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بسم الله الرحمن الرحيم

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Effect of Remineralizing Agents and Resin Infiltration on Resistance to Demineralization of Artificial Enamel Lesions

Thesis submitted to the Department of Operative Dentistry, Faculty of Dentistry, Ain Shams University in Partial Fulfillment of the Requirements for the master's degree in Operative Dentistry

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The development of white spot lesions (WSLs), as well as the durability and the ability of the chosen treatment to resist their progression, are considered a major problem worldwide.(1) WSLs are the first clinical sign of dental caries, known as hidden caries. They are susceptible to ongoing demineralization as a result of the continual imbalance between pathological and protective factors. These WSLs are considered reversible if early diagnosed and detected.(2) Consequently, early treatment including remineralization instead of restoring such WSLs is believed to be one of the important aspects of minimal intervention dentistry.(1)

Many materials aid in the remineralization and prevention of WSLs, such as those containing fluoride and calcium-based system, owing to the availability of calcium, phosphate and fluoride ions.(3,4) Nevertheless, the diffusion of these ions throughout the sub-surface lesion may be blocked. Thus, they may fail to reach the depth of the lesion, which in turn affects their protective action.(5)

On the other hand, resin infiltration is an adjunct therapeutic line. This system depends on filling the lesion pores and the formation of a diffusion barrier within the body of the lesion. Therefore, it could help in stopping carious lesion progression.(6) Meanwhile, the success of the resin infiltrant technique depends on their efficacy to penetrate within the depth of the lesion and the formation of a strong seal against acid attack.(6,7)

Furthermore, the long-term protection and stability of the different materials are usually related to their resistance to deterioration under the continuous changes caused by chemical and physical factors in the oral cavity. Thus, for their future evaluation and performance prediction, the conditions of the oral environment should be simulated, especially those concerning alterations in the pH.[8]

In addition, other attempts were made to evaluate the mineralization of the enamel surface. Enamel cross-sectional microhardness (CSH) affords an important indication regarding the mechanical in-depth resilience of the demineralized enamel. This might be indirectly related to the mineral content as it assesses the remineralizing and protective effect of different treatment modalities.[9] Accordingly, the study of the effect of resin infiltration, fluoride and CPP-ACP and their combined form on lessening the mineral loss and demineralization throughout the depth of the lesion upon exposure to the acidic medium will be valuable.

White spot lesions (WSLs) are clinically detectable, localized areas of enamel demineralization. They are the result of numerous attacks of demineralization and remineralization, rather than a unidirectional demineralizing process.[2] The main characteristics of these initial non-cavitated enamel lesions are the whitish opaque color with loss of the luster and numerous micro-porosities at the surface, which extend deeper into the enamel as a subsurface lesion.[10]

This whitish look owing to the refractive indices of the air or electrolytes contained in the porosities of the lesion compared with the adjacent sound enamel, resulting in light scattering. These features affect the patient's look and his esthetics appearance as well as the process of caries progression, even in the presence of the less acid-soluble surface zone.[10–12]

The surface zone remains relatively intact by the remineralizing effect of fluoride, phosphate, and calcium found in saliva. Meanwhile, subjacent to the surface zone is the body of the lesion. It is the most demineralized part of the lesion with a pore volume of 5% to 25%, which is demonstrated with polarizing microscopy as a dark brown color due to this loss of enamel.[13]

WSLs are often not self-limiting without proper care, so these lesions may be progressing, arrested or remineralizing depending on the state of demineralization and remineralization.[13,14] When the oral cavity is subjected to a low pH, the oral environment becomes under-saturated with mineral ions and the tooth undergoing demineralization.[2,4]

If the demineralization prolongs, excessive loss of minerals leads to the loss of the enamel structure and ends with cavitation. Meanwhile, in the event of pH rise, the remineralization via a natural repair process occurs, where calcium and phosphate ions are deposited into the crystal voids of the demineralized enamel. This results in a net mineral gain and the reverse take place.[2,4] So, when the demineralization is reduced or eliminated, white spot lesions may remineralize and not progress. This could be diagnosed by the size of the lesion, where the lesions not enlarging.[10,13]

Consequently, the main concern for the prevention of caries onset is understanding the role of remineralization in preventing caries progression and reestablishing a healthy balance when demineralization occurs. However, once the disease is present, we should determine the appropriate treatment to stop the consequences of the cariogenic process and resist the repeated acid attacks.[8,15,16]

In the oral cavity, the period of high and low pH alternating all over the day with a frequency that varies according to the cariogenic habit of the patient. It has been suggested that the acidic pH remains for some time after the neutralization of the acid in the plaque.[17] These alterations and the repeated acid attacks affect the progression of the WSLs as well as the anti-cariogenic effect of the different restorative materials.[8] Therefore to mimic these dynamic processes of mineral loss and gain, pH-cycling models were innovated. The origin of modern pH-cycling models was produced by **Ten Cate and Duijsters, (1982)**.[17]

PH-cycling models facilitate the production of sufficient quantitative data with high confidence to appropriately design the clinical trials, as verified by **Stookey et al.,(2011)**.[18] The pH-cycling models were designed to produce results that would parallel those generated in a human lesion formation model. Also, they allow the understanding of the anti-caries mechanism of different restorative materials.[19,20] **Sinfiteli et al., (2017)** conducted a study using the pH-cycling model to assess the effect of the different remineralizing agents in preventing enamel demineralization.[21]

Additionally**, Peng et al., (2016)** assess the resulting enamel microhardness and demineralization resistance after using fluoride varnish and resin infiltration, following the exposure to pH-cycling**.**[22] Simlarly, **Rodrigues et al., (2008)** used the pH-cycling model to verify the dose-response relationship of fluoride-releasing materials in their ability to reduce in vitro demineralization.[19] Moreover, **Vieira et al., (2005)** used different pH cycling models with different duration to evaluate the dose-response of fluoride.[23]

This model involves exposure of dental substrates to combinations of demineralization and remineralization. These combinations are involved in caries formation, which allows a high level of scientific control.[20] The exposure time in the demineralizing solution as well as the concentrations of ions may vary according to the focus of the study (de or remineralization). When the focus is more on remineralization, the samples stay less time in the demineralizing solution and this solution is prepared with a lower concentration of acid and ions and a higher pH.[5] Moreover, the time intervals of these cycles simulate the acidic changes that take place in the patient mouth, which depends on his cariogenic state. Each upward and downward of the pH would result in a rapid transformation of the formed reaction product.[17]

Nevertheless, pH-cycling models as all in vitro protocols have some limitations. Meanwhile, the success of these models would be achieved depending on the accurate consideration of the type of substrate, baseline artificial lesion as well as the statistical approaches.[5,20]

From another perspective, early intervention in the form of the non-invasive approach is one of the chosen treatment lines of WSLs. This approach relies on the ecological change in the oral environment, which in turn favors remineralization. [15] Meanwhile, the caries infiltration technique using a low-viscosity resin infiltrant is one of the micro-invasive approaches, which prevents enamel demineralization and restores the teeth' natural color. However, both approaches provided different results with positive outcomes, but clinical evidence is still limited and hinders obtaining definitive conclusions.[10,11,24]

Remineralization is a process of adding ions from an exterior source to the enamel surface to improve its hardness and wear resistance according to the concentration gradient of the ion. Calcium and phosphate ions are essential in the remineralization process because they are the natural constituents of the enamel hydroxyapatite.[25]

Consequently, saliva has a main role in the management of the caries process.[26] Saliva can act on the acids by neutralization or buffering.[27] Additionally, it has calcium and phosphate ions in a supersaturated state. Therefore, saliva could help small and superficial WSLs to regress by reestablishing pH levels with the actions of these ions. However, in most cases, the acid challenges may overcome this physiological remineralization process.[2,11,24] **Pulido et al., (2008)** documented the low inhibitory effect of artificial saliva on the progression of artificial enamel lesions. As it had the greatest increase in lesion depth after being exposed to pH cycling.[28] As a result, other therapeutic attempts are necessary to enhance remineralization and inhibit further demineralization.

The available evidence stated that fluoride is one of the effective caries preventive measures. Although most of the available evidence arises from fluoride-based products, other substances have also been proposed as remineralizing agents with an anti-cariogenic action, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[24,29]

Still, it is worth to note that the basic mechanism of remineralization involves the diffusion of calcium and phosphate ions from saliva and other topical sources aided by fluoride to build a hypermineralized, acid-resistant, fluorapatite like layer on the existing crystal remnants.[30] In the meantime, the ideal demand for a remineralizing agent is to diffuse and delivers calcium and phosphate throughout the depth of the lesion. It is also important to prevent delivering an excess of calcium as well as calculus formation and to be able to work at low pH. Additionally, it has to boost the remineralizing properties of saliva.[4]

Consequently, fluoride is considered the cornerstone of caries prevention. It is believed that fluoride decreases the progression, as well as the development of dental caries by 3 different mechanisms: including inhibition of the acidic bacterial production, inhibition of demineralization and promote enamel remineralization.[31] Besides, fluoride increases the driving force for apatite formation. It can be incorporated into the crystalline lattice of the tooth, which results in a less soluble fluorapatite ($Ca_{10}(PO_4)6F_2$) unit cell, that resists the acidic environment well.[2] Moreover, 10 calcium ions and six phosphate ions are essential for the formation of one unit cell of fluorapatite, thus the availability of calcium and phosphate ions affecting the occurrence of net enamel remineralization.[32]

Fluoride can be either self or professionally applied, fluoride varnish is one of the professionally applied forms. It acts as a physical barrier that