REVIEW

Neural crest derived stem cells from dental pulp and tooth-associated stem cells for peripheral nerve regeneration

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Abstract

The peripheral nerve injuries, representing some of the most common types of traumatic lesions affecting the nervous system, are highly invalidating for the patients besides being a huge social burden. Although peripheral nervous system owns a higher regenerative capacity than does central nervous system, mostly depending on Schwann cells intervention in injury repair, several factors determine the extent of functional outcome after healing. Based on the injury type, different therapeutic approaches have been investigated so far. Nerve grafting and Schwann cell transplantation have represented the gold standard treatment for peripheral nerve injuries, however these approaches own limitations, such as scarce donor nerve availability and donor site morbidity. Cell based therapies might provide a suitable tool for peripheral nerve regeneration, in fact, the ability of different stem cell types to differentiate towards Schwann cells in combination with the use of different scaffolds have been widely investigated in animal models of peripheral nerve injuries in the last decade. Dental pulp is a promising cell source for regenerative medicine, because of the ease of isolation procedures, stem cell proliferation and multipotency abilities, which are due to the embryological origin from neural crest. In this article we review the literature concerning the application of tooth derived stem cell populations combined with different conduits to peripheral nerve injuries animal models, highlighting their regenerative contribution exerted through either glial differentiation and neuroprotective/neurotrophic effects on the host tissue.

Key Words: *glial differentiation; human dental pulp stem cells; nerve regeneration; neural crest; neuroprotection; tooth*

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Introduction

Peripheral nerve injuries (PNI) are some of the most common types of traumatic lesions affecting the nervous system. PNI have an incidence of about 18 per 100,000 persons every year in developed countries, with a relatively higher impact in developing countries (Jiang et al., 2017). These damages result in highly invalidating for the affected patients, either physically or psychologically, representing an outstanding social burden. PNI can be related to either traumatic events or to different illness-related neuropathies, i.e., hereditary, toxic, metabolic, and immune-mediated neuropathies (Katona and Weis, 2017). PNI often cause the breakdown of neuronal circuit with following denervation of primary organs and functional limitations. It is well known that peripheral nervous system has a higher regenerative capacity than central nervous system. Following peripheral nerve damage, Schwann cells (SCs) undergo several changes needed to sustain axon outgrowth, such as transdifferentiating, losing the myelinating phenotype and shifting towards repair cells. SCs upregulate the growth-promoting genes, as well as adhesion molecules, neurotrophic factors and their receptors (You et al., 1997; Rahmatullah et al., 1998; Höke et al., 2002; Chen et al., 2007).

However, several factors, including patient's age, injury type and delayed intervention time – determine the degree of functional recovery after healing. Indeed, crushed nerves show better recovery than do transected nerves, with better outcomes of distal injuries when compared to proximal ones, since axons that need to cover a short gap to reach the target tissue have higher chances to reconnect (Sunderland, 1952; Woodhall and Beebe, 1956; Sunderland, 1978; Brushart, 2011). The lost function may not always be reverted because the regenerated axons are not able to reinnervate the areas formerly linked by them (Johnson et al., 2005). As a matter of fact, complete nerve transections, many of them being proximal lesions in the nerve or resulting in a huge gap, have poor chances of recovery, thus leading to decreased motor and sensory function (Wang and Sakiyama-Elbert, 2018) and life-long disabilities for the patients.

Based on the injury type, different therapeutic approaches have been investigated so far, ranging from suture for managing nerve discontinuities without a gap, up to nerve autografts for handling huge gap nerve lesions. Such methods would be although limited by a poor functional outcome or by scarce tissue graft availability and donor site morbidity. Furthermore, different synthetic conduits and acellular allografts have been investigated for their peripheral nerve regenerative potential and, although demonstrating the ability to recreate the nerve extracellular matrix, the lack of the mainly involved cellular component, i.e., the SCs, revealed to be critical for the regeneration (Sun et al., 2009; Moore et al., 2011; Saheb-Al-Zamani et al., 2013).

Cell based therapies might provide a suitable tool for peripheral nerve regeneration, in fact, the ability of different stem cell types to differentiate towards SCs and their regenerative potential in animal models of PNI have been widely investigated by several research groups in the last decade. Particularly, bone marrow mesenchymal stem cells, adipose derived stem cells and muscle derived stem cells have been studied for their potential application to PNI treatment (Shimizu et al., 2007, 2018; Razavi et al., 2012; Lavasani et al. 2014; Tamaki et al., 2016; Saller et al., 2018).

However, most of these investigated stem cell populations are characterized by an embryological origin differing from neuroectoderm. In light of the development of cellular therapies in compliance with ethics, it would be preferable to identify the most suitable cell source without involving an embryological transition. Starting from the characterization of Schwann cell physiology and their primary role in PNI regeneration, the aim of this article is to discuss the features of tooth derived stem cells in light of their shared peculiar embryological origin from neural crest and to review their contribution to peripheral nerve regeneration, and how their regenerative benefits might be extended to pre-clinical application.

Search Strategy and Selection Criteria

We searched on PubMed for articles published between 2000 and 2019, by using the terms "peripheral nerve regeneration" and "peripheral nerve injury" in combination with "tooth derived stem cells" or "dental stem cells" and the results were then selected according to their relevance within the scope of this review. Older publications regarded as highly relevant to the topic were included as well. Moreover, we also did a selection from the references listed in the articles resulting from our search on PubMed.

Neural Crest: the Fourth Germ Layer

The third week of embryo development is characterized by two fundamental processes: gastrulation and neurulation. During the first one, the three germ layers - ectoderm, mesoderm and endoderm - take origin, whereas in the second one, the development of nervous system occurs.

As far as neurulation is concerned, at the end of the third week of embryo development, the notochord induces the differentiation of part of ectodermal cells, which give rise to neural plate. At this time, ectoderm proceeds toward two different fates which will culminate in the formation of epithelial ectoderm and nervous ectoderm, respectively. Cells at the edges of neural plate shape their morphology forming the neural folds, that start growing, bulging up to converge with each other, converting the neural plate into the neural groove. The closing of neural groove begins on the 21st day in the neck region corresponding to the fourth pair of somites, by proceeding either in cephalic and caudal directions. Within 3 days, the neural groove is closed along the whole embryo axis, except for the ends of neural tube. The front end is the first one being closed, while the rear end being closed 2 days later, thus rendering the neural tube the primordium of the central nervous system. On the other hand, while the neural folds are converging to each other, at their edges some cells start proliferating without participating in the formation of the neural tube. These cells will indeed give rise to neural crest, which initially lays between the epidermis and the nervous tube and then starts migrating laterally to different directions (Le Douarin and Kalcheim, 1999). Neural crest cells migrate towards different districts, where they will differentiate in many different cell and tissue types, such as spinal ganglia and autonomic nervous system, SCs, pigmented cells, adrenal medulla, encephalic meninges and the mesenchyme of head and neck (Kulesa et al., 2010). Neural crest derived cells participate to the tooth development and reside within the dental pulp connective tissue up to adulthood, maintaining their stemness properties (Chai et al., 2000).

Schwann Cells: Development and Role in Nerve Injury Regeneration

SCs are the PNS glial cells, own myelinating functions and play a key role in survival and functionality of neurons. Besides producing myelin, SCs also exert a primary role in regenerating receptors and to receptors' functions (Bunge, 1993). There are a plenty of growth factors produced and released by SCs, such as neurotrophins, TGF-βs, glial cell line-derived neurotrophic factor (GDNF), epidermal growth factors (EGFs), and platelet-derived growth factor (PDGF). In the first phases of embryo development, SCs take origin from the neural crest which includes multipotent cells that migrate away from the dorsal neural tube (Le Douarin, 1986). SCs development takes place through three transitions if neural crest: 1) formation of a Schwann cell precursor; 2) establishment of an immature Schwann cell; 3) postnatal immature SCs can become either myelinating or non-myelinating SCs. For each phase, events are modulated by neuregulins (Bhatheja and Field, 2006). The primary function of SCs is to myelinate axons in the PNS. The production of myelin, a fatty layer isolating the axon, allows to increase the saltatory conduction of the neuron with one myelinating SC wrapped around a single axon. Large-diameter axons conduct impulses at the highest speed and become myelinated, whereas the thin, slow conducing fibers are pushed together and enclosed in massive, globular non-myelinating SCs (Voyvodic, 1989). Axon signals are critical in directing Schwann cell lineage. The expression of P0, a protein specific for Schwann cell myelin, returns to basal levels when the immature cell stops being associated with the axon, thus demonstrating an axon-dependent response. While mature SCs are able to survive without a neuronal signal, on the other hand, precursor Schwann cell cannot (Jessen and Mirsky, 2005; Bhateja and Field, 2006). Krox-20 (Erg2), Oct-6, and Sox-10 are transcription factors that regulate Schwann cell lineage. The Krox-20 transcription factor is important in transforming the immature Schwann cell into a myelinating Schwann cell, while also inhibiting cell death and proliferation (Topilko and Meijer, 2001). The POU transcription factor Oct-6, as well as Krox-20 is also responsible

for myelination with Krox-20 being present only in myelinating cells, whereas Oct-6 is found in all SCs (Jessen and Mirsky, 2005; Bhateja and Field, 2006). Peripheral nerves regeneration mostly relies on the plasticity of SCs. Indeed, after nerve injury, fully mature SCs undergo dedifferentiation towards a cell phenotype resembling different properties of immature SCs stage (Jessen and Mirsky, 2008; Shin et al., 2013; Hyung et al., 2019). During dedifferentiation, SCs downregulate the factors promoting myelination, start breaking down myelin and activate a repair program which provides a supportive environment for axonal regrowth: SCs start forming cellular conduits along which axons can regrow and express molecules favourable to the survival of injured neurons (Jessen and Mirsky, 2016; Hyung et al., 2019). Such features prove evidence for SCs to be the first and most widely used support cells in peripheral nerve regeneration (Guenard et al., 1992; Rodriguez et al., 2000; Mosahebi et al., 2001). Previous findings demonstrated the active role of SCs in nerve regeneration, by using a genetic labelling technique aimed to trace SCs after implantation into the nerve injury site (Mosahebi et al., 2002; Tohill et al., 2004; Gu et al., 2011). A genetic engineering method has been investigated to test whether SC-induced axonal growth in the spinal cord (Xu et al., 1995) might be optimized. Previous findings from Tuszynski et al. (1998) reported that cultured primary adult rat SCs were genetically engineered to secrete NGF. Following implantation into the midthoracic spinal cord of adult rats, these cells not only survived for one year but also were densely penetrated by primary sensory nociceptive axons, when compared to control implants. Schwan cells effectively myelinated axons either in NGF-secreting or in control implants (Tuszynski et al., 1998). When applied to the lesion cavity of a dorsal hemisection of the rat spinal cord, a significant increase in growth of spinal cord axons was observed in the implant area (Weidner et al., 1999). A denser network of coerulospinal axons and central processes of primary sensory afferents was detected in transduced implants, with respect to untransduced implants. Furthermore, these axons were ensheathed and, in some instances, remyelinated by SCs. Weidner et al. (1999) demonstrated that implanted SCs exhibited a phenotypic and temporal course of differentiation into a myelinating state while aligning spontaneously. Three days after implantation, SCs were still in an undifferentiated or non-myelinating state. After 2 weeks, they had upregulated the cell adhesion molecule L1, a marker for differentiated non-myelinating SCs. After 3 weeks, the major component of peripheral myelin, namely P0 protein, was detected, thus indicating that some SCs might have adopted a myelinating phenotype. As no differences in SC markers were found between NGF-secreting and control implants, it was argued that NGF itself did not modulate the SC myelinating phenotype. The observed time course of SC differentiation after grafting into a CNS injury site was the demonstration of the dedifferentiation process occurring in PNS after injury. The physiological response of SCs to injury appears to be retained following transplantation to an ectopic site, i.e., in the injured spinal cord (Weidner et al., 1999). SCs previously induced to BDNF secretion through retroviral vectors and then implanted as trails, in and distal to the transection site of the adult rat spinal cord, were able to attract more dorsal root ganglia and spinal and supraspinal fibers than control SC implants (Menei et al., 1998).

The SC track was maintained for at least 1 month and most of the fibers showed a germination phenomenon at the transection site. SCs that secrete BDNF, however, did not appear to myelinate regenerating axons, based on the absence of P0 expression, which was instead detected in normal SC and NGF-transduced SC. Such evidence might allow to state that, when BDNF is present, SCs retain a dedifferentiated phenotype favorable to fibers regeneration although not forming myelination (Menei et al., 1998). These studies show that the production of neurophysiological levels of neurotrophins by genetically modified SCs may increase the regenerative potential of injured spinal axons, but also that other characteristics of SCs, such as axon myelination (Ruitenberg et al., 2006), can be affected by the expression of a certain neurotrophic factor instead of another. The modulatory action of neurotrophins on the SCs can therefore be considered an interesting therapeutic approach for the PNI as much as it is for neurodegenerative diseases strictly correlated to demyelination, such as multiple sclerosis (Kocsis and Waxman, 2007) or peripheral denervation in the amyotrophic lateral sclerosis (Vallarola et al., 2018).

Dental Ecto-Mesenchymal Stem Cells

Teeth represent a suitable stem cell source due to their easy accessibility through routine procedures of wisdom teeth extraction and, principally, since they provide a huge quantity of quickly self-renewing, multipotent stem cells (Goldberg et al. 2004; d'Aquino et al., 2009; Tirino et al., 2012). So far, it has been well established that dental and periodontal tissues can be considered a reservoir of neural crest stem cells (Gronthos et al., 2000). The neural crest, which constitutes a peculiar type of mesenchymal tissue, namely the ectomesenchyme, gives origin to most of craniofacial structures, including dental pulp and periodontal ligament (Chai et al., 2000). Dental ectomesenchymal stem cells (EMSCs) own a common origin with neural crest cells, as a matter of fact the formation of oral muscles, bones, tongue, craniofacial nerves and teeth relies on ectomesenchyme (Janebodin et al., 2011). Nerve tissue regeneration approaches can take advantage from the use of dental and periodontal stem cells, since they own a neural crest phenotype. As far as mesoderm-derived MSCs are concerned, dental EMSCs constitutively express neural-progenitors markers yet under standard culture conditions (Davidson, 1994; Gronthos et al., 2002; Miura et al., 2003; Janebodin et al., 2011), thus suggesting that EMSCs might maintain the intrinsic ability to differentiate towards nerve cells. In particular, the fact that embryonic origin is shared with the peripheral nervous system allows to argue that dental EMSCs are much closer to nerve cells than other stem cells, such as mesodermal MSCs. Particularly, recent evidence from Kaukua et al. (2014) revealed a population of dental EMSCs that turned out to be derived from peripheral nerve-related glial cells, proposing a strong connection be-

tween SCs and dental EMSCs during tooth generation.

Dental EMSCs might represent an optimal choice to reach an effective neural and glial differentiation, under the appropriate conditions (Arthur et al., 2008; Janebodin et al., 2011).

Different stem cells populations were identified in dental associated tissues and components, with human dental pulp stem cells (hDPSCs) being first isolated by Gronthos et al. (2000); then, other stem cells populations were revealed in human exfoliated deciduous teeth (SHED), periodontal ligament (PDLSCs) and, finally, in the apical papilla (SCAP) (Miura et al., 2003; Seo et al., 2004; Sonoyama et al., 2006). For the characterization of these tooth-related stem cells populations, a comparison was made with the widely investigated bone marrow mesenchymal stem cells (BM-MSCs) and, as a source of MSCs, they were evaluated for the expression of typical mesenchymal surface antigens, such as CD44, CD73, CD90, CD105, CD271 and STRO-1, while they were expected not to express markers such as CD34, CD45, and HLA-DR (Uccelli et al., 2008). In spite of this immunophenotypical characterization, a specific, strict marker identifying DPSCs has not been outlined, thus allowing to define them as a heterogeneous population.

Data from different studies suggest that these dental tissue-derived stem cells not only show self-renewal and multiple differentiations potential but also display immunomodulatory properties and a promising regenerative potential towards different tissue injuries. **Table 1** reports the features of the different types of stem cells isolated from dental tissue. Here we will review the features of each tooth derived stem cell population, with particular focus on the translational and pre-clinical data concerning their application to peripheral nerve regeneration.

Dental Pulp Stem Cells

As earlier hinted, DPSCs were first identified and isolated from human dental pulp tissue by Gronthos et al. (2000). They are well characterized by a fibroblast-like morphology, clonogenic abilities and a high proliferation rate and express Oct-4, Nanog and Sox-2, besides nestin and vimentin, all of them being peculiar markers of undifferentiated embryonic stem cells (Govindasamy et al., 2011). After their original characterization and many parallels drawn between DPSCs and BM-MSCs through the years (Yamada et al., 2006), these stem cells were proved able to commit into different cytotypes, including osteogenic, chondrogenic, myogenic, adipogenic and neural lineages (Gronthos et al., 2002; Laino et al., 2005; d'Aquino et al., 2007; Arthur et al., 2008; Stevens et al., 2008; Armiñán et al., 2009; Pisciotta et al., 2018). It was recently demonstrated that DPSCs are also able to differentiate to insulin producing cells, thus suggesting that they can also be committed to the endodermal lineage (Carnevale et al., 2013). Moreover, another well-established property is their capability to promote angiogenesis *in vivo* (Pisciotta et al., 2012a, 2015b; Riccio et al., 2012; Maraldi et al., 2013).

To our knowledge, after isolation, human dental pulp stem cells are able to form colonies with different proliferation rates and showing different surface markers. In fact, hDPSCs consist in a heterogeneous cell population that cannot be defined by strictly specific markers. As well reported by Kawashima (2012), the existence of distinct hDPSCs subpopulations owning different biological properties was demonstrated by the use of different mesenchymal stem cell markers. To this regard, STRO-1 and c-Kit are two key surface markers whose expression is required to define the mesenchymal origin and the stemness of hDPSCs. Farther, our previous findings highlighted the presence of hDPSCs subpopulation expressing also CD34, in accordance with former evidence from Laino et al. (2005).

Although CD34 was shown to be a conventionally accepted marker identifying hematopoietic stem cells findings

Table 1 Tooth derived stem cells characterization, differentiation potential and role in PNI regeneration

Stem cell type	Immunophenotype/Surface markers expression	Differentiation potential	*In vivo* models of PNI	Contribution to PNI regeneration
DPSCs	Nanog, Oct-4, Sox-2, Nestin, Vimentin, CD44, CD105, CD73, CD90, CD117, CD34, STRO-1, CD271, Sox-10	Osteogenic, chondrogenic, adipogenic, myogenic, neural, β-pancreatic cells	Sciatic nerve injury (Askari et al., 2015; Kolar et al., 2017; Omi et al., 2017; Sanen et al., 2017; Carnevale et al., 2018) Facial nerve injury (Sasaki et al., 2008, 2011)	*In vivo* cell differentiation and neurotrophic factors release
SHED	Nanog, Oct-4, SSEA-3, SSEA-4, Nestin, CD44, CD105, CD73, CD90, STRO-1, CD146	Odontogenic, osteogenic, chondrogenic, adipogenic, myogenic, neural, hepatocytes	Sciatic nerve injury (Sagimura-Wakayama et al., 2015) Facial nerve injury (Pereira et al., 2019)	Neurotrophic factors in SHED-conditioned media In vivo cell differentiation and neurotrophic factors release
PDLSCs	Nanog, Oct-4, Klf4, Sox-2, Sox-10, Slug, CD271, Nestin, CD44, CD105, CD73, CD90, STRO-1	Osteogenic, chondrogenic, adipogenic, neural, β-pancreatic, hepatocytes	Mental nerve injury (Li et al., 2013) Optic nerve injury (Cen et al., 2018) Sciatic nerve injury (Kolar et al., 2017)	*In vivo* cell differentiation and neurotrophic factors release Neurotrophic factors release
SCAP	Nanog, Oct-4, Notch3, CD105, CD73, CD90, STRO-1, CD146	Odontogenic, osteogenic, chondrogenic, adipogenic, neural, hepatocytes	Sciatic nerve injury (Kolar et al., 2017)	Neurotrophic factors release

hDPSCs: Human dental pulp stem cells; PDLSCs: periodontal ligament stem cells; PNI: peripheral nerve injury; SCAP: stem cells from the apical papilla; SHED: stem cells from human exfoliated deciduous teeth.

from several research groups in the last decades reported the expression of CD34 also by mesenchymal stem cells isolated from different tissues, such as bone marrow (Dominici et al., 2006), adipose tissue (Suga et al., 2009) and dental pulp (Laino et al., 2006). Particularly, based on the expression of STRO-1, c-Kit and CD34, our research group recently identified a subpopulation of DPSCs able to differentiate not only towards the mesodermal lineages but also to the ectodermal neural lineage (Pisciotta et al., 2015a). Furthermore, this subpopulation demonstrated a strong tendency towards the neurogenic commitment, showing the expression, under floating 3D spheres culture conditions, of nestin, a cytoskeleton intermediate filament protein of neuronal stem cells, and of the surface antigen CD271 and SOX-10, which identify neural crest derived cells (Pisciotta et al., 2018). According to these findings and to previous reports from Laino and colleagues (Laino et al., 2006), hDPSCs expressing STRO-1, c-Kit and CD34 can be defined as a perivascular niche of neural crest derived stem cells. Taken together, these findings reveal that several and distinct stem cell subgroups are enclosed within dental pulp; in fact, stem cells isolated from dental pulp own a typical embryological origin from neuro-ectomesenchyme (Lumsden et al., 1988; Pierdomenico et al., 2005; Pisciotta et al., 2015a).

Previous findings from Askari et al. (2015) showed that hDPSCs, following transfection with oligodendrocyte lineage transcription factor 2 not only committed towards functional oligodendrocytes *in vitro* but also promoted regeneration in a mouse model of PNI. Over the years, multiple investigations have also proved the capability of hDPSCs-combined to different scaffold types-to favour the peripheral nerve regeneration and recover functionality in different animal models of PNI. Sasaki et al. (2008, 2011) highlighted the potential of hDPSCs/polylactic glycolic acid tubes complex to regenerate injured facial nerve and to improve functional recovery, similarly to autografts. Findings from Carnevale et al. (2016) revealed that the use of hDPSCs-injected collagen scaffolds in a rat sciatic nerve injury model contributed to axonal regeneration by promoting myelination, which was also reflected by a functional recovery. In particular, data from their *in vitro* experiments showed that after glial induction, hDPSCs secreted significant amounts of neurotrophic factors, such as BDNF, NGF and NT-3, which exert a key neuroprotective role during peripheral nerve regeneration. These findings confirmed the ability of hDPSCs to support axonal regeneration in PNI animal models either directly and via paracrine mechanisms, as previously reported by other groups (Sasaki et al., 2008; Martens et al., 2014). A later study from Sanen et al. (2017) further confirmed the regenerative potential of hDPSCs when applied to engineered collagen conduits for the repair of critical (15 mm) sciatic nerve gaps. Farther, a recent report from Omi et al. (2017) highlighted the contribution of hDPSCs in ameliorating the long-term diabetic polyneuropathy in rats; indeed, injected hDPSCs were able to improve the impaired sciatic nerve blood flow, to increase the sciatic motor-sensory nerve conduction speed and to increase the capillary number-to-muscle and intra-epidermal nerve fiber density ratio.

Stem Cells from Human Exfoliated Deciduous Teeth

Human exfoliated deciduous teeth represent an easily accessible source of multipotent MSCs able to differentiate towards different cell types (Gronthos et al., 2000). In comparison to DPSCs, SHED display multicytoplasmic processes and a higher proliferation rate (Miura et al., 2003).

SHED express the typical MSCs surface markers proposed by ISCT (Pivoriuūnas et al., 2010) and also express Oct4 and Nanog, SSEA-3 and SSEA-4, as embryonic stem cells antigens, and nestin, a neural stem cell marker (Miura et al., 2003; Liu et al., 2015). Similar to DPSCs they express STRO-1 and CD146, which characterize the cells in close proximity to pulp's blood vessels, suggesting that these cells reside in the perivascular environment.

These cells own the ability to form sphere-like cell clusters expressing glial and neuronal cell surface markers, such as nestin, when cultured in neurogenic medium, and a highly plastic differentiation potential when transplanted in different organs and tissues (Miura et al., 2003). Such a peculiar multipotent ability can be ascribed to the neural crest origin of dental pulp (Kerkis et al., 2006). Cordeiro et al. (2008) showed that SHED are able to differentiate into myogenic and chondrogenic lineages. Moreover, when cultured in hepatic induction medium, they were demonstrated to acquire morphological and functional properties of hepatocytes (Ishkitiev et al., 2010).

With regard to the osteogenic potential, SHED are distinct from DPSCs, since they are not able to differentiate towards osteoblasts or osteocytes, nevertheless, they are able to induce new bone formation through paracrine mechanisms. These findings demonstrate that deciduous teeth may not only guide the eruption of permanent teeth, but they may also be considered an immature form of DPSC, due to their odontogenic differentiation potential and osteogenesis promoting effect (Miura et al., 2003).

Moreover, SHED can promote new vascularization, differentiate into SCs and facilitate axonal regeneration. Most of them express indeed markers of neural progenitor cells, oligodendrocytes, and immature neural cells. As a matter of fact, studies demonstrated that SHED can be readily induced to differentiate to neuron-like cells and SCs-like cells (Ibarretxe et al., 2012). Such ability was then confirmed by different studies *in vivo*. It is noteworthy the capability of SHED to regenerate a facial nerve. In fact, several studies highlighted the ability of SHED to regenerate peripheral nerve directly or via secreting neurotrophic factors. Particularly, Pereira et al. evaluated facial nerve regeneration in rats treated with the combination of SHED with different types of conduits (Pereira et al., 2019). All the evaluated studies highlighted that the successfulness of the grafts was due to the contribution of SHED in restoring nerve function. Besides the evident contribution of SHED to commit into SCs for peripheral nerve regeneration, it is clear that also paracrine mechanisms exerted through neurotrophins secretion - NGF, BDNF, NT-3, CNTF, GDNF-played a key role in sustaining the regeneration process. As a matter of fact, SCs

exposed to SHED-conditioned medium (CM) *in vitro* exhibited a significant increase in proliferation, migration, and higher expression of neuron-, extracellular matrix-, and angiogenesis-related genes (Sugimura-Wakayama et al., 2015). Moreover, when applied to a rat model of sciatic nerve gap the SHED-CM group promoted sciatic nerve reinnervation and improved functional recovery (Sugimura-Wakayama et al., 2015).

Periodontal Ligament Stem Cells and Stem Cells from the Apical Papilla

The periodontal ligament (PDL) is a soft connective tissue enclosed between cementum and alveolar bone socket, which remodels itself continuously; therefore, it was hypothesized to contain progenitor cells. Early reports highlighted that PDL not only provides support to teeth, but it also represents a source for tooth nutrition, homeostasis and the regeneration of periodontal tissue. PDLSCs can be obtained from extracted teeth, either through explanted cultures or enzymatic digestion, and their features seem to depend on the harvest location, indeed cells isolated from the alveolar bone surface show a higher ability in regenerating the alveolar bone, compared to cells harvested from the root surface (McCulloch and Bordin, 1991). Similar to the tooth derived stem cells reviewed above, also PDLSCs exhibit a fibroblast-like morphology, colony forming abilities and a high proliferation rate, besides expressing STRO-1 and other MSCs markers. Furthermore, PDLSC subgroups may also express typical embryonic stem cell- and neural crest-related markers, as reported above for hDPSCs. PDLSCs have a functional role in maintenance of the homeostasis and regeneration of the periodontal tissue (Xu et al., 2009); later, they have also been demonstrated to be able to differentiate towards all the three germ layers when exposed to defined culture conditions (Huang et al., 2009; Xu et al., 2009; Dapeng et al., 2014; Lee et al., 2014; Ng et al., 2015).

The apical papilla, a soft tissue contributing to dental development, is located at the tip of growing roots in erupting permanent teeth and encloses a population of stem cells which are characterized by a notably higher proliferation rate and a superior mineralization ability, when compared to hDPSCs, while expressing the same typical mesenchymal markers of the latter (Sonoyama et al., 2006; Sonoyama et al., 2008, Ding et al. 2010) and the potential to commit in cell types derived from all the three germ layers (Ikeda et al., 2006; Abe et al., 2012; Patil et al., 2014; Kumar et al., 2017),

With regard to PDLSCs and SCAP, a few studies described their contribution in repairing nerve injuries. A valuable recent study from Kolar et al. compared the ability of PDLSCs, SCAPs and DPSCs to respond to glial induction *in vitro* and their direct contribution on sciatic nerve regeneration *in vivo* (Kolar et al., 2017). As reported, the secretion of neurotrophic factors was demonstrated either by PCR and ELISA analyses. These evaluations allowed the authors to highlight that SCAP had increased gene expression of neurotrophic factors, such as BDNF and GDNF, with respect to PDLSCs and DPSCs; on the other hand, significantly higher release of BDNF was observed in SCAP and DPSCs, when compared to PDLSCs, whereas no differences were detected with regard to NGF, NT-3 and GDNF in any of the analyzed dental stem cell populations. Moreover, the effect of neurotrophic factors released in culture media was reflected by the detection of neurite outgrowth in response to the induction with SCAP, PDLSCs and DPSCs, either unstimulated or stimulated. This event triggered the differentiated neuroblastoma SH-SY5Y cells to an increased percentage of neurite-producing cells and a greater general neurite outgrowth, with SCAP-conditioned media proving to be the most effective in inducing a significant increase in neurite length (Kolar et al. 2017). Then, Kolar et al. (2017) also evaluated the ability of SCAP, PDLSCs and DPSCs to participate in regeneration of a 10mm gap in rat sciatic nerve, when transplanted in combination with a fibrin glue conduit. According to their observations, the optimal result was obtained in the SCAP-treated group. Particularly, the authors conclusions suggest that the primary contribution of the investigated stem cells in sciatic nerve injury was attributable to their secretome rather than to a direct glial differentiation of the cells. As a matter of fact, cells were closely localised to the proximal regeneration front and BDNF was detected in proximity of the transplanted human stem cells, which however did not stain positively against the glial marker S-100. The main limitations of this study might be due to the small number of stem cells donors and to the 2-week experimental time.

Further studies confirmed the ability of SCAP and PDLSCs to participate either in a paracrine manner or directly to the regeneration of nerve injuries (Li et al., 2013; Cen et al., 2018).

Conclusions and Future Perspectives

This review aimed to focus on tooth-enclosed stem cells - DPSCs, SHED and SCAP - and dental-associated stem cells - PDLSCs - with regard to their contribution in promoting the regeneration of PNI.

These stem cells represent indeed a valuable tool for cell therapy approaches due to their easy harvesting procedures with low invasiveness for the patients, to their ease of expansion *in vitro* and, in particular, for their embryological origin from neural crest - a peculiar feature shared with SCs - which confers them multipotency and makes them the ideal candidate as a source/reservoir of glial cells. SCs are the primary cell component required and involved in the regeneration process, following PNI; however, SCs transplant is limited by the difficulty to keep them proliferating *in vitro* and by the potential morbidity at donor site.

Many research groups have highlighted that tooth-derived stem cells participate to the regeneration of PNI by secreting neurotrophic factors such as BDNF, NGF, NT-3 and GDNF, which exert a neuroprotective effect and improves nerve regeneration, as widely shown by the findings reviewed above. Based on the reviewed literature and on our previous findings as well, it is noteworthy how tooth derived stem cells sequentially contributed to nerve repair, first by secreting neu-

rotrophins that play a fundamental role in the earliest phases and then, by directly differentiating *in vivo* towards SC-like cells at later experimental times providing the survival of injured neurons, axonal regeneration and target reinnervation.

These findings underline that axonal guidance and alignment in nerve regeneration is a key event operated by SCs. Therefore, the ideal candidate stem cells for peripheral nerve repair are supposed to support the regeneration process either directly and via paracrine mechanisms.

Most of the reviewed studies highlight the ability of stem cells to participate in nerve regeneration in animal models, but the same studies neglect an important biological aspect which is peculiar of stem cells, i.e., the immunomodulatory properties. Indeed, the stem cell transplantation is carried out under immunosuppressive regimen. A deep characterization of the immunomodulatory properties of tooth derived stem cells would provide several advantages: 1) a niche of stem cells that might offer promptly SC-like differentiating cells; 2) the release of cytokines that can promote host SCs proliferation or inhibit host SC apoptosis.

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