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A LITERATURE REVIEW ON THE EFFECTS OF SMOKING ON TERTIARY DENTIN FORMATION

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A THESIS

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Abstract

PURPOSE. The tooth is a complex structure composed of two anatomical regions, a crown and root. Each region is comprised of two, highly mineralized tissue layers that surround and protect the vital pulp chamber. The crown is visible in the oral cavity and covered externally with enamel. The outer layer of the root is covered in cementum. Dentin is the hard tissue that makes up the bulk of the tooth and gives structural support to the overlying enamel and cementum. Dentin also provides protection to the cellular and neurovascular elements in the pulp chamber. Dental trauma and carious lesions can induce the deposition of tertiary dentin as a protective response to localized sites of injury. The extent of injury and the cellular response of the pulp determines whether a reparative or reactionary type of tertiary dentin will be deposited. Smoking is considered a contributing factor to several oral diseases; however, little is known about the effect of smoking on the reparative process of the pulp chamber. The purpose of this review is to investigate the impact of smoking on tertiary dentin formation. METHODS. A literature review on tertiary dentin and the effect of smoking was performed. Peer reviewed research articles, published between 1994 through 2016, were obtained through PubMed. RESULTS. This review includes information on tertiary dentin formation and the proposed mechanisms that initiate this process. The effect of smoking on teeth and gingiva is also discussed to demonstrate the impact of smoking on the formation of tertiary dentin. SIGNIFICANCE. Demonstrating a relationship between smoking and tertiary dentin formation could have a significant impact on the treatment of patients. Documentation of the effect of smoking on the inhibition of tertiary dentin formation could lead to the development of new treatment plans and ultimately preventive measures resulting in improved oral health.
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1 - Introduction

The effect of smoking has many health implications often seen in modern day medicine. In the oral cavity, smoking has a significant impact ranging from periodontal disease [6], to changes in the fundamental structure and development of the teeth [3, 4]. These conditions can have significant clinical implications when developing treatment plans for patients. Teeth have a mechanism of repair known as tertiary dentin formation [2, 24]. This repair allows the tooth to protect itself from carious lesions that can infiltrate the pulp chamber resulting in the death of the tooth. In light of the number of mechanisms that play a role in tertiary dentin formation, there are several elements which can negatively affect the repair process such as smoking. One of the most important aspects of the repair process is the function of the dentin-pulp complex [1, 28, 29]. Smoking clearly impacts the differentiation of pulp cells from embryogenesis through adulthood [3-6]. Smoking affects both prenatal development as well as the adult structures of the tooth. These factors could lead to problems with an individual’s ability to form tertiary dentin. For this project, we reviewed the literature for information regarding the negative effects of smoking on tertiary dentin formation. The results of this comprehensive review found that very little is known about the relationship between tertiary dentin formation and the effects of smoking. The potential effects of smoking on tertiary dentin formation is important as abnormal tertiary dentin formation can result in diminished repair capabilities and possible tooth loss. Finding a link between smoking and its effects on tertiary dentin could lead to potential clinical implications for dental treatment in smokers.
1.1 - Human Tooth Anatomy

Human teeth have a complex anatomical structure (See Figure 1). The tooth can be divided into two anatomical regions: the crown and the root. The crown is the portion of the tooth that visible in the oral cavity; it is comprised of a hard tissue known as enamel. This is a highly mineralized layer that aids in protection and functionality. The mineral content of enamel is 95-96% hydroxyapatite crystals, which makes it the hardest tissue in the human body [1]. As for the root, the outer layer is comprised of a substance known as cementum. This is a much thinner layer than the enamel in the crown of the tooth.

The cementum aids in anchoring the tooth in the alveolar socket with the help of the periodontal ligament (PDL). Dentin is the hard tissue that makes up the bulk of the tooth and gives structural support to the overlying enamel and cementum. The calcified matrix that comprises dentin is similar to bone, but it has a higher proportion of hydroxyapatite crystals. The percentage of hydroxyapatite crystals in dentin is 70% while bone and cementum is slightly less than dentin [1]. Hydroxyapatite crystals make up the inorganic material of dentin as well as the other hard tissues in the body [1]. The inner portion of the tooth is known as the pulp chamber. This area consists of specialized supportive connective tissue along with the vascular supply and nerve supply for the
tooth. The pulp also consists of cells such as odontoblasts, fibroblasts, and a stem cell population [2].

The tooth is held in place by the periodontium which is comprised of the PDL, alveolar bone, and cementum [1]. The periodontal ligament is composed of a fibrous collagenous connective tissue that aid in anchoring and securing the tooth in place by connecting the cementum of the tooth to the alveolar bone. The PDL fibers that insert into the cementum and to the alveolar bone are known as Sharpy’s fibers. The alveolar bone is bone that encompasses and houses the tooth within the mandible or maxilla [1].

1.2 - Tooth Development

Tooth development encompasses a series of sequential complex signaling events that lead to the adult tooth structure. All of these stages are highly regulated due to strict epithelial-ectomesenchymal interactions. Both tissues signal back and forth to regulate odontogenesis [1]. There are different stages of tooth development which occur during odontogenesis. The beginning of tooth development begins with an initiation stage, which appears as the condensation of the oral ectoderm or oral epithelium. This stage initiates the beginning of tooth formation and specifies the location of the tooth within the oral cavity [1]. The following stages of tooth development help determine the type of tooth and cause differentiation of the different cells that are responsible for the development of the tooth. The stage that follows the initiation step of tooth development is the bud stage, which is a further condensation of the mesenchymal cells. This is followed by cap stage which is the first stage the tooth germ is visible. The tooth germ is comprised of the enamel organ, dental papilla, and dental follicle [1]. The enamel organ eventually gives rise to ameloblasts, which form enamel. The dental papilla gives rise to
the cells of the pulp and odontoblasts which form the dentin. The dental follicle gives rise to the periodontal ligament, cementoblasts, which form cementum, and the alveolar bone.

The next stage is the bell stage which is the first stage that hard tissue is deposited. Predentin and dentin are first deposited in late bell stage by odontoblasts through a process which is known as dentinogenesis. Crown stage follows the bell stage. This stage is demarcated by the beginning of the differentiation of the enamel organ into ameloblasts which then deposit enamel. Root formation is the final stage of tooth development which leads to the differentiation of the dental follicle and formation of the periodontium [1].

1.3 - Dentin

The deposition and formation of dentin is known as dentinogenesis. Odontoblasts initially deposit an unmineralized matrix known as predentin from the odontoblastic process found at the apices of the cell. As the odontoblasts mineralize the predentin matrix, they form dentinal tubules around the odontoblastic processes. The tubules in the crown extend from the dentinoenamel junction (DEJ) to the pulp chamber. The tubules exhibit a primary “S” curvature (See Figure 2), which is the characteristic shape of dentinal tubules and represents the path of the odontoblast [1]. Mineralized dentin is composed of 70% inorganic hydroxyapatite crystals, 10% water, and 20% organic substance such as collagen fibers and proteins. Based on its composition, dentin
is more resilient than enamel and this allows it to absorb occlusal forces without fracturing the enamel [1].

There are three types of dentin: primary, secondary, and tertiary. Primary dentin is deposited throughout tooth development and constitutes the bulk of the dentin in the tooth. Primary dentin is put down first at the dentinoenamel junction. There are two specific regions of primary dentin visible in the adult tooth, mantle and circumpulpal. The first is the mantle dentin. This dentin is less mineralized and thinner than circumpulpal dentin.

Secondary dentin is deposited after the tooth is in occlusion and helps protect the pulp from normal occlusal wear [2]. It is deposited at a slower rate than primary dentin along the pulpal border throughout the life of the tooth. Histologically, secondary dentin appears different as the dentinal tubules lose their “S” shape curvature and appear to extend straight towards the pulp [1].

Tertiary dentin is known as a reparative or reactionary dentin and may be deposited at a fast or slow rate. Many factors can stimulate tertiary dentin formation ranging from attrition, abrasion, caries, or restorations [1]. Reparative dentin (See Figure 3) is rapidly deposited in response to severe trauma such as exposure of the pulp chamber. The odontoblasts die due to the trauma and the stem cell population of the pulp chamber must

![Figure 3. Tooth cut in cross section in the coronal 2/3 of the root. Reparative tertiary dentin is in the field of view.](image-url)
differentiate to form new odontoblasts [24].

Histologically, reparative dentin appears quite different compared to primary, secondary, or even reactionary tertiary dentin. There are no dentinal tubules seen in reparative dentin. This is due to the high rate at which the dentin is being deposited [1]. The second type of tertiary dentin is reactionary dentin (See Figure 4). This type of dentin is formed in response to a mild injury. Odontoblasts do not die and form tertiary dentin that is similar to primary or secondary dentin [1]. The appearance may vary. Odontoblasts may appear stuck within the new dentin matrix that is deposited. This reactionary tertiary dentin is osteodentin since it resembles bone more so than dentin, since bone also has cells stuck in the matrix. Reactionary dentin may also have an irregular appearance of the dentinal tubules. Additionally, there may be a combination of both irregular tubules as well as cells that are stuck within the dentin matrix. This extra layer of protection that is added to the pulpal side (See Figure 3 and 4) helps block any surface pathogens from entering the pulp chamber resulting in the death of the tooth [1].

1.4 - Dentin-Pulp Complex

The pulp chamber and dentin are very closely related in maintaining the health of the tooth. The pulp’s most important function is to supply nutrients, blood supply, and nerve supply to the tooth [1]. The pulp consists of many types of cells. Fibroblasts are the
most numerous and they produce the collagen fibers and ground substance seen in the pulp. Odontoblasts found along the perimeter of the pulpal border are responsible for the deposition of dentin. The pulp also has nerve cells and different immunological cells such as lymphocytes and macrophages [2]. This is important for the longevity and life of the tooth. The pulp also functions as an inductive signal to initiate tooth formation [1]. The pulp plays a major role in protection of the vital neurovascular supply that is supplying the tooth.

Inflammation is an important aspect in the pulp chamber. Since the pulp is highly vascularized, inflammation is often present within the pulp chamber. Inflammation is the body’s natural defense to pathogens or microorganisms that cause disease [30]. The immune system has two different responses, an innate response and an adaptive response. The innate response is the natural and inherent defense the body has to any pathogen. This includes physical defenses such as epithelial barriers, chemical mediators such as cytokines, and cellular defenses that include natural killer cells, macrophages, dendritic cells, and neutrophils. These cells engulf and phagocytose any foreign body. These are also the direct link to the adaptive immune system [30]. After phagocytosis, monocyte derived cells are known as antigen presenting cells. The antigen presentation of the phagocytosed pathogen alerts cells known as helper T cells to aid in mediating an immune response. These T cells also recruit B cells and create memory cells that will cause a faster reaction to the pathogen if seen again in the body. Cytokines are also released that will recruit more immunological cells that will aid in inflammation and the overall effectiveness to protecting the body from the intruding pathogen [30]. Inflammation in the pulp can lead to pain and cause changes to the pulp. Pain can be
stimulated due to a theory known as hydrodynamic theory. This suggests that since the pulp is fluid filled, the increased pressure that is caused by inflammation can lead to the stimulation of the nerve endings found in the pulp chamber and dentinal tubules [1].

The pulp can also sense different stimuli such as heat or attrition and can cause cellular mechanisms to aid in protection of the tooth itself [1]. It is important for the pulp to have normal functional ability to ensure the repair processes of the tooth will work properly. The dental pulp can cause odontoblasts to form new reactionary dentin, or cause differentiation of stem cells to form a reparative type of dentin [28]. This is critical to how the tooth would be able to repair itself. If there were damage to the cells of the pulp, the repair process would become inhibited. Odontoblasts are known to aid in this repair process by not only laying down tertiary dentin, but also by releasing growth factors such as BMP [1,8], TGFβ [8], Wnt [1,10], and VEGF [11, 12, 13] to continue the repair process [29]. These growth factors can initiate the formation of tertiary dentin and aid in the mineralization of the dentin deposited. The cascade of growth factors helps perpetuate the pulp’s signaling mechanism to alert the cells to aid in this repair function. Without the pulp functioning properly, the tooth would be unable to defend itself by way of its defense mechanisms. Other pulpal responses to injury include other dentin changes and connective tissue changes of the pulp [1]. A lesion or any area of damage to the enamel or dentin will cause the pulp to initiate the formation of sclerotic dentin [1]. Sclerotic dentin is the hypermineralization of the dentinal tubules. This causes the blocking of the dentinal tubules so that microbes or other debris will not travel through the dental tubule and reach the pulp chamber. Injury can also cause a fibrosis to occur. Fibroblasts will begin making more collagen fibers in response to injury as well as the
tooth is aging [1]. These changes cause the pulp chamber to shrink in size over the life of the tooth because of the fibrosis as well as the continual deposition of dentin.

2 - Methods

A thorough literature review was performed on the effect of smoking on tertiary dentin formation. Peer-reviewed articles published from 1994 through 2016 were obtained using PubMed. Search terms included tertiary dentin, smoking, nicotine, reparative dentin, disease, wound healing, inflammation, microvascular, autonomic nervous system, sympathetic nervous system, dentin-pulp complex, and different combinations of the above searched terms. Thirty sources were used to write this paper.

3 - Results

3.1 - Effects of Nicotine and Smoking

Nicotine alters the autonomic nervous system by stimulating the sympathetic nervous system. Nicotine causes stimulation of the sympathetic nervous system by triggering the release of catecholamines from nicotine acetylcholine receptors [14]. When the body has an increase of catecholamines in the plasma it has been shown that it increases heart rate and blood pressure [14]. This increase of heart rate and blood pressure negatively affects the flow of blood and oxygen levels as well as increases the stress put on the heart and vessels. Increases in norepinephrine and epinephrine have been also associated with smoking [20]. These hormones are directly related to stimulating the sympathetic nervous system [15]. This supports the proposed relationship between smoking and an increased stimulation of the sympathetic nervous system since the hormones involved with sympathetic stimulation have been shown to be increased during smoking [15].
Smoking also affects immune function and inflammation. Studies have shown that smokers exhibit a decrease in neutrophil activity [16], higher levels of T cell activity [17], and a decreased number of dendritic cells [18]. These cells play an important role in the immune response and determine how an individual can fight infection.

The higher levels of T cell activity seen in smokers leads to chronic inflammation. In the oral cavity, high levels of T cell activity are a risk factor for periodontitis, which is chronic inflammation of the gingival tissue. As shown in the study by Loos et al., the increased number of T cells seen in the smoking population leads to an increase breakdown of the PDL fibers and alveolar bone [17]. These researchers had a total population of 112 adult subjects. These individuals were classified into four separate groups: non smokers, former smokers whom have quit within the last 10 years, light smokers (less than 10 cigarettes a day), and heavy smokers (over 10 cigarettes a day). Loos et al. obtained venous blood to observe the number of leukocytes. They performed lymphocyte immunophenotyping for CD3+ T cells, CD4+ and CD8+ T cells, and CD19+ B cells. They found that T cell proliferation was highest in smokers compared to non-smokers. Smokers had an increased number of CD3+, CD4+, and CD8+ T cell populations. They conclude that the higher number of T cells caused increased periodontal damage. This was due to the destruction of the periodontal ligament and alveolar bone structure. Smokers had higher incidences of periodontal disease which was linked to these higher number of T cells [17].

Neutrophils, a principle cell involved in the innate immune response, are the predominant cellular defense mechanism against microorganisms in the gingiva and periodontium [16]. Once a pathogen is recognized, the neutrophils move to that location
and phagocytose the pathogen. Srinivas et al. conducted research on the effects of smoking and neutrophil migration on 120 different patients. Patients were divided into smokers and non-smokers, and the groups were then further classified into a healthy periodontium group, mild gingivitis, and chronic periodontitis. Three milliliters of venous blood was collected from their participants, and a neutrophil chemotaxis assay was performed. The samples were then prepped onto histological slides and stained. The results indicated that neutrophil chemotaxis was significantly decreased in smokers when compared to non-smokers in all three of the subsets of smokers and non-smokers. The decrease in chemotaxis seen in smokers allows pathogens to survive causing destruction of the periodontium [16]. Srinivas et al. found that smoking causes destruction of the periodontium as well as downregulating the immune response the body has to any bacterial pathogen. Peripheral vasoconstriction from smoking is an additional factor to consider for these results since smoking decreases peripheral blood flow [16].

Dendritic cells greatly affect the immunological response an individual can mount against a pathogen since they are the main antigen presenting cells that stimulate T-cells as part of an adaptive immune response [18]. Souto et al. obtained gingival samples from 24 smokers and 21 non-smokers. They classified patients based on smoking habits, number of cigarettes per day, periodontal status, number of teeth, and analyzed it against inflammatory markers such as the number of dendritic cells and cytokines. Histological slides were prepared and cell counts were taken to determine the number of inflammatory cells such as the dendritic cells. Cytokine levels were also examined and measured by flow cytometry. Their results showed that smoking decreases the number of mature dendritic cells and cytokines released. The higher number of cigarettes smoked per day
correlated to lower numbers of dendritic cells. The lower number of teeth seen in the patient also correlated to a lower number of dendritic cells. The fewer teeth, due to extraction, necrosis or other forms of tooth loss, also showed a decrease in the number of dendritic cells. This observed decrease in dendritic cells is important due to the decrease in immune function and connection between the innate and adaptive immune system [18].

Another study conducted by Alrashdan et al. demonstrated a decrease in macrophage counts in a particular pathology known as oral lichenoid lesions [19]. These are chronic, immune mediated lesions affect the oral mucosa. Redness, blistersing and ulceration are common symptoms observed. Smokers were seen to have a much lower number of macrophages in the area of these lesions in comparison to non-smokers [19]. Alrashdan et al. conducted an experiment with 53 total patients and placed them into either a smoking or non smoking group. Both groups had been diagnosed with oral lichenoid lesions and they compared immune expression of macrophages. The researchers used immunohistochemical reactions to assess any change in the number of inflammatory cells known as macrophages. They demonstrated that smoking lowers the number of macrophages compared to the control group of non-smokers. Alrashdan et al. found this to be important with how smoking affects natural immune function but they were concerned about other confounding variables that were not considered, such as the use of alcohol [19]. Smoking changes the inflammatory response, and therefore alters normal function that healthy individuals typically express. Smoking negatively impacts the overall function and effectiveness of the immune system [19].
In addition to the effect smoking has on the sympathetic nervous system and immune system, it also has been shown that smoking impacts wound healing capabilities [20]. It has been proven that nicotine as well as carbon monoxide, which is formed as the cigarette burns, are vasoconstrictors that decrease peripheral blood flow [20]. Nicotine also influences wound healing by causing an increase in platelet adhesiveness that leads to micro occlusion of the capillaries. This drastic decrease in blood flow correlate with the decrease in wound healing capabilities. Clinical studies have been reported to show a decrease in wound healing capabilities that range from healing of extraction sockets [21], a higher rate of failure in implant surgery [22], and basic self-healing capability of periodontal tissues [23].

Ozkan et al. conducted an experiment using 84 male rats and looked at how smoking affected the healing of extraction sockets. The rats were placed into 3 groups. Group 1 was placed in smoking chambers before and after surgery while Group 2 was only exposed to smoking prior to surgery. The final group represented normal controls not exposed to any smoke. Tissue in the healing tooth socket was analyzed. Smoke exposure followed the extraction of the central incisor. Rats were euthanized at days 3, 7, 15, and 28 after the maxillary right central incisor was removed. Ozkan et al. also examined the amount of type I collagen antibody and found that the first two groups did not have any significant change, but day 15 and day 28 groups had much less than the control group, which was not exposed to smoke. Normal healing is associated with an increase amount of type I collagen which is a key supportive fiber in connective tissue. Their results concluded that smoking negatively influences the healing process [21].
Levin and Schwartz-Arad (2005) conducted a literature review that examined the current research of smoking and the effects on dental implants. They found due to the effects of smoking on wound healing, bone grafts were unsuccessful. Although bone grafts are not always utilized when performing implant procedures, a bone graft will expedite the time and efficiency of the healing process. Levin and Schwartz-Arad found through the literature that implant failures were very high in smokers whether the dentist used bone grafts or not. This was due to the heat of cigarettes, and the toxic byproducts such as carbon monoxide and hydrogen cyanide [22]. These toxins affect both the hard tissue as well as decrease blood supply to the area. Implant surgeries showed numerous complications in studies that compared smokers and non-smokers [22].

Benatti et al. conducted an experiment on the effects of cigarette smoke and nicotine on periodontal tissue repair. Forty-two rats were placed into three groups: a control, a group that was exposed to cigarette smoke, and a group that was given injections of nicotine. The mandible, the first mandibular molar, and surrounding gingiva was damaged using a high speed drill and healing process was assessed. Histological results showed a decrease in total bone volume created due to the impaired repair process in the group of smoking rats. The group in which nicotine was administered without inhalation showed no difference compared to the control group, suggesting it is the cigarettes that inhibit the self healing properties of the periodontium rather than the nicotine itself [23].

3.2 - The Effects of Smoking on Teeth

Many different dangers threaten the health of teeth, whether from a physical injury that cracks the tooth to sugary drinks. One of the biggest culprits of tooth decay
and tooth loss is smoking. Smoking is linked to many health issues and the health of the oral cavity is no stranger to the negative effects that smoking can have on both the gingival tissue as well as the teeth.

Yanagita, et al. looked at the effects that nicotine had on human dental pulp cells. The teeth used in this study were healthy first premolars that were extracted for orthodontic treatment [3]. Therefore, these teeth represented to be healthy with normal pulp functionality. These researchers looked specifically at nicotinic acetylcholine receptors since nicotine acts as an agonist on these receptors. Many different subunits of these receptors have been found and may reside on human dental pulp cells [3]. Yanagita et al. cultured these human dental pulp cells and treated them with nicotine to observe the effects. The group of researchers used a culture medium to grow multiple new lineages of the dental pulp cells. They replaced it with a mineralization medium afterwards to observe the function of these cells. Every third day they changed the mineralization medium to a fresh medium either treated with nicotine or not for the control group. mRNA was tested to observe the expression of certain proteins. The results showed that nicotine treated pulp cells exhibited a large decrease in alkaline phosphatase activity, along with a decrease in acidic phosphoprotein-1 and bone sialoprotein. This is important due to the function of alkaline phosphatase activity. Alkaline phosphatase is responsible for the mineralization of hard tissue such as bone and dentin [1]. Therefore, nicotine appears to act through these nicotinic acetylcholine receptors and cause decrease mineralization and pulp cell function [3].

Dong et al. [4] performed a study to observe the effects of passive maternal smoking on rat pups. Not only does smoking affect fully formed teeth, but it also shown
to affect odontogenesis [4]. The study involved 60 healthy adult pregnant rats. Tobacco smoke was given in two hour intervals to the experimental group in smoking chambers. They studied three groups specifically: rat pups on day 20 of gestation, 3rd day after birth, and 10th day after birth. The researchers specifically used the tissue thickness and mineral density to compare how smoking affected the development of teeth on the rat pups. Dong et al. used micro CT scans, dispersive X-ray spectroscopy, and scanning electron microscopy to observe the mineral density and volume of mineralized material. The rat pups that were exposed to smoke during embryogenesis saw a decrease in both total volume of mineralized material on their mandibular first molars, as well as a decrease in the mineral density [4].

Smoking clearly has effects on the hard tissues of teeth such as enamel and dentin as seen in the study by Dong et al [4]. It also has a secondary effect on hard tissue through the process of periodontal disease. Periodontal disease is a chronic inflammation of the gum and supportive tissues around the tooth. This disease is largely seen throughout the world due to smoking as the main risk factor in individuals with this chronic disease. Bacteria in the dental plaque cause inflammation, which destroys the periodontal ligament as well as the alveolar bone [5]. Cojocaru et al. [6] looked into the effects that periodontitis has on the hard tissues of the tooth, rather than the support system that keeps the tooth in place. They specifically looked at only the effects of periodontitis on hard tissues rather than how smoking in addition to periodontitis would affect them. They found differing results when it came to the amount of radicular dentin damage in the tooth. Radicular dentin damage depended on the length of time the patients had the disease, the total damage that occurred in the PDL, and how intense the immune
reaction was for each individual when it came to the total amount of inflammation [6]. Therefore, smoking and periodontal disease affects the hard tissue structures in the tooth. This can complicate issues when clinicians and researchers are trying to discern smoking affects on the teeth in the oral cavity.

3.3 - Dentin Tissue Repair

Smoking can damage many different systems in the body and cause a multitude of problems ranging from lung cancer to COPD [7]. The oral cavity is very much affected, however, the question remains whether smoking also damages the reparative response of tertiary dentin formation. There are several different pathways that have been studied that lead to tertiary dentin formation. First, differentiation of pulpal stem cells into odontoblasts that create reparative dentin is similar to the way dentin is formed during embryogenesis [2]. In multiple studies, researchers have tried to induce reparative dentin formation with different signals that are known to be present while the embryo is forming teeth. Both TGFβ and BMP have been shown to be active during dentinogenesis [8]. Researchers attempted to use both of these signals in animal studies to induce dentin formation. Specifically, TGFβ-1 was administered in animal studies as a pulp capping agent. Pulp capping is performed by placing a material to help create an environment that would cause the death of any microbial life [10]. This induced a variable amount of reparative-like dentin to normal tubular dentin that resembled primary or secondary dentin [8]. When BMP was used in addition to TGFβ-1, there were significant amounts of a reparative dentin matrix seen. However, it was unclear whether it was actually causing odontoblast differentiation or if these signaling molecules caused a non-specific bone-like matrix that also resembled dentin. The addition of BMP resulted in a
histologically different matrix of mineralized material when compared to the dentin TGFβ-1 formed when used alone [8]. These results suggest that different types of tertiary dentin could be formed depending on the growth factor signal.

Additional studies have looked at the actual pathway that may lead to the start of tertiary dentin formation. One study showed a relationship between sympathetic stimulation and downregulation of tertiary dentin formation. Gu et al. [9] specifically looked at a specific adrenergic receptor, the beta-2 adrenergic receptor. Norepinephrine released from postganglionic sympathetic neurons activates these receptors. This receptor has been found to be present on odontoblasts as well as osteoblasts. Previous studies have demonstrated that a beta-2 antagonist may lead to increased bone mass among rats [9]. Therefore, Gu et al. applied the same concept to the formation of tertiary dentin. Gu et al. used a total of 20 healthy male rats that were 9 weeks old. They prepared the rat’s maxillary first molars with a groove shaped cavity on the medial aspect of the tooth. Then propranolol was administrated to Group 1 and Group 2 in two different concentrations, Group 3 was given a saline treatment, and Group 4 was administered nothing. They found that propranolol, a sympathetic antagonist, caused an increase in the tertiary dentin deposition compared to the control groups [9]. These data suggest that activation of the sympathetic nervous system could cause a slower rate or completely halt the formation of tertiary dentin [9]. According to the researchers, this is the first time that beta adrenergic antagonists up-regulated the amount of tertiary dentin that is formed. Gu et al. also hypothesize that this sympathetic antagonist effect could cause an increase in bone mass and mineralization as well as in the teeth [9].
Another signaling mechanism that has been shown to affect reparative dentin formation involve Wnt’s. Wnt proteins are small lipid-modified proteins that have been shown to control stem cell differentiation and cell renewal [10]. Hunter et al. found that there are specific Wnt proteins found in the pulp chamber. These researchers induced pulpal injuries and found that if they amplified the Wnt signaling, a higher level of healing could be achieved in the tooth. They did this through a process known as pulp capping. Hunter et al. engineered a Wnt protein compound called WNT3A. They found that not only does this protein cause the pulpal cells to differentiate into secretory odontoblasts, but it also induces pulp stem cells to increase significantly in their mitotic rate. WNT3A also reduced pulpal cell apoptosis caused by injury. These outcomes overall lead to an increase in tertiary dentin deposition [10].

Finally, an additional study showed vascular endothelial growth factor (VEGF), a signaling pathway, could aid in pulp healing. VEGF is a family of growth factors known as vascular endothelial growth factors. These growth factors have been shown to be one of the most important factors in the regulation of tertiary dentin formation [11]. VEGF’s are an endothelial specific protein that is secreted to aid in tissue repair from endothelial cells. They have been found in the pulp chamber and shown to be secreted by pulpal stem cells [25]. The amount of VEGF is also dependent on the amount of blood flow observed in the pulp [25]. Inflammation of the pulp upregulates these growth factors but chronic inflammation may actually cause a decrease [25].

Zhang et al. looked at how VEGF may affect the rate of formation and amount of tertiary dentin. These researchers created a vector to deliver hVEGF into the dental pulp cells of the maxillary first molars of rats. Observations of the mineralization and gene
expression were used to evaluate their results. RT-PCR was used to measure the RNA expression of genes involved in osteogenesis and dentinogenesis. They also used histological slides to observe the pulp chamber and amount of tertiary dentin deposited. When they introduced the pulp to the VEGF vector, they found a significant increase in the mineralization of dentin. There were additionally higher levels of alkaline phosphatase found in the pulp chamber. VEGF also increased the expression of genes that are involved in osteogenesis and odontogenesis [11]. This group suggested that using VEGF’s could have some significant clinical applications to help with the body’s natural repair function.

An additional study looked into the use of a biomolecule known as iloprost and the effects this biomolecule would have on tertiary dentin formation. Prostacyclin (PGI$_2$) aids in vasodilatation as well as increasing angiogenesis, or the formation of new blood vessels [12]. PGI$_2$ has also been seen to be released by osteoblasts, as well as osteocytes in response to stress [26]. PGI$_2$ is not a stable molecule, therefore Nakalekha et al. used a synthetic substitute called iloprost in their experiment. Iloprost has been used to treat hypertension and prevent bone necrosis [12]. Nakalekha et al. designed an experiment to look at the effects of iloprost on the pulp chamber. Rats were prepared with pulp injuries in vivo and were given treatments of iloprost. The results show that iloprost significantly increased the blood flow into the pulp chamber, resulting in an increased expression of VEGF [12].

Later the same group of researchers looked closely at the direct effect iloprost has on tertiary dentin formation. Nakalekha et al. conducted an experiment both on human pulp cultured cells and an animal model using rat molars in which they induced pulpal
injury by exposing the pulp chamber. In the *in vitro* model using human pulp cells, it was found that iloprost, resulted in a significant increase in alkaline phosphatase activity [13]. There was also a large increase in the expression of VEGF [13], supporting their previous work [12]. In the animal model, 30 days after treatment with iloprost they observed the effects of the biomolecule on the pulp chamber. The researchers concluded that iloprost significantly induced the formation of tertiary dentin. This was due to the fact that it also increased the VEGF protein expression [13]. This biomolecule could potentially be used as a way to treat pulp diseases as well as help the tooth repair itself when used in a clinical approach to treating dental disease.

**4 - Discussion**

Based on the literature, it appears there is a gap in the information known about the effect of smoking on tertiary dentin formation. However, some conclusions can be made to help direct the research. Smoking affects hard tissue of the oral cavity both prenatally [4] as well as in the mature tooth [3, 5, 6]. The demineralization of dentin could affect the formation of tertiary dentin due to the decrease of alkaline phosphatase activity. The pulpal cells showed a decrease in the production of alkaline phosphatase, which is responsible for the mineralization of hard tissues such as dentin. This shows that smoking has an effect on the hard tissue and the pulpal cells [3].

The cellular mechanisms of tertiary dentin formation are another key aspect to investigate. The different growth factors that are observed to be responsible for the formation of tertiary dentin such as BMP [1,8], TGFβ [8], Wnt [1,10], and VEGF [11, 12, 13] could be affected by smoking. If smoking does inhibit or affect any of the mechanisms, that could lead to a decrease or inhibition of the repair process of tertiary
dentin formation. Sympathetic stimulation has also been shown to affect tertiary dentin formation [9]. One study showed an increase in the total amount of tertiary dentin formed by odontoblasts when using a sympathetic antagonist. Since smoking stimulates the sympathetic nervous system [14], one could assume that tertiary dentin may be decreased or inhibited. The impact of the sympathetic stimulation and modulation on tertiary dentin repair is another area for additional research.

Finally, it appears that pulpal blood flow is a very important contributing factor to tertiary dentin formation. Both VEGF’s and iloprost aided in increasing blood flow as well as increasing tertiary dentin formation [11, 12, 13, 25]. The increased blood flow stimulated the differentiation of the pulpal stem cells, therefore causing an increase in the amount of tertiary dentin being formed. Smoking on the other hand decreases the amount of blood flow through sympathetic stimulation [20]. Sympathetic vasoconstriction of the capillaries and decrease the blood flow to the pulp. This could inhibit the differentiation of the pulpal stem cells. The effects of smoking clearly influence the healing process [20, 21, 22, 23]. Since tertiary dentin formation is a reparative process, smoking could also affect healing of the dentin in a similar manner.

Overall, it appears that there is a connection between smoking and tertiary dentin formation.

Clinically there could be further research done to aid in treatment. Since VEGF’s and iloprost were seen to increase blood flow and tertiary dentin formation [11, 12, 13, 25], clinicians could begin to use iloprost to aid in their treatment plan for patients. Iloprost could be used in addition to other pulp capping agents to help the progression of treatment. Calcium hydroxide based agents are normally used to help fight bacteria [27],
but the addition of iloprost could also lead to better healing due to the increase of blood supply and improved rate of tertiary dentin formation. In addition to iloprost, the different growth factor mechanisms could also be used in treatment plans. The amplification of Wnt’s [10], TGFβ-1, and BMP [8] could also lead to better repair of the pulp chamber.

In order to come to a definitive conclusion about the role of smoking and tertiary dentin formation additional research is necessary. Additional research is also important given the clinical implications that smoking has on wound healing and pulpal repair. The treatment of smokers could change if dentists can find a connection between smoking and the formation of tertiary dentin. It is also important to know this information so dentists can educate their patients on the health hazards caused by smoking, particularly in the oral cavity.
5 - References


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