

● REVIEW

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

Alessandra Pisciotto¹, Laura Bertoni¹, Antonio Vallarola², Giulia Bertani¹, Daniela Mecugni^{1,3}, Gianluca Carnevale^{1,*}

1 Department of Surgery, Medicine, Dentistry and Morphological Sciences with Interest in Transplant, Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy

2 Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

3 Azienda USL - Institute and Health Care (IRCCS) di Reggio Emilia, Reggio Emilia, Italy

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*

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 (Kato 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).

*Correspondence to:

Gianluca Carnevale, PhD,
gianluca.carnevale@unimore.it.

orcid:

0000-0002-5348-5991
(Gianluca Carnevale)

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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 pri-

mordium 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- β s, 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 conducting 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; Bhatheja 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. Schwann 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 coeruleospinal 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 (Ruitenberget 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 (Janebodinet 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; Janebodinet 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	<i>In vivo</i> 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)	<i>In vivo</i> 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 <i>In vivo</i> 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)	<i>In vivo</i> 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/poly(lactic 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 multicyttoplasmic 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-

retroprophins 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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References

- Abe S, Hamada K, Miura M, Yamaguchi S (2012) Neural crest stem cell property of apical pulp cells derived from human developing tooth. *Cell Biol Int* 36:927-936.
- Armiñán A, Gandía C, Bartual M, García-Verdugo JM, Lledó E, Mirabet V, Llop M, Barea J, Montero JA, Sepúlveda P (2009) Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. *Stem Cells Dev* 18:907-918.
- Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells* 26:1787-1795.
- Askari N, Yaghoobi MM, Shamsara M, Esmaeili-Mahani S (2015) Tetracycline-regulated expression of OLIG2 gene in human dental pulp stem cells lead to mouse sciatic nerve regeneration upon transplantation. *Neuroscience* 305:197-208.
- Bhatheja K, Field J (2006) Schwann cells: Origins and role in axonal maintenance and regeneration. *Int J Biochem Cell Biol* 38:1995-1999.
- Brushart TM (2011) *Nerve Repair*. Oxford, New York: Oxford University Press.
- Bunge RP (1993) Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. *Curr Opin Neurobiol* 3:805-809.
- Carnevale G, Pisciotta A, Riccio M, Bertoni L, De Biasi S, Gibellini L, Zordani A, Cavallini GM, La Sala GB, Bruzzesi G, Ferrari A, Cos-sarizza A, de Pol A (2018) Human dental pulp stem cells expressing STRO-1, c-kit and CD34 markers in peripheral nerve regeneration. *J Tissue Eng Regen Med* 12:e774-785.
- Carnevale G, Riccio M, Pisciotta A, Beretti F, Maraldi T, Zavatti M, Cavallini GM, La Sala GB, Ferrari A, De Pol A (2013) In vitro differentiation into insulin-producing β -cells of stem cells isolated from human amniotic fluid and dental pulp. *Dig Liver Dis* 45:669-676.
- Cen LP, Ng TK, Liang JJ, Zhuang X, Yao X, Yam GH-F, Chen H, Cheung HS, Zhang M, Pang CP (2018) Human periodontal ligament-derived stem cells promote retinal ganglion cell survival and axon regeneration after optic nerve injury. *Stem Cells* 36:844-855.
- Chai Y, Jiang X, Ito Y, Bringas P, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 127:1671-1679.
- Chen ZL, Yu WM, Strickland S (2007) Peripheral Regeneration. *Annu Rev Neurosci* 30:209-233.
- Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nör JE (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J Endod* 34:962-969.
- d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endothelial cells: a pivotal synergy leading to adult bone tissue formation. *Cell Death Differ* 14:1162-1171.
- d'Aquino R, Rosa AD, Laino G, Caruso F, Guida L, Rullo R, Checchi V, Laino L, Tirino V, Papaccio G (2009) Human dental pulp stem cells: from biology to clinical applications. *J Exp Zool B Mol Dev Evol* 312B:408-415.
- Dapeng L, Xiaojie L, Ping G, Yan D, Gang S (2014) Erk1/2 signalling is involved in the differentiation of periodontal ligament stem cells to Schwann cells in dog. *Arch Oral Biol* 59:487-491.
- Davidson RM (1994) Neural form of voltage-dependent sodium current in human cultured dental pulp cells. *Arch Oral Biol* 39:613-620.
- Ding G, Liu Y, An Y, Zhang C, Shi S, Wang W, Wang S (2010) Suppression of T cell proliferation by root apical papilla stem cells in vitro. *Cells Tissues Organs* 191:357-364.
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315-317.
- Douarin NL (1986) Cell line segregation during peripheral nervous system ontogeny. *Science* 231:1515-1522.
- Douarin NL, Kalchauer C (1999) *The Neural Crest* Cambridge University Press.
- Goldberg M, Smith AJ (2004) Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med* 15:13-27.
- Govindasamy V, Ronald VS, Abdullah AN, Nathan KRG, Ab. Aziz ZAC, Abdullah M, Musa S, Kasim NHA, Bhonde R (2011) Differentiation of dental pulp stem cells into islet-like aggregates. *J Dent Res* 90:646-652.
- Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, Den-Besten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. *J Dent Res* 81:531-535.
- Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 97:13625-13630.
- Gu X, Ding F, Yang Y, Liu J (2011) Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. *Prog Neurobiol* 93:204-230.
- Guenard V, Kleitman N, Morrissey TK, Bunge RP, Aebischer P (1992) Syngeneic Schwann cells derived from adult nerves seeded in semi-permeable guidance channels enhance peripheral nerve regeneration. *J Neurosci* 12:3310-3320.
- Höke A, Gordon T, Zochodne DW, Sulaiman OAR (2002) A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. *Exp Neurol* 173:77-85.
- Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 88:792-806.
- Hyung S, Im SK, Lee BY, Shin J, Park JC, Lee C, Suh JF, Hur EM (2019) Dedifferentiated Schwann cells secrete progranulin that enhances the survival and axon growth of motor neurons. *Glia* 67:360-375.
- Ibarretxe G, Crende O, Aurrekoetxea M, García-Murga V, Etxaniz J, Unda F (2012) Neural crest stem cells from dental tissues: a new hope for dental and neural regeneration. *Stem Cells Int* 2012:103503.

- Ikeda E, Hirose M, Kotobuki N, Shimaoka H, Tadokoro M, Maeda M, Hayashi Y, Kirita T, Ohgushi H (2006) Osteogenic differentiation of human dental papilla mesenchymal cells. *Biochem Biophys Res Commun* 342:1257-1262.
- Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, Haapasalo M (2010) Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. *J Endod* 36:469-474.
- Janebodin K, Horst OV, Ieronimakis N, Balasundaram G, Reesukumal K, Pratumvinit B, Reyes M (2011) Isolation and characterization of neural crest-derived stem cells from dental pulp of neonatal mice. *PLoS One* 6:e27526.
- Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. *Nat Rev Neurosci* 6:671-682.
- Jessen KR, Mirsky R (2008) Negative regulation of myelination: Relevance for development, injury, and demyelinating disease. *Glia* 56:1552-1565.
- Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. *J Physiol* 594:3521-3531.
- Jiang L, Jones S, Jia X (2017) Stem cell transplantation for peripheral nerve regeneration: current options and opportunities. *Int J Mol Sci* doi: 10.3390/ijms18010094.
- Johnson EO, Zoubos AB, Soucacos PN (2005) Regeneration and repair of peripheral nerves. *Injury* 36:S24-29.
- Katona I, Weis J (2018) Chapter 31-Diseases of the peripheral nerves. In: *Handbook of clinical neurology* (Kovacs GG, Alafuzoff I, eds), pp 453-474. New Jersey: Wiley-Blackwell.
- Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, An Z, Wang L, Hultman I, Ahrlund-Richter L, Blom H9, Brismar H, Lopes NA, Pachnis V, Suter U, Clevers H, Thesleff I, Sharpe P, Ernfors P, Fried K, et al. (2014) Glial origin of mesenchymal stem cells in a tooth model system. *Nature* 513:551-554.
- Kawashima N (2012) Characterisation of dental pulp stem cells: A new horizon for tissue regeneration? *Arch Oral Biol* 57:1439-1458.
- Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Massironi SMG, Pereira LV, Caplan AI, Cerruti HF (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. *Cells Tissues Organs* 184:105-116.
- Kocsis JD, Waxman SG (2007) Schwann cells and their precursors for repair of central nervous system myelin. *Brain* 130:1978-1980.
- Kolar MK, Itte VN, Kingham PJ, Novikov LN, Wiberg M, Kelk P (2017) The neurotrophic effects of different human dental mesenchymal stem cells. *Sci Rep* 7:12605.
- Kulesa PM, Bailey CM, Kasemeier-Kulesa JC, McLennan R (2010) Cranial neural crest migration: new rules for an old road. *Dev Biol* 344:543-554.
- Kumar A, Kumar V, Rattan V, Jha V, Pal A, Bhattacharyya S (2017) Molecular spectrum of secretome regulates the relative hepatogenic potential of mesenchymal stem cells from bone marrow and dental tissue. *Sci Rep* 7:15015.
- Laino G, Carinci F, Graziano A, D'Aquino R, Lanza V, Rosa AD, Gombos F, Caruso F, Guida L, Rullo R, Menditti D, Papaccio G (2006) In vitro bone production using stem cells derived from human dental pulp. *J Craniofac Surg* 17:511-515.
- Laino G, D'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G, Papaccio G (2005) A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). *J Bone Miner Res* 20:1394-1402.
- Lavasani M, Thompson SD, Pollett JB, Usas A, Lu A, Stolz DB, Clark KA, Sun B, Péault B, Huard J (2014) Human muscle-derived stem/progenitor cells promote functional murine peripheral nerve regeneration. *J Clin Invest* 124:1745-1756.
- Lee JS, An SY, Kwon IK, Heo JS (2014) Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage. *Cell Biochem Funct* 32:605-611.
- Li B, Jung HJ, Kim SM, Kim MJ, Jahng JW, Lee JH (2013) Human periodontal ligament stem cells repair mental nerve injury. *Neural Regen Res* 8:2827-2837.
- Li Z, Liang Y, Pan K, Li H, Yu M, Guo W, Chen G, Tian W (2017) Schwann cells secrete extracellular vesicles to promote and maintain the proliferation and multipotency of hDPCs. *Cell Prolif* doi: 10.1111/cpr.12353.
- Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S (2015) Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. *Stem Cells* 33:627-638.
- Lumsden AG (1988) Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 103:155-169.
- Maraldi T, Riccio M, Pisciotta A, Zavatti M, Carnevale G, Beretti F, La Sala GB, Motta A, De Pol A (2013) Human amniotic fluid-derived and dental pulp-derived stem cells seeded into collagen scaffold repair critical-size bone defects promoting vascularization. *Stem Cell Res Ther* 4:53.
- Martens W, Bronckaers A, Politis C, Jacobs R, Lambrichts I (2013) Dental stem cells and their promising role in neural regeneration: an update. *Clin Oral Invest* 17:1969-1983.
- McCulloch CA, Bordin S (1991) Role of fibroblast subpopulations in periodontal physiology and pathology. *J Periodont Res* 26:144-154.
- Menei P, Montero-Menei C, Whittemore SR, Bunge RP, Bunge MB (1998) Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord. *Eur J Neurosci* 10:607-621.
- Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 100:5807-5812.
- Moore AM, MacEwan M, Santosa KB, Chenard KE, Ray WZ, Hunter DA, Mackinnon SE, Johnson PJ (2011) Acellular nerve allografts in peripheral nerve regeneration: a comparative study. *Muscle Nerve* 44:221-234.
- Mosahebi A, Fuller P, Wiberg M, Terenghi G (2002) Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. *Exp Neurol* 173:213-223.
- Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G (2001) Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. *Glia* 34:8-17.
- Ng TK, Yung JSY, Choy KW, Cao D, Leung CKS, Cheung HS, Pang CP (2015) Transdifferentiation of periodontal ligament-derived stem cells into retinal ganglion-like cells and its microRNA signature. *Sci Rep* 5:16429.
- Omi M, Hata M, Nakamura N, Miyabe M, Ozawa S, Nukada H, Tsukamoto M, Sango K, Himeno T, Kamiya H, Nakamura J, Takebe J, Matsubara T, Naruse K (2017) Transplantation of dental pulp stem cells improves long-term diabetic polyneuropathy together with improvement of nerve morphometrical evaluation. *Stem Cell Res Ther* 8:279.
- Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ, Lee YM, Park BW, Byun JH, Ahn CS, Kim JW, Rho GJ (2014) Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. *Exp Cell Res* 320:92-107.
- Pereira LV, Bento RF, Cruz DB, Marchi C, Salomone R, Oiticiccia J, Costa MP, Haddad LA, Mingroni-Netto RC, Costa HJZR (2019) Stem cells from human exfoliated deciduous teeth (SHED) differentiate in vivo and promote facial nerve regeneration. *Cell Transplant* 28:55-64.
- Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V, Staffolani N, Franchina M, Grossi A, Bagnara GP (2005) Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation* 80:836-842.
- Pisciotta A, Bertoni L, Riccio M, Mapelli J, Bigiani A, La Noce M, Orciani M, de Pol A, Carnevale G (2018) Use of a 3D floating sphere culture system to maintain the neural crest-related properties of human dental pulp stem cells. *Front Physiol* 9:547.
- Pisciotta A, Carnevale G, Meloni S, Riccio M, De Biasi S, Gibellini L, Ferrari A, Bruzzesi G, De Pol A (2015a) Human Dental pulp stem cells (hDPCs): isolation, enrichment and comparative differentiation of two sub-populations. *BMC Dev Biol* 15:14.
- Pisciotta A, Riccio M, Carnevale G, Beretti F, Gibellini L, Maraldi T, Cavallini GM, Ferrari A, Bruzzesi G, De Pol A (2012a) Human serum promotes osteogenic differentiation of human dental pulp stem cells in vitro and in vivo. *PLoS One* 7:e50542.
- Pisciotta A, Riccio M, Carnevale G, Lu A, De Biasi S, Gibellini L, La Sala GB, Bruzzesi G, Ferrari A, Huard J, De Pol A (2015b) Stem cells isolated from human dental pulp and amniotic fluid improve skeletal muscle histopathology in mdx/SCID mice. *Stem Cell Res Ther* 6:156.

- Rahmatullah M, Schroering A, Rothblum K, Stahl RC, Urban B, Carey DJ (1998) Synergistic regulation of Schwann cell proliferation by heparin and forskolin. *Mol Cell Biol* 18:6245-6252.
- Razavi S, Ahmadi N, Kazemi M, Mardani M, Esfandiari E (2012) Efficient transdifferentiation of human adipose-derived stem cells into Schwann-like cells: A promise for treatment of demyelinating diseases. *Adv Biomed Res* 1:12.
- Riccio M, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, Motta A, Migliaresi C, De Pol A (2012) Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. *Tissue Eng Part A* 18:1006-1013.
- Rodriguez FJ, Verdú E, Ceballos D, Navarro X (2000) Nerve guides seeded with autologous Schwann cells improve nerve regeneration. *Exp Neurol* 161:571-584.
- Ruitenbergh MJ, Hendriks WTJ, Boer GJ, Verhaagen J (2006) CHAPTER 21 - Gene Therapy for Spinal Cord Injury. In: *Gene Therapy of the Central Nervous System* (Kaplit MG, Doring MJ, eds), pp 273-288. Amsterdam: Academic Press.
- Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, Stewart SA, Johnson PJ, Mackinnon SE (2013) Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. *Exp Neurol* 247:165-177.
- Saller MM, Huettl R-E, Mayer JM, Feuchtinger A, Krug C, Holzbach T, Volkmer E (2018) Validation of a novel animal model for sciatic nerve repair with an adipose-derived stem cell loaded fibrin conduit. *Neural Regen Res* 13:854-861.
- Sanen K, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J (2017) Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med* 11:3362-3372.
- Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, Okano T, Ando T (2011) PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. *J Tissue Eng Regen Med* 5:823-830.
- Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, Ogiuchi H (2008) Tubulation with dental pulp cells promotes facial nerve regeneration in rats. *Tissue Eng Part A* 14:1141-1147.
- Seo BM, Miura M, Gronthos S, Mark Bartold P, Batouli S, Brahim J, Young M, Gehron Robey P, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 364:149-155.
- Shimizu M, Matsumine H, Osaki H, Ueta Y, Tsunoda S, Kamei W, Hashimoto K, Niimi Y, Watanabe Y, Miyata M, Sakurai H (2018) Adipose-derived stem cells and the stromal vascular fraction in polyglycolic acid-collagen nerve conduits promote rat facial nerve regeneration. *Wound Repair Regen* 26:446-455.
- Shimizu S, Kitada M, Ishikawa H, Itokazu Y, Wakao S, Dezawa M (2007) Peripheral nerve regeneration by the in vitro differentiated-human bone marrow stromal cells with Schwann cell property. *Biochem Biophys Res Commun* 359:915-920.
- Shin YK, Jang SY, Park JY, Park SY, Lee HJ, Suh DJ, Park HT (2013) The Neuregulin-Rac-MKK7 pathway regulates antagonistic c-jun/Krox20 expression in Schwann cell dedifferentiation. *Glia* 61:892-904.
- Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Shi S, Wang S (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One* 1:e79.
- Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GTJ (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 34:166-171.
- Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, Formstecher P, Bailliez Y, Polakowska RR (2008) Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. *Stem Cells Dev* 17:1175-1184.
- Suga H, Matsumoto D, Eto H, Inoue K, Aoi N, Kato H, Araki J, Yoshimura K (2009) Functional implications of CD34 expression in human adipose-derived stem/progenitor cells. *Stem Cells Dev* 18:1201-1210.
- Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Saka-guchi K, Hibi H (2015) Peripheral nerve regeneration by secretomes of stem cells from human exfoliated deciduous teeth. *Stem Cells Dev* 24:2687-2699.
- Sun XH, Che YQ, Tong XJ, Zhang LX, Feng Y, Xu AH, Tong L, Jia H, Zhang X (2009) Improving nerve regeneration of acellular nerve allografts seeded with SCs bridging the sciatic nerve defects of rat. *Cell Mol Neurobiol* 29:347-353.
- Sunderland S (1952) Factors influencing the course of regeneration and the quality of the recovery after nerve suture. *Brain* 75:19-54.
- Sunderland S (1978) *Nerves and nerve injuries*. 2nd ed. New York: Churchill Livingstone.
- Tamaki T, Hirata M, Nakajima N, Saito K, Hashimoto H, Soeda S, Uchiyama Y, Watanabe M (2016) A long-gap peripheral nerve injury therapy using human skeletal muscle-derived stem cells (Sk-SCs): an achievement of significant morphological, numerical and functional recovery. *PLoS One* 11:e0166639.
- Tirino V, Paino F, De Rosa A, Papaccio G (2012) Identification, isolation, characterization, and banking of human dental pulp stem cells. *Methods Mol Biol* 879:443-63.
- Tohill MP, Mann DJ, Mantovani CM, Wiberg M, Terenghi G (2004) Green fluorescent protein is a stable morphological marker for schwann cell transplants in bioengineered nerve conduits. *Tissue Eng* 10:1359-1367.
- Topilko P, Meijer D (2001). Transcription factors that control Schwann cell development and myelination. In: *Glial cell development*. 2nd ed. (Jessen KR, Richardson WD, eds), pp 223-244. Oxford, UK: Oxford University Press.
- Tuszynski MH, Weidner N, McCormack M, Miller I, Powell H, Conner J (1998) Grafts of genetically modified Schwann cells to the spinal cord: survival, axon growth, and myelination. *Cell Transplant* 7:187-196.
- Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 8:726-736.
- Vallarola A, Sironi F, Tortarolo M, Gatto N, De Gioia R, Pasetto L, De Paola M, Mariani A, Ghosh S, Watson R, Kalmes A, Bonetto V, Bendotti C (2018) RNS60 exerts therapeutic effects in the SOD1 ALS mouse model through protective glia and peripheral nerve rescue. *J Neuroinflammation* 15:65.
- Voyvodic JT (1989) Target size regulates calibre and myelination of sympathetic axons. *Nature* 342:430-433.
- Wang ZZ, Sakiyama-Elbert SE (2018) Matrices, scaffolds & carriers for cell delivery in nerve regeneration. *Exp Neurol* doi: 10.1016/j.expneurol.2018.09.020.
- Weidner N, Blesch A, Grill RJ, Tuszynski MH (1999) Nerve growth factor-hypersecreting Schwann cell grafts augment and guide spinal cord axonal growth and remyelinate central nervous system axons in a phenotypically appropriate manner that correlates with expression of L1. *J Comp Neurol* 413:495-506.
- Woodhall B, Beebe GW (1956) *Peripheral nerve regeneration: a follow-up study of 3,656 World War II injuries* (U.S. Government Printing Office, 1956).
- Xu J, Wang W, Kapila Y, Lotz J, Kapila S (2009) Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. *Stem Cells Dev* 18:487-496.
- Xu XM, Guénard V, Kleitman N, Aebischer P, Bunge MB (1995) A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Exp Neurol* 134:261-272.
- Yamada Y, Fujimoto A, Ito A, Yoshimi R, Ueda M (2006) Cluster analysis and gene expression profiles: A cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. *Biomaterials* 27:3766-3781.
- You S, Petrov T, Chung PH, Gordon T (1997) The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. *Glia* 20:87-100.