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RESEARCH ARTICLE

NONFLUORIDATED REMINERALIZING AGENTS

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Abstract

Early diagnosis of dental caries is of paramount importance. If we are able to intercept caries lesion at subsurface level with the help of remineralizing agents, we can actually achieve a caries free mouth.Remineralization was ruled by fluorides from the 1960s. Since then it has been successfully remineralizing carious lesions & has certain disadvantages like it is dependant on calcium and phosphate from saliva to accomplish this. Also overuse of fluorides can cause dental and skeletal fluorosis. This made researchers to explore nonfluoridated remineralizing agents.ACP compounds are considered prime candidates for remineralization therapy due to their high solubility under oral conditions and ability to rapidly hydrolyze to form apatite. Casein phospho-peptides are responsible for the high bioavailability of calcium from milk and other dairy products. CPP have the ability to bind and stabilize calcium and phosphate in solution, as well as to bind to dental plaque and tooth enamel. CPP stabilize ACP, localize ACP in dental plaque, thereby maintaining a state of supersaturation with respect to tooth enamel, reducing demineralization. TCP is a new hybrid material & when TCP comes into contact with the tooth surface and is moistened by saliva, the protective barrier breaks down making calcium, phosphate and fluoride ions available to the tooth. Xylitol, sugar substitute especially in chewing gums which increases salivary flow rate and enhances the protective properties of saliva. Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica which are all elements naturally found in the body. Ozone can shift microbial flora from acidogenic bacteria to normal commensals allowing remineralization to occur. Nanohydroxyapatite, a bioactive and biocompatible material, functions by directly filling up the micropores in early caries lesions.On the basis of the available data authors have given an insight into different nonfluoridated remineralizing agents in this article.

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Introduction:-

The human tooth is the highly mineralized tissues of the body containing hydroxyapatite as the primary constituent. According to Miller's Chemoparasitic theory caries is brought about by acid dissolution of mineral phase of teeth, the acid being produced by metabolism of dietary carbohydrates by oral bacteria. This theory emphasizes that there is an association between mineral content of teeth and dental caries.

Dental caries is a dynamic process which occurs when demineralization exceeds remineralization. But progression of dental caries is a slow process and during early stages non-invasive intervention can convert the lesion to inactive state from an active state .A drop in pH of oral cavity results in demineralization .The reversal can occur if pH rises resulting in deposition of caicium, phosphate and fluoride.

Demineralization starts at the enamel surface as it is the most mineralized tissue of the tooth. This subsurface demineralization increases porosity and changes the optical properties of the enamel. Thus early diagnosis of dental caries is of paramount important. If we are able to intercept caries lesion at subsurface level with the help of remineralizing agents, we can actually achieve a caries free mouth.

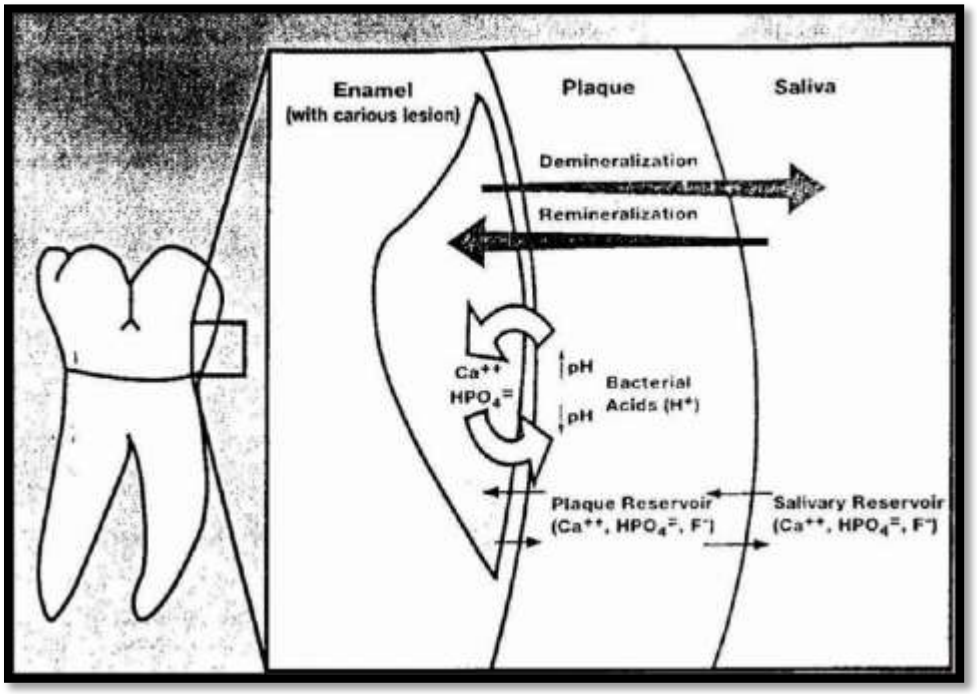

Fig 1:- Cycle of demineralization and remineralization in enamel.

Remineralizing agents are of two types, fluoridated and non fluoridated.Fluoridated remineralizing agents are being used since ages. Fluoride increases caries resistance when applied both topically and systematically. Fluoride level of 3 parts per million is needed to shift demineralization into remineralization Fluoride is known to promote remineralization but they have certain disadvantages like it is dependant on calcium and phosphate ions from saliva to accomplish this .Also improper use of fluoride can lead to adverse effects like fluorosis and fluoride is highly effective on smooth surface caries but not on pit and fissure caries.

Thus Non fluoridated remineralizing agents are gaining popularity in the field of remineralization because of the harmful effects of fluoridated agents.Non fluoridated remineralizing systems are not dependent on variable like quality of saliva and doesn't have a strict regime of topical application like that of fluoridated remineralizing agents.. Non fluoridated remineralizing agents are highly effective on both pit and fissure and smooth surface areas. Moreover these systems are based on improving efficacy of fluoride and reducing the concern regarding fluoride toxicity. With newer nonfluoridated techniques, we can re establish the health of oral tissues without adverse effects of fluorides.

Ideal Requirements Of Non Fluoridated Remineralizing Agent

- It should deliver calcium and phosphate into the subsurface.
- It should not deliver any excess of calcium.
- It should not favor calculus formation.
- It should work at an acidic pH so as to stop demineralization during a carious attack.
- It should work in xerostomic patients also, as saliva cannot effectively stop the carious process.
- It should be able to boost the remineralizing properties of saliva.
- The novel materials should be able to show some benefits over fluoride.[1]

Classification

Non-fluoride-based remineralizing agents can be classified as either calcium-based or non-calcium-based compounds or compositions. The non-calcium-based technologies focus primarily on pH modification through micro environmental buffering effects within the saliva, surface biofilm, and/or enamel pellicle.[2]

Nonfluoridated remineralizing agents are classified into:

I. Calcium based

a) Amorphous Calcium Phosphate (ACP)
b) Casein Phosphopeptides-Amorphous Calcium Phosphate
c) Tricalcium Phosphate (TCP)
d) Cavistat
e) Bioactive Glass

II. Non calcium based

a) Nano Hydroxy apatite
b) Sugar substitutes (xylitol)
c) Ozone

Indication

1. An adjunct to preventive therapy for reducing caries in high-risk patients
2. Reduce dental erosion in patients with gastric reflux or other disorders
3. To reduce decalcification in orthodontic patients
4. To repair enamel in cases involving whitespot lesions
5. For fluorosis, before and after teeth whitening and to desensitize sensitive teeth[1]

Contraindication

1. Teeth with cavitated lesions.
2. Teeth with pulpal involvement

AMORPHOUS CALCIUM PHOSPHATE (ACP)

Amorphous calcium phosphate (ACP) is a noncrystalline form of calcium phosphate which remineralizes the tooth structures and aid in the prevention of tooth decay. Amorphous Calcium Phosphate compounds (ACPs) are considered prime candidates for remineralization therapy due to their high solubility under oral conditions and ability to rapidly hydrolyze to form apatite.

Mechanism of action

Amorphous calcium phosphates (ACP) is the reacted complex of calcium and phosphate ions that precipitate and can grow on the tooth surfaces in the oral environment. These initial Amorphous calcium phosphates complexes are thermodynamically unstable under the conditions normally prevalent in the oral environment ; pH , temperature etc.

Transformation mechanism of ACP to apatite at physiological pH has been described as followings: firstly ACP dissolution happens and then a transient Octacalcium phosphate solid phase reprecipitation happens through nucleation growth, and finally hydrolysis of the transient Octacalcium phosphate phase into the thermodynamically more stable apatite by a topotactic reaction occurs.

Advantages

1. Application can be done both professionally and at home.
2. Can be used by patients of all age because of its ease of application.

3. Cosmetic improvements in dimpled, abraded or etched tooth enamel.[3]
4. ACP is Biocompatible and no side effects have been reported.

Disadvantages
1. Amorphous calcium phosphate is an unstable compound.
2. ACP has poor mechanical properties due to its unstable nature.

CASEIN PHOSPHOPEPTIDES-AMORPHOUS CALCIUM PHOSPHATE
Casein, a bovine milk phospho-protein is known to interact with calcium and phosphate and is a natural food component. Its technical name is casein phospho-peptides-amorphous calcium phosphate or CPP-ACP. Other than fluoride, CPP-ACP is the most extensively researched remineralization technology.

Casein phosphor-peptides (CPP) are naturally present in cows milk and is responsible for the high bioavailability of calcium from milk and other dairy products.. Calcium phosphate is normally insoluble that is, it forms a crystalline structure at neutral pH. However, in the presence of CPP calcium and phosphate remain in an amorphous, no crystalline state. In this amorphous state, calcium and phosphate ions can enter the tooth enamel. Moreover CPP have the ability to bind and stabilize calcium and phosphate in solution, as well as to bind to dental plaque and tooth enamel . Thus CPP keeps calcium and phosphate in close proximity to tooth surface and plaque for a longer duration of time. The high-concentration of calcium and phosphate ions in dental plaque have been extensively researched and proven to reduce the risk of enamel demineralization and promote remineralization of tooth enamel. CPP stabilize ACP, localize ACP in dental plaque, thereby maintaining a state of supersaturation with respect to tooth enamel, reducing demineralization and enhancing remineralization. The CPPs have been shown to keep fluoride ions in solution too, thereby enhancing the efficacy of the fluoride as a remineralizing agent.[4]

Advantages
1. CPP-ACP can be used both professionally and personally due to its ease of application.
2. Can be used in patients of all age group.
3. CPP-ACP has a pleasant taste and comes in different flavours.

Disadvantages
1. CPP-ACP is costly to be used regularly.
2. CPP-ACP products are hard to find in all stores because of its restricted availability

TRI CALCIUM PHOSPATE (TCP)
Another calcium phosphate technology is Tri-calcium phosphate (TCP) which provides bioavailable calcium and phosphate ions to the teeth generating significant remineralization even when used in small amounts.

Mechanism Of Action
Tricalcium phosphate is milled with simple organic materials to create a functionalized tricalcium calcium phosphate ingredient. This process ensures that prior to use, the active calcium sites are protected from premature interactions with fluoride, which could otherwise render both calcium and fluoride inactive.Tricalcium phosphate,when comes in contact with tooth surface and moistened by saliva, the protective barrier breaks making available calcium, phosphate and fluoride.(fig 2)

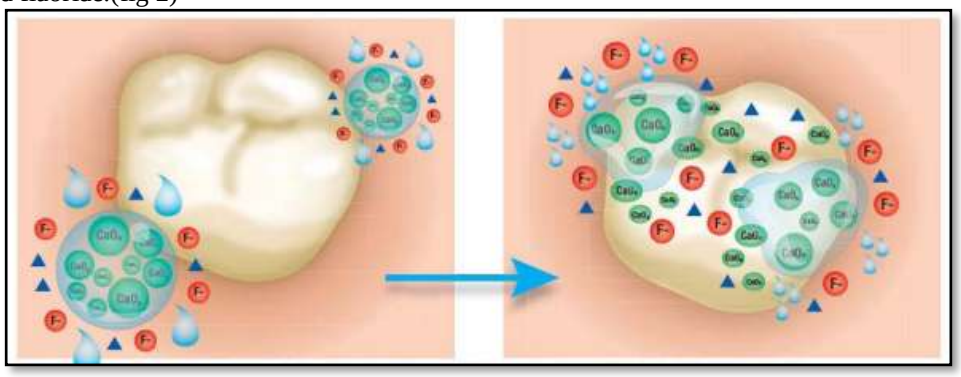

Fig 2:- Saliva activates the Ca, braking down the protective layer.

Since the structure of tricalcium calcium phosphate is similar to hydroxyapatite, once the functionalized calcium ions are released, they readily interact with the tooth surface and subsurface. While other calcium phosphate additives may require an acidic pH, which could limit the benefits to the tooth, functionalized Tricalcium calcium phosphate can offer optimal benefits when delivered in a neutral pH environment. Thus it can be safely recommended that Tricalcium phosphate in conjuction with fluoride increases the efficacy and decreases the required concentration of fluoride needed.

Advantage

1. It is biocompatible and bioactive in the oral cavity.
2. Only low levels of functionalized tricalcium phosphate are needed to produce strong, acid-resistant mineral nucleation.
3. Additionally, tri-calcium phosphate can be custom-tailored for a variety of oral care products like tooth paste , varnishes etc.

Disadvantage

Production of TCP and incorporation of it in toothpaste is costly.

XYLITOL

Another caries preventive method is the consumption of food with sweetening content; Xylitol(fig:3) is the most frequently used sugar variable in this area. As regards oral and dental health, it is essential to use this sugar alcohol which minimizes dental caries risk in candies, beverages, and gums to replace sucrose. Xylitol which cannot be fermented by S. mutans shows antiacidogenic and anticariogenic features. Another role of xylitol in oral health is that it reduces the amount of plaque accumulation in the mouth.[5]

Fig 3:- Xylitol gum.

Xylitol works in three major ways.

1. Xylitol acts on the mitochondria of streptococcus mutans and inhibit the process of glycolysis of these microrganisms, thus interfering their growth and metabolism.
2. Xylitol works by making the pH alkaline. When S. mutans bacteria ferment sugar, they produce acids, which lower the pH level below 7 and slowly weakens the protective tooth enamel by increasing the dissolution of calcium and phosphate ions. When xylitol is ingested, the acid attack is reduced because the caries producing bacteria are unable to ferment xylitol. As a result the growth of these bacteria and simultaneous production of acid is reduced and oral pH stays higher.
3. At the high pH, the hydrophilic molecule of xylitol is able to form complexes with calcium in solution, thus stabilizing the calcium and phosphates found in saliva thus promoting remineralization.[6]

Advantages
1. Decrease in caries rates, increment, and/or onset
2. Maternal consumption of xylitol reduce the acquisition of mutans streptococci (MS) and dental caries by their children."
3. Have pleasant taste and a sweetness which equals that of sucrose.
4. It does not effect blood pressure or blood glucose levels as most sugar substitutes.

Disadvantages
At higher dose, xylitol use is associated with symptoms like gas and osmotic diarrhoea because of slow rate of absorption of xylitol through the gut wall.

BIOACTIVE GLASS MATERIALS
Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica , which are all elements naturally found in the body. Bioactive glass materials have been used in medicine and dentistry for years. This unique material has numerous novel features, including the ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits, while also affecting cell signals in a way that benefits the restoration of tissue structure and function. Bioactive glass is considered a break-through advance in remineralization technology.

Mechanism Of Action
When bioactive glass comes in contact with saliva, there is a rapid release of sodium, calcium, and phosphorous ions into the saliva that are available for remineralization of the tooth surface. The released ions form hydroxycarbonate apatite directly. They also attach to the tooth surface and continue to release ions and remineralize the tooth surface after the initial application. These particles have been shown to release ions and transform into HCA for up to 2 weeks.

Bioactive glass formulation commonly used in research studies contains 45 wt% SiO_2 4.5 wt% Na_2O and CaO and 6 wt% P_2O_5.[7] The network breakdown of silica depends upon the concentration of SiO_2 and is time dependent. Thus, keeping the silica below 60 wt% and maintaining a high CaO/ P_2O_5 ratio guarantees a highly reactive surface.

Novamin®, a trade name for bioactive glass, is manufactured by Novamin Technologies Inc.. It has been demonstrated that, fine particulate bioactive glasses incorporated into an aqueous dentifrice has the ability to clinically reduce the tooth hypersensitivity through the occlusion of dentinal tubules by the formation of the CAP layer.[7]

Advantages
1. It strengthens and revitalizes your teeth with ionic calcium and phosphates.
2. Biosilicates are Biocompatible
3. They are acceptable in taste and available in different flavours.
4. Easy and safe to use as regular toothpaste.

Disadvantages
1. It is not cost effective on regular basis.
2. Its availability is restricted.

OZONE
Ozone is a naturally occurring compound consisting of three oxygen atoms. It is found in nature, in the form of a gas in the stratosphere in a concentration of 1–10 ppm. The reliable microbiologic and metabolic properties of ozone, in either the gaseous or aqueous phases, make it a useful disinfectant with a wide range of activity.

Ozone, in the gaseous or aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses.

Mechanism of action
Ozone has proven to be effective against bacteria as well as viruses and fungi. It is a strong oxidant, highly bactericidal. It disrupts the integrity of the bacterial cell envelope by oxidation of phospholipids and lipoproteins and

thus is used to treat primary root caries or occlusal caries as well as dentinal hypersensitivity.Ozone at low concentration of 0.1 ppm, is sufficient to inactivate bacterial cells including their spores.

Ozone is also capable of remineralizing lesions within the dentin. It opens dentinal tubules within carious lesion and, thus enhances remineralization by increasing perfusion of remineralizing agents.

It has been reported that an exposure of about 60 s exhibited 99.9% killing efficiency against cariogenic bacteria such as Actinomyces naeslundii, Streptococcus mutans and Lactobacillus casei.[8]

Advantages

1. It is effective in fighting decay-causing bacteria.
2. It supports gum health and healing. Ozone has been used very successfully to treat periodontal disease.
3. It's noninvasive.It can be used in plaque control, help decrease sensitive tooth necks, and assist in cavity preventions without any invasive tools or discomfort.
4. It's all-natural. Dentists are able to remove harmful bacteria without resorting to the use of other products that can have toxic and unhealthy side-effects.
5. There is no injection involved in ozone treatment and the ozone delivery system is portable. Therefore, elderly patients who have limited access to the dental services can highly benefit from this treatment.[9]

Disadvantages

People should never inhale ozone. If it enters the mouth, nose, or eyes, it can burn and cause coughing, nausea, vomiting, or headaches. More severe exposure can lead to respiratory complications.

CAVISTAT

An arginine bicarbonate and calcium carbonate complex has been capable of preventing the development of dental caries and it prevents caries upto 50%. It is one of the most prevalent and economical method of caries reduction.

Mechanism Of Action

This technology is based on the mechanism that when the toothpaste is used, the amino acids will be deaminated by the arginine deaminase system in saliva, producing ammonia, which is highly alkaline and causes a rise in pH within the oral environment, thus presenting an ideal condition for remineralization as well as modifying and reducing the pathogenicity of the cariogenic plaque .

CaviStat is also comprised of bicarbonate, the main buffer in saliva for neutralizing plaque acid and calcium carbonate serves as the calcium source for remineralization to occur.

Advantages

1. It Can be used in patients of all age group.
2. It is cost effective.
3. Patients can apply it easily.

Disadvantages

1. Arginine has minor side effects which includes abdominal pain, bloating, diarrhea and gout
2. It can cause worsening of breathing in people.

NANOHYDROXYAPATITE

Hydroxyapatite is the main component of enamel, which gives an appearance of bright white and eliminates the diffuse reflectivity of light by closing the small pores of the enamel surface.[10]

Hydroxyapatite is the main constituent of the mineral part of bone and teeth. Hydroxyapatite is also an important source of calcium and phosphate, very important for the remineralization of demineralized enamel areas.

The recently developed interest for nanotechnology in many fields, is producing interesting and imminent applications in dentistry for nano-hydroxyapatite, which presents crystals ranging in size between 50 and 1000 nm.

Mechanism of action

Nano-hydroxyapatite has a size range between 50 and 1000 nm. The nano-hydroxyapatite has a strong ability to bond with proteins, as well as with fragments of plaque and bacteria, when contained in toothpastes. This ability is due to the size of nanoparticles, which considerably increase the surface area to which proteins can bind. Besides, nano-hydroxyapatite also acts as filler because it repairs small holes and depressions on enamel surface, a function enhanced by the small size of the particles that compose it.

The biomimetic hydroxyapatite function is to protect the teeth with the creation of a new layer of synthetic enamel around the tooth, rather than hardening the existing layer with fluorine. A concentration of 10% nano-hydroxyapatite is considered to be optimal for remineralization of early enamel caries.

Advantages

1. Nano hydroxyapatite is nearly alike in the chemical composition of human teeth and is safe & biocompatible.
2. It can be used by patients of all age because of its ease of application.

Disadvantages

Nanohydroxyapatite containing toothpaste are expensive and cannot be used on daily basis.

Conclusion:-

In the last few decades, advances in technologies, changes in lifestyle, modifications in the diet, and longer life expectancy are some of the many factors which have affected the health and esthetics of tooth enamel and dentin. With a clearer understanding of the implementation of these remineralizing agents and new technologies accessible to dentists, we can create a more favorable relationship in which remineralization occurs more often than demineralization.

Historically remineralization was ruled by fluorides from the year 1960s. Since then it has been successfully remineralizing incipient carious lesions. It has certain disadvantages like it is dependant on calcium and phosphate ions from saliva to accomplish this. Also overuse of fluorides can cause dental and skeletal fluorosis. This made researchers to explore and study non fluoridated remineralizing agents.

The ACP technology was developed by Dr. Ming S. Tung. In 1999, ACP was incorporated into toothpaste called Enamelon and later reintroduced in 2004 in Enamel Care toothpaste by Church and Dwight. ACP compounds are considered prime candidates for remineralization therapy due to their high solubility under oral conditions and ability to rapidly hydrolyze to form apatite.

CPP–ACP is the acronym for a complex of casein phosphopeptides and amorphous calcium phosphate. Casein phospho-peptides are responsible for the high bioavailability of calcium from milk and other dairy products. CPP have the ability to bind and stabilize calcium and phosphate in solution, as well as to bind to dental plaque and tooth enamel. CPP stabilize ACP, localize ACP in dental plaque, thereby maintaining a state of supersaturation with respect to tooth enamel, reducing demineralization and enhancing remineralization.

Tricalcium phosphate is a new hybrid material created with a milling technique that fuses beta TCP and sodium lauryl sulfate or fumaric acid. When TCP comes into contact with the tooth surface and is moistened by saliva, the protective barrier breaks down making calcium, phosphate and fluoride ions available to the tooth. TCP has also been considered as one possible means for enhancing the levels of calcium in plaque and saliva.

Xylitol is a commonly used sugar substitute especially in chewing gums. A non-fermentable sugar alcohol act as a carrier for calcium and phosphates.
The use of chewing gum carrying xylitol increases salivary flow rate and enhances the protective properties of saliva. This is because the concentration of bicarbonate and phosphate is higher in stimulated saliva, and the resultant increase in plaque pH and salivary buffering capacity prevents demineralization of tooth structure. Moreover, the higher concentration of calcium, phosphate, and hydroxyl ions in such saliva also enhances remineralization

Bioactive glass is made of synthetic mineral containing sodium, calcium, phosphorous and silica, which are all elements naturally found in the body. When in contact with saliva or water, first releases sodium ions. This elevates

the pH into the range essential for HAP formation. This increase in ionic concentration, combined with an increase in pH, causes the ions to precipitate onto the tooth surface and form calcium hydroxycarbonate apatite (HCA) to remineralize the defect and to occlude open tubules.

Ozone is a chemical compound which is a powerful oxidizing agent. Ozone acts by attacking thiol groups of cysteine amino acid and destroys the cellular membrane of carious bacteria. Ozone can shift microbial flora from acidogenic bacteria to normal commensals allowing remineralization to occur.

An arginine bicarbonate and calcium carbonate complex has been capable of preventing the development of dental caries and it prevents caries upto 50%. It is one of the most prevalent and economical method of caries reduction.

Nanohydroxyapatite, a bioactive and biocompatible material, functions by directly filling up the micropores in early caries lesions, where it act as a template in the remineralization process by continuously attracting large amount of calcium and phosphate ions from the oral fluids into the lesion, thus promoting crystal growth. A concentration of 10% nano-HA may be optimal for remineralization of early enamel caries.

References:-

1. Naveena P, Nagarathana C, Sakunthala BK. Remineralizing agent—Then and now—An update. Dentistry. 2014;4(9):1-5.
2. Jefferies SR. Advances in remineralization for early carious lesions: a comprehensive review. Compend Contin Educ Dent. 2014 Apr 1;35(4):237-43.
3. Walsh LJ. Contemporary technologies for remineralization therapies: A review. Int Dent SA. 2009 Jan;11(6):6-16.
4. Kalra DD, Kalra RD, Kini PV, Prabhu CA. Nonfluoride remineralization: An evidence-based review of contemporary technologies. Journal of Dental and Allied Sciences. 2014 Jan 1;3(1):24.
5. Aras A, Celenk S, Dogan MS, Bardakci E. Comparative evaluation of combined remineralization agents on demineralized tooth surface. Nigerian Journal of Clinical Practice. 2019 Nov 1;22(11):1546-52.
6. Mäkinen KK. Xylitol-associated remineralization of caries lesions. Oralprophylaxe & Kinderzahnheilkunde. 2009;2:66-71.
7. Nagarathana C, Sakunthala BK, Naveena Preethi P. An update on current remineralizing agent. OHDM. 2015;14(4):183-7.
8. Tiwari S, Avinash A, Katiyar S, Iyer AA, Jain S. Dental applications of ozone therapy: A review of literature. The Saudi Journal for Dental Research. 2017 Jan 1;8(1-2):105-11.
9. Baysan A, Lynch E. Management of root caries using ozone. InKaVo Dental Ltd website. URL: http://www. kavo. com/forum/upfiles/4/Db84539. pdf. Accessed April 2004 (Vol. 37).
10. Jeong SH, Jang SO, Kim KN, Kwon HK, Park YD, Kim BI. Remineralization potential of new toothpaste containing nano-hydroxyapatite. InKey Engineering Materials 2006 (Vol. 309, pp. 537-540). Trans Tech Publications Ltd.