

## **Tooth Innervation and Neuro-Regenerative Therapies: A Review**

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### **Abstract**

Innervation of the tooth germ involves 6 cellular processes. They are myelinated or unmyelinated, and classified as mammalian nerve fibres A, B and C. The major target area of trigeminal nerve endings is the odontoblast region of the crown. Nerve endings are coiled around the cell bodies and the processes within the dentinal canaliculus. The maximum penetration of a nerve fiber into the dentin was 125  $\mu\text{m}$ .

Calculations point out that adrenergic nerve endings in the dental pulps of mouse molars specify a mean value of  $35.5 \pm 5.2$  in the pulp horn,  $26.1 \pm 2.4$  in the central coronal,  $5.6 \pm 0.7$  in the bifurcation and  $5.6 \pm 0.9$  in the root pulp per tooth. Neurotransmitters such as calcitonin gene-related peptide, enkephalin, neuropeptide Y, VIP, substance P, somatostatin, serotonin, acetylcholine and norepinephrine, have been reported to be associated with nerves innervating the mature dental pulp. Serotonin and dopamine are implicated in stem cell differentiation into odontogenic stem cells, platelets, and neurons.

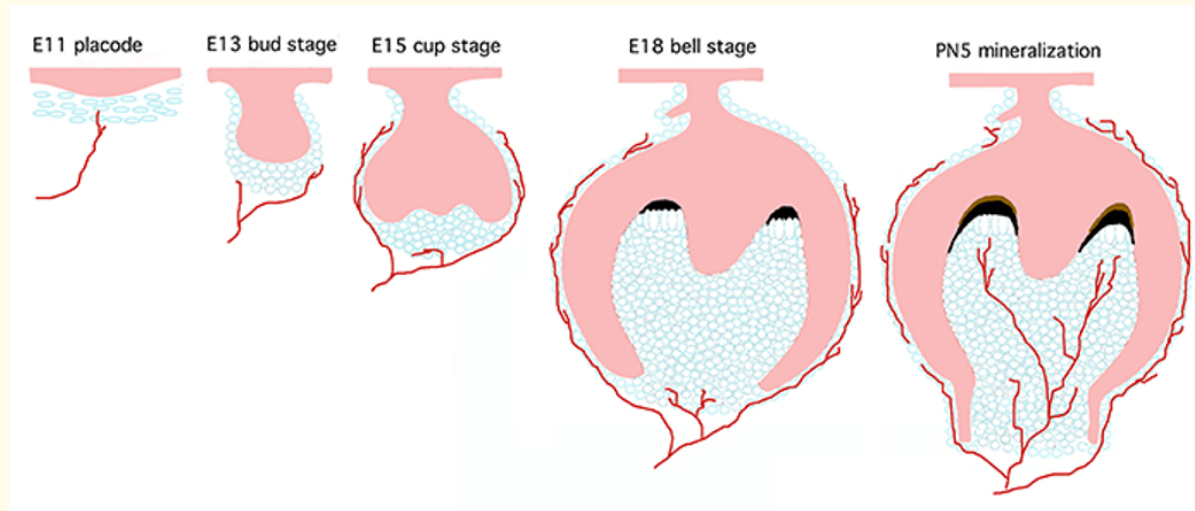
**Keywords:** *Tooth Innervation; Neuro-Regenerative Therapies*

### **The development of tooth innervation**

In the developing mouse tooth, the initial innervation begins during the crown formation. Neuronal development implies a sequence of 6 cellular processes. The 6 stages include (1) neurogenesis, (2) cell migration, (3) cell differentiation, (4) synaptogenesis, (5) neuronal cell death (apoptosis), and (6) synaptic rearrangement. The nerve development results from a basal plexus below the dental papilla during the bud stage whereas a more complex plexus appears during the early cap stage. Nerves enter the dental papilla after the crown formation began (bell stage) [1].

In the central pulp, nerves are arising from the apex (as branches of the Vth cranial nerve, also named trigeminal nerve). Two branches innervate the dental pulps and dentin. One is sensitive (the maxillary nerve) and the other sensory-motor (the mandibular nerve). The third branch is issued from the trigeminal ganglion and innervates the upper third of the face. It is the ophthalmic branch of Willis.

Nerves arise from the nerves main trunks of the Vth cranial pair (primarily the maxillary and mandibular nerves), and they are growing in the direction of tooth buds. When the dental lamina ends its formation, a stage implicating underdeveloped tooth buds, the dental nerves in grow into the tooth buds. At the bell and cap stages, small nerve bundles enter the dental pulp mesenchyme [2].



**Figure 1:** Developing innervation inside a tooth germ [2].

The nerves fan out and colonize the mesenchymal pulp. Located below the primordium area, they innervate the dental papilla. There is a relationship between the nerve sprouting and neural induction playing part of the initiation of odontogenesis. Nerves form a plexus. Innervation of the follicle is followed by nerve development within the papilla during tooth early maturation. Nerves enter the dental papilla when the crown formation reach completeness [1]. DPSCs already express neural markers enhancing and guiding axonal outgrowth [3].

During tooth development, Nerve Growth Factor (NGF) and brain-derived neurotrophic factor (BDNF) were detected in the dental papilla /pulp of post-natal rats. NGF is involved in the guidance of trigeminal axons to embryonic teeth. In post-natal teeth, NGF is involved in local sprouting and establishment of the final innervation pattern of the dental papilla and dentin. The expression pattern of neurotrophin receptors suggest that the neurotrophin system may also serve non-neuronal functions during tooth development [4]. Neurotrophin 3 and 4 were predominantly epithelial. Neurturin (NTN) is a member of the glial cell line-derived neurotrophic factor (GDNF). GDNF promote the survival of certain populations of neuronal cells in the peripheral and central nervous systems [5]. GDNF was mainly mesenchymal, observed in the odontoblast layer and subodontoblastic zone [6]. The receptors Ret, GFRA-1, and GFRA2 were expressed in the dental mesenchymal cells during the bud and cap stages having non-neuronal organogenetic functions during tooth morphogenesis [6,7]. The physical confinement of dental pulp and its innervation within the tooth and the high incidence of polymodal A-delta and C-fibers in pulp and dentin allow to study the neuroinflammatory interactions. A-fibers (myelinated) and C- fibers (unmyelinated) [8] pass through the radicular pulp. Unmyelinated axons are found in the periphery of the root pulp. In the coronal pulp nerve bundles fan out and reach the pulp-dentin border. Sympathetic innervation forms plexus, usually around pulpal arterioles. Adrenergic endings display the greatest percentage in the pulp horn.

Several lines of evidence have demonstrated that odontoblasts express mechano- and/or thermosensitive transient receptor potential ion channels (TRPV1, TRPV2, TRPV3, TRPM3, KCa3, TREK-1) that are likely to sense teat hot or cold or movements of dentinal fluid within tubules [9].

Sensory afferent from trigeminal ganglia are classified into fast conducting A-β, Aδ fibers (lightly myelinated, medium diameter) and C fibers (unmyelinated, small diameter).

Human premolars receive about 2300 axons at the root apex, which about 13% are myelinated and 87% non-myelinated fibers. Myelinated axons in the apex are fast-conducting A-δ fibers located at the pulpal periphery and inner dentin. Most C-fibers are slow-conducting fine sensory afferents. Intradental sensory axons play regulatory role in the maintenance and repair of the dentin-pulp complex [10].

|     |            |                                    |
|-----|------------|------------------------------------|
| A-α | 12 - 22 mm | Motor proprioception               |
| A-β | 5 - 12 mm  | Sensory touch pressure             |
| A-g | 3 - 6 mm   | Motor                              |
| A-δ | 2 - 5 mm   | Sharp pain                         |
| B   | < 3 mm     | Preganglionic autonomic            |
| C   | < 2 mm     | Dull pain postganglionic autonomic |

**Table 1:** Classification of mammalian nerve fibers.

*A and B fibers are myelinated. A is subdivided into α, β, γ and δ fibers. Group C are the smallest, non-myelinated axons*

Nerve fibers enter teeth via apical foramina and form bundles. The axons pass through the root canal and form the sub-odontoblastic Rashkow’s plexus. The axons lose the myelin sheath and some reach the inner dentin as non-myelinated axons. The greatest innervation is found adjacent to the tip of the pulp horn, and fewest in the dentin of the root. Nerves are growing in permanent teeth between the ages of 10 to 15 years. Myelinated nerves in human premolars have an average of  $312 \pm 149$  and non-myelinated have an average of  $2000 \pm 1023$  in the juxta-apical level.

**Dentin sensitivity**

No nerve fibers have been found in the middle and outer dentin. They are present only in the inner part of dentin. The conduction theory proposes that nerve endings are directly activated by external stimuli. The transduction theory postulates that the odontoblastic processes mediate external stimuli to the nerve endings located in the peripheral pulp. Junctional complexes exist between the processes and nerve fibers. The hydrodynamic theory suggests that fluid movements move rapidly through external stimuli.

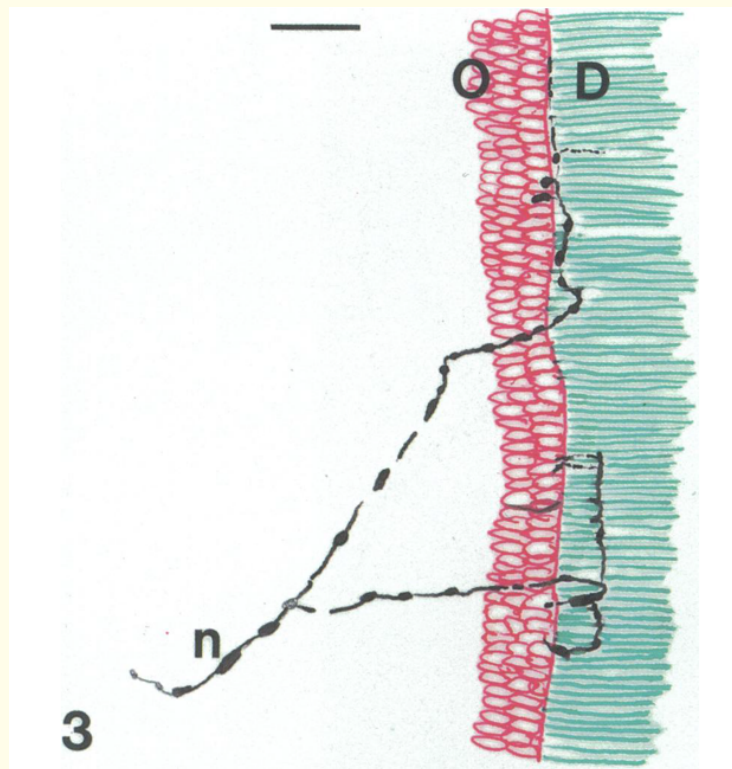
There are many A-δ - and C-fibers in the tooth-pulp, with the A-δ -axons ending in the inner third of the dentinal tubules and the C-fibers ending mostly in the pulp itself. Among the few A-β-fibers some might be nociceptive, but most are mechanoreceptors implicated in pre-pain [11].

In addition to sensory components, sympathetic primary afferents and possible parasympathetic endings are found. No cholinesterase activity was found in the odontoblast cell process per cell body, nor were cholinergic nerves found in the zone of pulp immediately below the odontoblasts. Adrenergic endings are located throughout the pulp and among the odontoblasts. The cholinergic free endings were implicated in synaptic connections in the pulp, however the transmission of the impulse was not apparently mediated by cholinergic activity. Fine nerve filaments were found ascending in the pulp [12]. Cholinergic endings were also located in this zone and may be sensory [13].

The 5-OH-DA localizes only in an adrenergic terminal. The location and number of endings was evaluated. Calculations point out that adrenergic nerve endings in the dental pulps of mouse molars specify a mean value of  $35.5 \pm 5.2$  in the pulp horn,  $26.1 \pm 2.4$  in the

central coronal,  $5.6 \pm 0.7$  in the bifurcation and  $5.6 \pm 0.9$  in the root pulp per tooth [14]. Neurotransmitters such as calcitonin gene-related peptide, enkephalin, neuropeptide Y, VIP, substance P, somatostatin, serotonin, acetylcholine and norepinephrine, have been reported to be associated with nerves innervating the mature dental pulp.

Growth-Associated protein (GAP-43) is associated with the reaction products in their axonal spaces [15]. GAP-43 positive nerve fibers entered the root pulp through the apical foramen and ascended through the center of the root pulp toward the coronal pulp. They form a sub-odontoblastic plexus (Raschkow plexus).



**Figure 2:** Innervation of deciduous pulp and dentin form complex terminals in the dentin [16].

Force applied at the top of dentinal tubules is transmitted to the sensory transduction apparatus deep inside by mechanical displacement (flow) of the fluid filling the tubules [17]. Positive PGP 9.5 are demonstrated in the pulp and in the inner 100 mm of dentin. Neurofilament proteins (NFP) were distributed in the predentin throughout the coronal region, while a few positive fibers penetrate only a short distance into the dentin [18].

The major target area of trigeminal nerve endings is the odontoblast region of the crown. Nerve endings are coiled around the cell bodies and the processes within the dentinal canaliculus. The maximum penetration of a nerve fiber into the dentin was 125 mm [16].

Dental pulp stem cells expressed some adipogenic, myogenic, neurogenic, osteogenic (osteonectin, osteocalcin, osteopontin, Runx-2, and type I collagen) and chondrogenic markers. Embryonic stem cell markers were also identified. The differentiation potential of human

natal dental pulp and human bone marrow mesenchymal stem cells to adipogenic, osteogenic, chondrogenic, myogenic and neurogenic was confirmed [19].

Axonal transport of radioactive <sup>3</sup>H proline was followed by radioautography. Numerous myelinated and unmyelinated axons formed a few terminal branches in the roots but primarily branched in the crown to form the peripheral plexus of Raschkow and terminate as free endings in the odontoblast layer, and as far as 120 µm in the inner part of dentinal tubules.

During physiological root resorption, axonal degeneration was prominent. This was accompanied by myelin degradation and progressive loss of myelinated axons [20].

The transported protein was confined to sensory axons and endings. Odontoblasts and dentin matrix were not significantly labeled. Dentinal sensory nerve endings in primate teeth can be profuse, sparse, or absent, depending on the location and structure of dentin and the adjacent pulp. When dentin is innervated, the tubules were straight and contained odontoblast processes. Odontoblast cell bodies are relatively columnar, with an adjacent cell-free zone and pulpal nerve plexus (Byers and Dong 1983).

### Regeneration

With respect to innervation, it is likely that regenerated pulp contains nerve fibers. DPSCs have been shown to either produce neurotrophic factors or possess neural differentiation potential. The reason why dentin is so sensitive to various irritations is because of the hydrodynamic activities of the dentinal tubules in association with the sensory A-δ fibers extending into the dentinal tubules. Since the newly generated dentin does not appear to have well-organized dentinal tubules, it may not cause the normal dentin sensitivity that natural teeth display (Huang 2009).

hDPSCs grew out of the neurospheres *in vitro*. Neurogenic differentiated hDPSC culture were characterized by the increased expression of neuronal markers. Neurogenic maturation of hDPSC demonstrate that these cells are capable of neuronal commitment following neurosphere formation, The perikaryon of DPSCs was characterized by a large central nucleus with a prominent nucleolus. Abundant organelles associated with the metabolic activity and protein synthesis were present in the cytoplasm of DPSCs. These organelles included an extended Golgi apparatus and RER, indicating increased packing of proteins in membrane-bound vesicles (Gervois., *et al.* 2015).

### Neuroregeneration

Dental pulp stem cells expressed some adipogenic, myogenic, neurogenic, osteogenic and chondrogenic markers. Embryonic stem cell markers were also identified. The differentiation potential of human natal dental pulp and human bone marrow mesenchymal stem cells to adipogenic, osteogenic, chondrogenic, myogenic and neurogenic was confirmed [19].

Human dental pulp stem cells can differentiate into Schwann cells and support neural outgrowth *in vitro* [3]. In addition, neurites were myelinated in a 3-dimensional collagen type I hydrogel neural tissue construct. DPSCs have the potential to differentiate along the neural lineage. To evaluate the expression of glial markers, immunocytochemical staining was performed and both Schwann cells and DPSCs showed a positive immune reaction for laminin, p75, GFAP, and CD104. Furthermore, expression of the early neural marker nestin decreased in differentiated cell cultures compared to DPSCs and was not detected in Schwann cells.

Bone marrow-derived mesenchymal stem cells (BMMSCs) are multipotent stem cells and have been the most studied MSCs. Mesenchymal stem cells can be differentiated into endothelial cells *in vitro* [21]. They are clonogenic and express multilineage differentiation following appropriate stimulation. Bone Marrow stromal cells are progenitors of skeletal tissue components. They may also contribute to the formation of neural, myogenic cells, and vascular walls (Bianco., *et al.* 2001). Studies on the transplant ability of marrow stromal cells are inscribed into the general problem of bone marrow transplantation (BMT). Transplantation of BMT allows for an ectopic development of hematopoietic tissue at the site of transplantation.

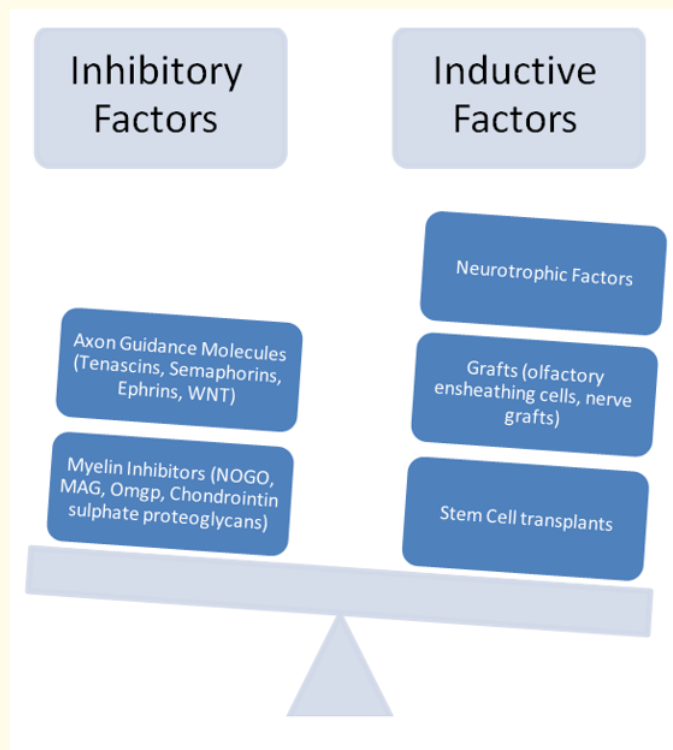
Regardless of whether genomic or cytoplasmic sequences are the target of gene therapy, the efficacy of all of these new technologies depend on: the efficiency at which the reagents are incorporated into BMSCs in the *ex-vivo* environment; the selection of specific targets, and the maintenance of the ability of BMSCs to function appropriately *in vitro*.

They undergo osteogenic differentiation when stimulated by osteogenic reagents *in vitro*. When the cells are transplanted into immunocompromised mice, they are capable of forming bone and inducing hematopoietic marrow. This colony-forming cell population showed a high uptake rate for bromodeoxyuridine, indicative of cell proliferation, exhibiting over 70 population doublings *in vitro*.

Brain-derived neurotropic factor (BDNF) and gamma-aminobutyric acid (GABA)-ergic transmission are related to both neuro-degeneration and neuro-regeneration. We summarize here the accumulating evidences that suggest a pathogenic role of BDNF and GABAergic transmission, their underlying mechanisms, and the relationship between BDNF and GABA in neuro-degeneration and neuro-regeneration [22].

### Neurogenesis

Endogenous axon guidance was induced by adult human dental pulp stem cells (DPSCs) [23]. Implanted into the pulp, they were mediated via the chemokine CXCL12. DPSCs differentiate into functionally active neurons, influencing endogenous recruitment of neural stem cells, and generating neurospheres. CXCL12, interacting via its cognate receptor CXCR4, has been shown to possess axon guidance activities in the nervous system and allows the recruitment of dental pulp cells after injury. PPAR $\alpha$  is a promising therapeutic target.



**Figure 3:** Stem cells for neuro-regeneration [24].

### Conclusion

As a conclusion, stem cells may be instrumental for neuro-regeneration. Implanted into the pulp, stem cells were mediated via the chemokine CXCL12. DPSCs differentiate into functionally active neurons, influencing endogenous recruitment of neural stem cells, and generating neurospheres. The population of pulp stem cells is heterogenous. The differentiation potential of human natal dental pulp and human bone marrow mesenchymal stem cells into adipogenic, osteogenic, chondrogenic, myogenic and neurogenic was confirmed experimentally.

To evaluate the expression of glial markers, immunocytochemical staining was performed and both Schwann cells and DPSCs showed a positive immune reaction for laminin, p75, GFAP, and CD104. Neurotransmitters such as calcitonin gene-related peptide, enkephalin, neuropeptide Y, VIP, substance P, somatostatin, serotonin, acetylcholine and norepinephrine, have been reported to be associated with nerves innervating mature dental pulp cells. Serotonin and dopamine are implicated in stem cell differentiation into odontogenic stem cells, platelets, and neurons [25]. Furthermore, expression of the early neural marker nestin decreased in differentiated cell cultures compared to DPSCs and was not detected in Schwann cells. They were implicated into cells differentiation and ultimately, they were implicated into neuroregeneration.

Neural stem cells are reported to lie in a vascular niche, but there is no direct evidence for a functional relationship between the stem cells and blood vessel component. We show in this review that endothelial cells but not vascular smooth muscle cells release soluble factors that stimulate the self-renewal of neural stem cells, inhibit their differentiation, and enhance their neuron production. Both embryonic and adult neural stem cells respond to these stimuli, allowing extensive production of neuron and interneuron types *in vitro*. Endothelial coculture stimulates neuroepithelial cell contact, activating Notch and Hes 1 to promote self-renewal. These findings identify endothelial cells as a critical component of the neural stem cell niches [26,27].

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