treatment of age-related diseases and for promoting repair of the damaged tissues (Zhang *et al*., 2016). These vesicles are enriched with diverse proteins, lipids, messenger RNAs (mRNAs), and non-coding RNAs (ncRNAs) such as miRNAs, that are associated with immune system regulation (Robbins and Morelli, 2014).

However, despite the considerable progress in the last decade, little is known about the mechanisms underlying senescence-related phenomena in the dental field. Whether differences between senescence-associated factors secreted by both adult and young DPSCs exist is a key open question. The involvement of the immune system in age-related dental and oral pathological manifestations might be further investigated. DPSCs could be used to better assess all these open questions, since they are easily accessible and play a pivotal role in dental pulp tissue regeneration, angiogenesis, neurogenesis and mineralisation.

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Discussion with reviewers

Javier Catón: I have been wondering as I read this M/S if there are there any known plans to use the extracellular material discussed here in any medical trials? Also, do the authors expect any possible issues with rejection by the host? Very interesting field of research!

Authors: Studies performed in other compartments show that exosomes can indeed exert significant effects on target-cells behaviour, including rejuvenation, without the need for cell transplantation (Colao *et al.*, 2018).

Based on these indications, a handful of studies have either been undertaken or are currently ongoing. These include the use of autologous, modified dendritic-derived exosomes for maintenance immunotherapy (Besse *et al.*, 2015), allogeneic MSC-derived exosomes for the treatment of chronic kidney disease (Nassar *et al.*, 2016), type 1 diabetes mellitus (clinical trial NCT02138331), acute ischaemic stroke (clinical trial NCT03384433), and autologous plasma-derived exosomes for cutaneous wound repair (clinical trial NCT02565264) (Colao *et al.*, 2018).

As exosomes have proved to be excellent carriers *in vivo*, they appear as optimal and easy candidates for rejuvenation of aged dental (and possibly non-dental) tissues by local injection or incorporation in appropriate biomaterials. As of now, however, no studies aiming at DPSCs-exosome-mediated rejuvenation are under trial. Studies concerning the exact properties of exosomes isolated from dental pulp cells of young and adult individuals are needed before we can envisage any therapeutic application.

Recent studies showed that exosomes released by allografts actually contribute to the host's immune response leading to their rejection (Benichou and Prunevieille, 2018). At the same time, however, abundant evidence indicates that exosomes, and particularly MSC-derived exosomes, exert important immunomodulatory effects (*e.g.* Zhang *et al.*, 2018). Allogeneic MSC-derived exosomes were even used to treat a single patient with graft *versus* host disease (Kordelas *et al.*, 2014). Much effort is, therefore, being spent in optimising methods that would allow clinical-grade exosomes purification and selection, so as to obtain reliable therapeutic tools with predictable effects on the recipient's immune system (Colao *et al.*, 2018).

Michel Goldberg: What about the turnover of pulp stem cells. Are they really ageing? or renewed?

Authors: As discussed in the review, dental pulp stem cell isolated from aged patients display a significant decrease in their differentiation and proliferative potential (Yi *et al*., 2017; Feng *et al*., 2014; Iezzi *et al*., 2019). This indicates that these cells themselves are significantly affected by the ageing process. This can be driven by age-dependent cell-intrinsic alterations, such as *e.g*. accumulation of mutations or misfolded proteins, but *in vivo* this effect can be exacerbated by the ageing of the surrounding microenvironment. For example, among other age-related alterations, the reduced vascular supply observed in dental pulps from old patients (Bernick and Nedelman, 1975; Burke and Samarawickrama, 1995) can clearly affect dental pulp stem cells functionality, as these cells (like other stem cell types) strictly depend on vessel-derived trophic support as well as angiocrine signals to function properly (Rafii *et al*., 2016). To date, no clear evidence supports or shows increased apoptosis within the dental pulp of aged patients. The latter question is still open; but, according to the existing literature, stem cell apoptosis does not seem to be the primary reason for age-dependent decline in tissue homeostasis and regeneration in several other organs (Schultz and Sinclair 2016; Krimpenfort and Berns 2017).

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