REVIEW

Angiogenic mechanisms of human dental pulp and their relationship with substance P expression in response to occlusal trauma

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Abstract


Angiogenesis is the formation of new blood vessels based on a pre-existing vasculature. It comprises two processes, sprouting of endothelial cells and the division of vessels due to abnormal growth of the microvasculature. It has been demonstrated that substance P (SP) can induce angiogenesis either by modulating endothelial cell growth (direct mechanism) or by attracting cells with angiogenic potential to the injury site (indirect mechanism). Therefore, the purpose of this article is to review the angiogenic mechanisms that regulate mineralized tissue formation in human dental pulp tissue and their relationship with SP expression as a defence response to stimuli such as the masticatory function and occlusal trauma. Articles included in this review were searched in PubMed, Scopus and ISI Web of Science databases, combining the following keywords: human dentine pulp, angiogenesis, angiogenic growth factors, neuropeptides, substance P, neurogenic inflammation, dentine matrix, dentinogenesis, occlusal trauma and dental occlusion. It is concluded that human dental pulp tissue responds to occlusal trauma and masticatory function with a neurogenic inflammatory phenomenon in which SP plays an important role in the direct and indirect mechanisms of angiogenesis by the action evoked via NK1 receptors at different cells, such as fibroblasts, endothelial and inflammatory cells, leading to new blood vessel formation which are needed to stimulate mineralized tissue formation as a defence mechanism.

Keywords: angiogenesis, angiogenic growth factors, human dental pulp, masticatory function, neurogenic inflammation, occlusal trauma, substance P.

Received 13 October 2015; accepted 3 March 2016

Introduction

The human dental pulp is a loose connective tissue containing different types of cells, collagen fibres, extracellular matrix, blood vessels and nerve endings. During mastication, it undergoes mechanical stress, and whenever the masticatory function exceeds the tolerance and adaptive capacity of both the pulp and the periodontal ligament, occlusal trauma takes place, stimulating the pulp nerve fibres to release neuropeptides, which are substances that take part in the process of transmission and modulation of pain and inflammatory processes as a defence mechanism (Mattuella et al. 2007a, Caviedes-Bucheli et al. 2008,
This response is necessary for pulp defence and repair to take place in cases such as pulpitis, degenerative pulp calcification and necrosis.

When the pulp is submitted to mechanical stress, it undergoes an ageing process where perfusion decreases, affecting the tissue nutrition and oxygenation. Simultaneously, in an attempt to adapt to its new condition, the pulp creates new blood vessels. This process is known as angiogenesis and it can occur by direct and indirect mechanisms. This phenomenon is also regulated by numerous growth factors as well as by endogenous substances such as substance P (SP), which is necessary to fulfill the needs for nutrients and oxygen of the areas that require them (Ikeda et al. 1998, Caviedes-Bucheli et al. 2008, Killough et al. 2009, Saghiri et al. 2015).

Although it has been demonstrated that SP is released when thermal, chemical or mechanical stimuli such as occlusal trauma, masticatory function and when orthodontics movements are applied to the tooth (Caviedes-Bucheli et al. 2005, 2010, 2011a,b, Javed et al. 2015), little is known about its relationship with angiogenesis as a response of pulp tissue to occlusal trauma.

Occlusal trauma has been associated with pulp calcification phenomena. The mechanism by which the pulp produces tertiary dentine is preceded by the formation of new blood vessels. As perfusion of the pulp decreases in the presence of harmful stimuli, the tissue should be resupplied with essential fluids in order that the repair processes take place optimally. Vascular neoformation will carry out the function of providing oxygen and nutrients to the damaged tissue to make the dental pulp viable in spite of the dentine apposition that characterizes the calcification phenomena (Ikeda et al. 1998, Tran-Hung et al. 2008, Marchionni et al. 2009, Zhang et al. 2011).

The purpose of this review was to analyse the role of SP in the angiogenic mechanisms that regulate mineralized tissue formation in the human dental pulp as a defence response to stimuli such as the masticatory function and occlusal trauma.

Materials and methods

The methodology followed in this article includes the search and review of articles published about the role of SP in the induction of the angiogenic mechanisms of the human pulp tissue response to occlusal trauma and the masticatory function. The articles were searched in PubMed, Scopus and ISI Web of Science databases using keywords listed in MeSH Terms, Index key Words and Key Word Plus of each databases.

Search strategy

In the three databases used, the keywords chosen were human dental pulp in combination with angiogenesis, angiogenic growth factors, neuropeptides, substance P, neurogenic inflammation, dentine matrix, dentinogenesis, occlusal trauma and dental occlusion. Two reviewers carried out the evaluation of the eligibility and search for relevant data independently; a third reviewer was selected for the resolution of disagreement during the evaluation process.

Inclusion and exclusion criteria

The inclusion criteria considered were:

- Review articles and research studies about the human dental pulp, either in vivo or in vitro;
- Articles that meet the quality standards required by AMSTAR (Shea et al. 2007) and PRISMA (Urrutia & Bonfill 2010) guides, published in English journals with impact factors Q1 and Q2 from January 1990 to August 2015 and
- Articles that evaluate the angiogenic mechanisms of the human dental pulp and the role of substance P prior to the formation of mineralized tissue as a response to the masticatory function and occlusal trauma.

Articles about animal models or cell lines studies on other tissues that do not refer to human dental pulp as well as studies where medicaments are used as endodontic therapy to produce angiogenesis were excluded.

Literature review

Dental pulp microcirculation and innervation

The dental pulp is a loose connective tissue of mesenchymal origin that lies inside the rigid walls of the dentine, which together form the so-called pulp–dentine complex. This complex is considered a functional and structural unit that acts as a sensorial system which can respond to different stimuli (Mattuella et al. 2007a). The dental pulp has several functions, including the formation of hard tissues such as dentine, the production of tertiary dentine to compensate the loss of hard tissues caused by mechanical and
chemical trauma and providing cell nutrition and tissue vitality (Arana-Chavez & Massa 2004, Cooper et al. 2010).

In the presence of occlusal trauma several cells induce defensive and repairing processes within the pulp-dentine complex. Amongst these cells, the odontoblasts are responsible for producing the reactive tertiary dentine depending on the intensity and duration of the stimulus; secondly, the dental pulp stem cells (DPSCs) take part in the process of tertiary dentine production; and finally, the human dental pulp fibroblasts (HDPF) that can be found in a greater proportion are responsible for the collagen matrix that becomes mineralized just before reparative tertiary dentine formation (Arana-Chavez & Massa 2004, Kilough et al. 2009, Rosa et al. 2011, Saghiri et al. 2015).

The cells that take part in the pulp-repairing process respond to signals from molecules in the bloodstream and the dentine matrix, which reach the pulp due to a microcirculatory system characterized by the presence of arterioles, capillaries and venules whose main functions are the regulation of dental pulp local interstitial medium and the elimination of waste products (Li et al. 2011, Zhang et al. 2011). A singular characteristic of pulp microvasculature is the presence of arteriovenous anastomoses (AVA), which participate in the regional control of the blood flow due to its dilation or contraction in the presence of thermal, chemical or mechanical irritation (Matthews & Andrew 1995, Heyeraas & Berggreen 1999, Rodd & Boissonade 2002), such as occlusal trauma, where an alteration in the vasculature takes place, thereby increasing vascular permeability and vascular stasis and decreasing the resistance of the vessels and causing oedema. All these changes are caused not only by the local control mechanisms of the blood vessels (presence of pre- and post-capillary sphincters) but also by the main control performed by the nervous system in the regulation of pulp blood flow due to the release of neurotransmitters by the sympathetic, parasympathetic and somatosensory fibres such as SP (Kim 1990, Olgart 1996, Rodd & Boissonade 2003, Caviedes-Bucheli et al. 2008, Lee et al. 2013).

The pulp tissue is innervated by fibres from the sympathetic, the parasympathetic and the somatosensory systems. The sympathetic nervous system reduces pulp blood flow (vasoconstriction) due to the release of neurotransmitters as neuropeptide Y (NPY). The parasympathetic fibres stimulate vasodilation due to the release vasoactive intestinal peptide (VIP).

Somatosensory fibres are responsible for the production of several neuropeptides that regulate the pulpal blood flow such as SP, calcitonin gene-related peptide (CGRP) and neurokinin A (NKA) which inhibit the sympathetic activity through vasodilation (Wakisaka 1990, Olgart 1996, Rodd & Boissonade 2003, Caviedes-Bucheli et al. 2008).

Substance P is a neuropeptide found in large quantities in immunoreactive nerve fibres and has been also found in both the human dental pulp and dentine in immunohistochemical studies (Wakisaka 1990). In the pulp centre, reactive SP nerve fibres are associated with large blood vessels, whereas in the periphery they are associated with smaller ones. On the other hand, nerve fibres not related to the blood vessels, pass into the subodontoblastic layer and branch out in the pre-dentine, some of them crossing into the dentine (Rodd & Boissonade 2003). SP is released into the dentine–pulp complex by neurons as a response to thermal, chemical or mechanical noxious stimuli, including orthodontic movement, masticatory function and occlusal trauma (Caviedes-Bucheli et al. 2010, 2011a,b).

Moreover, SP is not able to cross cell membranes; therefore, target cells must express the correspondent receptor on their plasma membrane, which must have a binding site on the extracellular surface of the cell. These receptors bind the natural agonist when present in the extracellular environment and generate intracellular signals to regulate other functions within the cell (Caviedes-Bucheli et al. 2007). Substance P receptor (NK1) is expressed in most inflammatory cells such as mast cells and macrophages as well as in other connective tissue cells (Caviedes-Bucheli et al. 2008). It has been demonstrated that SP receptor expression in human pulps is significantly greater during clinical inflammatory phenomena (Table 1) (Awawdeh et al. 2002, Bowles et al. 2003, Caviedes-Bucheli et al. 2006, 2007).

Occlusal trauma and neurogenic inflammation

The pulp suffers physiological modifications throughout its life due to phenomena such as mastication. It also reacts in the presence of pathological situations such as caries, cavity preparation, dental materials and occlusal trauma (Tziafas 1994, von Bohl et al. 2012, Lee et al. 2013), which stimulate the nerve fibres of the periodontal ligament and the pulp releasing neuropeptides to regulate all the vascular and cellular processes that occur in the premature pulp.
ageing forming tertiary reparative dentine as a response to harmful stimuli (Rodd & Boissonade 2003, Caviedes-Bucheli et al. 2011a, Lee et al. 2013).

Occlusal trauma is the condition in which the masticatory function exceeds the tolerance of the ligament and the dental pulp, causing damages on both tissues. Studies have shown that the application of excessive occlusal loads disturbs pulp metabolism generating changes in the pulp and periodontal fibres, as well as other inflammatory reactions and vascular alterations. This response depends on the intensity and magnitude of the force and the pulp tissue condition. After this form of stimuli takes place, the defence mechanisms of the pulp include the production of tertiary dentine and inflammation (Ikeda et al. 1998, Caviedes-Bucheli et al. 2011a, Lee et al. 2013).

Occlusal trauma can be classified as primary or secondary. The former takes place in the pulp and in the periodontal ligament due to the application of a higher-than-normal force on the dental structures with a periodontal support system in optimal conditions. This case includes orthodontic movements, ‘high’ or overextended restorations and parafunctional habits such as bruxism. Secondary occlusal trauma takes place when normal and higher-than-normal forces are applied on teeth with previous supporting tissue alterations caused by periodontal disease or previous trauma (Jin & Cao 1992, Poiate et al. 2009).

There is evidence that human fibroblasts can produce SP and that neuropeptides regulate the expression of angiogenic growth factors in fibroblasts, suggesting that such cells also play a role in neurogenic inflammation (Tran-Hung et al. 2006, El Karim et al. 2009). There is a close relationship between the periodontal ligament and the pulp which has been considered as synergic; therefore, once the periodontal tissues become inflamed due to mechanical loads, the inflammation is quickly transferred to the dental pulp. Likewise, any inflammation that initially affects the pulp will be transferred to the periodontal tissues. This type of inflammation is neurogenic in nature because the nervous system controls both the vascular and the immune system responses. This process describes the stimulation of peripheral neurons which results in neuropeptides release, altering the vascular processes causing vasodilatation, increasing the permeability of the vessels and facilitating the accumulation of leucocytes (Kim 1990, Ikeda et al. 1998, Caviedes-Bucheli et al. 2008, 2011a).

Substance P release takes place when mechanical stimuli are applied to the pulp-dentine complex. It plays an important role in inflammation due to its interaction with the endothelial cells that cause the opening of the intercellular pores allowing fluid release. Additionally, it causes the production of histamine by the granulocytes favouring plasma extravasation. It also activates the immune system and promotes chemotaxis by recruiting and regulating other inflammatory cells, an effect that occurs after the activation of cells through the receptors present on their surface. These receptors are called neurokinin 1, 2 and 3 (NK1, NK2 and NK3). When the SP is found in high concentrations, it can stimulate receptors NK2 and NK3 in some peripheral tissues, but it binds with more affinity to NK1 (Awawdeh et al. 2002, Caviedes-Bucheli et al. 2007, Nakashima et al. 2009).

However, on the other hand, SP plays an important role in pulp repair after injury. Tissue repair induced by SP occurs due to the chemotactic properties of the neuropeptide, which favours the recruitment of several cellular lineages such as myeloid, lymphoid, dendritic cells, macrophages, epithelial progenitor cells and DPSCs from the blood. Finally, SP promotes the differentiation and proliferation of endothelial cells, which are needed for tissue repairing process to take place (El Karim et al. 2009, Kohara et al. 2010). Several studies indicate that tissue repair involves the formation of new vessels. During occlusal trauma,

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**Table 1** Summary of vascular and inflammatory effects of substance P

<table>
<thead>
<tr>
<th>Substance P</th>
<th>Dental pulp target cell (expressing NK1 receptor)</th>
<th>Vascular effects</th>
<th>Inflammatory effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Mast cell</td>
<td>• Blood flow regulation</td>
<td>• Immune system stimulation</td>
</tr>
<tr>
<td></td>
<td>• Macrophage</td>
<td>• Vasodilation</td>
<td>• Chemotaxis</td>
</tr>
<tr>
<td></td>
<td>• Mesenchymal cell</td>
<td>• Plasma extravasation Kim (1990), Olgart (1996), Caviedes-Bucheli et al. (2008)</td>
<td>• Increased macrophages action</td>
</tr>
<tr>
<td></td>
<td>• Endothelial cell</td>
<td></td>
<td>• Increased collagen formation</td>
</tr>
<tr>
<td></td>
<td>• FibroblastTakahashi et al. (1992)</td>
<td></td>
<td>Trantor et al. (1995), Caviedes-Bucheli et al. (2008)</td>
</tr>
</tbody>
</table>

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there is a decrease in the nutritional and oxygen contribution in the pulp followed by the apposition of tertiary dentine as a defence and repairing mechanism. It is possible to infer from the literature that such a process is preceded by a vascular neoformation, a process known as angiogenesis (Derringer et al. 1996, Roberts-Clark & Smith 2000, Tran-Hung et al. 2008, Kohara et al. 2010, Zhang et al. 2011).

**Angiogenesis**

Angiogenesis is the formation of new vessels based on a pre-existing vasculature. It comprises two processes, the sprout of endothelial cells and the division of vessels by the abnormal growth of the microvasculature. The organs derived from the ectoderm and the mesoderm are vascularized by angiogenesis, a process which occurs physiologically during embryogenesis and remains as a normal condition during inflammation and tissue repair (Derringer et al. 1996, Tran-Hung et al. 2006, Marchionni et al. 2009, Saghiri et al. 2015).

The stages of angiogenesis (Fig. 1) involve the breaking of the vascular membrane which consists of the fragmentation of the basal layer and the extracellular matrix of the blood vessels due to the proteolytic action of some enzymes such as metalloproteinases (MMPs) and plasminogen activators, which are segregated by the endothelial cells and the macrophages after the stimulation. After this vasculature breaking, cell migration starts due to chemotactic signals and multiplication (by mitosis) of the endothelial cells at the perivascular space. Then, there is enlargement of the sprout of the future blood vessel that will be located near the pre-existing blood vessels. Blood capillaries start to sprout and fold themselves to form the lumen of the vessel and then join each other at their tips until forming handle-shape ramifications. Blood flow starts to circulate after the formation of such ramifications which will also produce new sprouts until the formation of a new capillary plexus. Finally, the apposition of the pericytes and the smooth muscle cells takes place to give support to the newly synthet-

![Figure 1](https://example.com/angiogenesis_figure.png)

**Figure 1** Phases of angiogenesis: the breaking of the vascular membrane through the proteolytic action of enzymes such as metalloproteinase and plasminogen activators secreted by the endothelial cells and macrophages after stimulation. The macrophages, granulocytes and fibroblasts produce growth factors with angiogenic potential, such as VEGF, PDGF and bFGF. Consequently, migration and multiplication of the endothelial cells begin at the perivascular space, and enlargement of the sprout of the future blood vessel occurs. Finally, the apposition of pericytes and smooth muscle cells takes place which will function as a support to the recently synthesized vessels.

The extracellular matrix surrounding the vessels constitutes a scaffold for the new vascular endothelium, as it favours proliferation, migration and adhesion of the endothelial cells in three dimensions. In addition, as a consequence of the generation of contractile mechanical forces in the extracellular matrix, the endothelial cells establish some form of orientation based on tension. This process allows the formation of networks of interconnected blood vessels just where the injury is located, where collagen type 1 intervenes on the endothelial cells shape change. The main angiogenic factor is the vascular endothelial growth factor (VEGF), which induces angiogenesis and also favours the expression of integrins on the microvascular endothelial cells (Derringer et al. 1996, Artese et al. 2002, Tran-Hung et al. 2008, Krishnan & Davidovitch 2009, Nakashima et al. 2009).

The lack of collateral perfusion in the pulp makes it one of the most sensitive tissues in humans. There is evidence indicating that in the presence of occlusal trauma, the oxygen levels in the pulp decrease so that cell damage, vascular disorders and inflammation may occur. However, hypoxia-inducing factor-1α (HIF-1α) and VEGF expression in DPSC and HDPF are then enhanced, and therefore, angiogenesis takes place. This explains why vascular neoinformation plays a fundamental role in repairing the pulp tissue as these new vessels will be in control of fulfilling the metabolic and oxygenation needs of the pulp by the mediation of the pulp tissue nervous system through neuropeptides such as SP (Derringer et al. 1996, Derringer & Linden 1998, Aranha et al. 2010, Kim et al. 2014, Zhang et al. 2014).

Therefore, SP can induce angiogenesis either by direct mechanisms such as the growth modulation of the endothelial cells or by indirect mechanisms such as the attraction of the cells with angiogenic potential to the location of the injury (Fig. 2) (Kohara et al. 2010).

**Indirect mechanism**

Several studies confirm that SP performs chemotactic activities to carry granulocytes from the blood flow to the location of the inflammatory injury (Kohara et al. 2010, Murakami et al. 2013). SP binds to granulo-
cytic cells through NK1 and makes them release proinflammatory cytokines that have angiogenic and angiostatic functions. Table 2 summarizes the action of some of these cytokines, including the vascular endothelial growth factor (VEGF), the basic fibroblastic growth factor (bFGF), the transforming growth factor-beta (TGF-β), angiogenin, leptin, angiopoietin 2 (ANG-2), the epidermal growth factor (EGF), the heparin-binding epidermal growth factor (HB-EGF) and the platelet-derived growth factor (PDGF) (Derringer & Linden 2007, Mattuella et al. 2007a,b, El Karim et al. 2009, Kohara et al. 2010, Virtej et al. 2013).

Vascular endothelial growth factor is a heparin-binding protein with specific affinity to endothelial cells and plays a key role in angiogenesis. This glycoprotein family includes six members, VEGF-A, B, C, D and E, and PDGF, which have autocrine and paracrine effects in the dental pulp. Factors VEGF-A and VEGF-B are closely related to the angiogenic phenomenon as they stimulate endothelial cellular proliferation and increase vascular permeability (Mattuella et al. 2007a). VEGF also seems to induce the differentiation of human dental pulp cells into endothelial cells, and their ligands are expressed in DPSCs and HDPFs in hypoxic conditions. It is also known that blood vessels and immune cells are equipped with VEGFR-2 and VEGFR-3 (Mattuella et al. 2007a,b, Marchionni et al. 2009, Dissanayaka et al. 2012, Janebodin et al. 2013, Virtej et al. 2013).

Angiogenin and ANG-2 have functions related to the proliferation of endothelial cells. In the presence of VEGF, ANG-2 causes proliferation and migration of endothelial cells and stimulates the formation of new vessels, whereas angiogenin causes the neovascular formation from bFGF and VEGF. Factor HB-EGF and leptin also have angiogenic potential inducing the formation of structures similar to blood vessels (El Karim et al. 2009).

Moreover, the activation of macrophages by SP adds to its indirect angiogenic function. Macrophages are key stimulators of angiogenesis as they have several functions during the process of vascular neoformation. One of these functions is the production of metalloproteinases which degrade the basal membrane and the extracellular matrix of the vessel walls to allow migration of endothelial cells in the early stages of angiogenesis. They also promote the release of a large number of growth factors which can induce the proliferation and migration of cells containing that capillary endothelium as well as the release of more angiogenic factors such as the tumoral growth factor alpha (TGF-α), the granulocyte–monocyte col-

Table 2 Angiogenic growth factors and proteins released from granulocytes and macrophages attracted by SP-induced chemotaxis

<table>
<thead>
<tr>
<th>Dental pulp cells attracted by chemotaxis</th>
<th>Growth factors and proteins with angiogenic functions</th>
<th>Biological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages Metalloproteinases TGF-α GM-CSF bFGF IGF PDGF VEGF</td>
<td>Degradation of the basal membrane during initial stages of angiogenesis (Saghiri et al. 2015) Proliferation and migration of endothelial cells and increase release of more angiogenic factors Murakami et al. (2013)</td>
<td></td>
</tr>
</tbody>
</table>
Angiogenesis and substance P  Caviedes-Bucheli et al.

Angiogenesis can be induced by growth factors released during the masticatory function of the PDL. These factors include vascular endothelial growth factor (VEGF), bFGF, fibroblast growth factor-2 (FGF-2), PDGF, and IGF (Table 2) (Murakami et al. 2013, Saghiri et al. 2015). Some of these growth factors, including the hepatocyte growth factor (HGF), the placental growth factor (PIGF), and bFGF, are stimulated by other neuropeptides such as the CGRP. This peptide has some functions during the early stages of angiogenesis, suggesting that the mechanism for the formation of new vessels is complex and involves more than one neuropeptide. It is also important to mention that SP rarely acts alone. Quite the opposite, it coexists along with other nerve fibres that produce CGRP and NKA. It has also been claimed that NKA and SP have similar synergic effects because they share the same receptors (Caviedes-Bucheli et al. 2006, El Karim et al. 2009).

Direct mechanism

It has been reported that the biological mechanism for the direct induction of angiogenesis by SP is caused by its interaction with the endothelial cells and the fibroblasts that take part in the repairing process of the connective tissue (El Karim et al. 2009, Kohara et al. 2010). SP increases the differentiation and proliferation of endothelial cells because they express NK1 receptors on their surface. It also induces the formation of structures similar to capillaries thus increasing vascular density (Fig. 2). Angiogenesis is affected by the concentration of neuropeptides as the angiogenic phenomenon produced by SP is dose dependent. In other words, the more intense and prolonged the occlusal trauma, the higher the SP release, and therefore a greater differentiation and proliferation of the vascular endothelium will occur (Derringer & Linden 2007, Caviedes-Bucheli et al. 2008, 2011a, Kohara et al. 2010).

It has also been observed that in the presence of interleukin-1 (IL-1), SP acts in a synergic way and can cause vascular neoformations. The presence of IL-1 in pulp inflammation has been widely described (Killough et al. 2009, Kohara et al. 2010). Some studies confirm also the presence of NK1 receptors on the surface of the fibroblasts. These cells can produce growth factors with angiogenic potential such as VEGF, bFGF, fibroblastic growth factor-2 (FGF-2), placental-like growth factor (PIGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) (Tran-Hung et al. 2006, El Karim et al. 2009).

SP-induced angiogenic changes in human dental pulp and periodontal ligament in response to masticatory function and occlusal trauma

It has been reported that changes in the pulp and periodontal tissues vary according to the intensity and force of the masticatory stimuli. The application of force generates compression and tension zones on the periodontal ligament. The compression zones are associated with an increase in cellular activity, disorganization of the fibres of the ligament and bone resorption. In the tension zones, there is enlargement of such fibres as well as formation of bone and apical cement. Additionally, in the compression zones, damage to the periodontal ligament, venous thrombosis, haemorrhage, necrosis and hyalinization of the periodontal ligament occur (Jin & Cao 1992, Poiate et al. 2009, Caviedes-Bucheli et al. 2011a).

The presence of SP in the periodontal ligament after excessive occlusal load has been demonstrated. It can therefore be inferred that due to the presence of the neuropeptide, the cells that express NK1 receptors on their surface release numerous angiogenic factors in order to reverse the hypoxia caused by the decrease of blood flow in occlusal trauma (Fig. 3). Therefore, angiogenic changes take place when elastic cells in the compression zone respond to SP with root resorption, whilst blastic cells in the tension zone respond to SP with tissue apposition, as a compensatory defence mechanism of PDL that depends on duration and direction of the force created by occlusal trauma and masticatory function (Motohira et al. 2007, Poiate et al. 2009, Caviedes-Bucheli et al. 2011a).

The changes that take place in the dental pulp and PDL potentiate each other as both are mesenchymal in origin and are related via the apical foramen and the lateral canals (Ikeda et al. 1998, Caviedes-Bucheli et al. 2011a). However, very little is known about the response of the pulp to excessive occlusal loads. In spite of the lack of studies on the subject, it is well known that occlusal trauma causes neurogenic inflammation in the pulp characterized by changes similar to pulp ageing (Caviedes-Bucheli et al. 2008, Lee et al. 2013).

In the presence of occlusal loads, the pulp becomes inflamed in a localized manner. By the chemoattractant properties of the SP, it reacts by adding cellular components to the cell-free zone in the tissue adjacent to the injury. Most of these cells have morphologic characteristics of fibroblasts and undifferentiated cells, and only a few of them are inflammatory. The formation of new capillaries induced by SP is evident by the
presence of erythrocytes produced by small blood extravasations (Table 3) (El Karim et al. 2009, Kohara et al. 2010, Caviedes-Bucheli et al. 2011a).

In addition, vascular alterations mediated by SP also take place, such as arteriosclerotic changes of the blood vessels in which the tunica intima thickens whilst the adventitia is calcified lowering the blood flow towards the pulp tissue. Nerve fibres also undergo a progressive mineralization of all their membranes, and fat deposits are also observed around the odontoblastic layer, the cell-rich zone and the walls of the capillaries. The accumulation of interstitial liquid provokes the presence of vacuoles of odontoblasts coming from the separation of these cells from the dentine wall. There is a net of atrophic reticular fibres related to the high content of interstitial liquid and to the decrease in the number of pulp cells. As ageing progresses, the pulp continues to lose cellular content, whereas the number of collagen fibres continues to rise. The remaining odontoblasts have a smaller size and are flat in shape. The number of capillaries and nerve fibres has a tendency to decrease, and simultaneously, collagen is produced to form a completely fibrous pulp (Morse 1991, Goga et al. 2008, Cooper et al. 2010, Lee et al. 2013).

Latent odontoblasts may become activated by SP signalling, and therefore, the production of tertiary dentine starts with a mixture of dentine deposits and direct deposits of mineral crystals inside the dentine tubules which reduces their permeability (Fig. 4). This process is therefore called tubular or sclerotic dentine and is the result of increasing dentine deposits and the occlusion of the tubules due to the precipitation of crystals in a short period of time. In order to produce reactive tertiary dentine, it is necessary that the effect of the harmful stimulus be much longer than that needed for sclerotic dentine formation (Roberts-Clark & Smith 2000, Arana-Chavez & Massa 2004, Zhang et al. 2011).

After a longer period of time or after increasing the occlusal load, the reaction will be characterized by a noticeable cellular infiltration observed on the injury site and the formation of microabscesses due to the compartmentalization capacity of the pulp. Polymophonuclear and mononuclear leucocytes are also observed in the affected area. The nuclei of the odontoblasts can be seen in the tubules due to the increase in fluid pressure and numerous vascular neformations surrounding the intense cellular infiltration (Mattuella et al. 2007a, Li et al. 2011, Lee et al. 2013). There is also evidence of FGF-2, PDGF and VEGF in the extracellular matrix. These are released in a neurogenic inflammatory process by a SP stimulus in order to contribute to the repairing response of

If the damage persists, odontoblasts will not be able to defend the pulp as they are post-mitotic cells. It is at this stage, and due to the action of different types of cells, that the repairing dentine starts forming under the damaged tubular region by increasing the normal collagen portion of the pulp and decreasing the cellular content. The harmful stimulus can destroy the subjacent odontoblasts, and the pulp might need a repopulation of the destroyed odontoblast layer based on the differentiation of cells which can be undifferentiated perivascular cells, pulp fibro-
lasts or cells formed by the odontoblastic lineage but had not been exposed to final epithelial influence during dentinogenesis (Tziafas 1994, Cooper et al. 2010, Smith et al. 2012).

The changes that take place in the connective tissue as a response to both occlusal trauma and masticatory function are mediated mainly by the fibroblasts. These cells respond to mechanical stimuli due to a cascade of signals which come from the extracellular matrix and are received by the receptors located on their surface such as the NK1 specific for the SP. Such signals also affect the morphology as well as the proliferation and differentiation of fibroblasts, which contain proteins called kinases that are sensitive to the stress caused by the extracellular matrix. These proteins activate phosphorylation sites recognized by several molecular signals that modify the operation of the fibroblasts (El Karim et al. 2009, Krishnan & Davidovitch 2009, Kohara et al. 2010).

Once stimulated by SP, the fibroblasts express angiogenic factors that contribute to the formation of new blood vessels by either direct or indirect mechanisms which are active participants in tissue remodelling associated with occlusal trauma. Under normal and pathological conditions, the new vessels supply the periodontal ligament, the pulp and their surrounding tissues with oxygenation and nutrients necessary for their metabolic activities. They also provide tissues with hormones and a wide variety of endogenous substances (Tran-Hung et al. 2008, Killough et al. 2009, Krishnan & Davidovitch 2009).

If the stimulus persists after the inflammation process is diagnosed, the vascular neoformation cannot supply oxygen and nutrients to the tissues, the vascular return system also collapses, and the result will be the accumulation of mediators, liquid and plasma, as this system was in charge of removing the inflammatory mediators. This would provoke a vicious cycle in which the pulp located inside a cavity with limited expansion capacity will not be able to defend itself, and therefore, pulp necrosis will occur by coagulation. This type of necrosis is characterized by the coagulation of intracellular proteins due to a low level of oxygen. It is important to highlight that only 7% of root canals that become calcified will develop pulp necrosis (Holcomb & Gregory 1967). This low percentage of necrotic pulps is probably due to the continuous formation of vessels induced by the constant release of SP which provides the tissue with the capacity to remain viable (Killough et al. 2009, Levin et al. 2009).

**Conclusion**

Human dental pulps respond to occlusal trauma and to masticatory function via a neurogenic inflammatory process in which SP plays an important role in the direct and indirect mechanisms of angiogenesis by triggering the NK1 receptors of cell populations such as fibroblasts, endothelial and inflammatory cells, leading to the formation of new blood vessels which are needed to form mineralized tissue as a defence mechanism.

**Acknowledgements**

Authors would like to express gratitude to Mr. Wilson Guacame Rodriguez, from the Biblioteca General Pontificia Universidad Javeriana, for his assistance and collaboration in the use of library databases.

**Conflict of interest**

The authors have stated explicitly that there are no conflict of interests in connection with this article.

**References**


