Nano-hydroxyapatite for Dental Enamel Remineralisation

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A number of 24 enamel specimens were prepared from bovine teeth and demineralized with H3PO4 gel - 37% for 100 s. Before and after demineralization, specimen surfaces were examined using SEM and then stored by immersion in NaCl 0.9% solution at 37 degrees C. The 24 enamel specimens were randomly assigned to 4 groups (n=6) and brushed twice a day for 2 min using a hand piece (Nsk Ti-max Z25L) and curve hand piece bowl dental brushes on half of their surface. The specimens were brushed with: group 1: HA hydrogel synthesized at 40 degrees C; group 2: nano-HA + nano-calcium-silicate (galvanometrical proportion 9:1); group 3: nano-HA + nano-calcium-silicate (g.p. 8:2); group 4: nano-HA + nano-calcium-silicate + calcium-carbonate (g.p. 8:1:1). After a period of 2 weeks, specimens were analyzed using SEM. A significant mineral deposition was observed at the top of all exposed enamel prisms thanks to the nano-HA remineralization capacity.

Keywords: nano-hydroxyapatite, remineralisation, dental enamel, SEM

Hydroxyapatite (HA), is a natural occurring mineral form of calcium apatite with the formula Ca10(PO4)6(OH)2, but is usually written Ca5(PO4)3(OH), to denote that the crystal unit cell comprises two entities. It crystalizes in the hexagonal crystal system. Up to 70% of the bone weight is a modified form of hydroxyapatite. 96% percent of dental enamel and 67% of dentine consists of mineral, the majority being HA. It’s primary mineral: HA yield enamel’s strength and brittleness.

Although dental enamel is the hardest tissue in the human body, its high mineral content makes it susceptible to the demineralization process, which often occurs in the mouth. Some foods such as: candies, soft drinks, citric juices, and others, may lead to the enamel destruction when interacting with intraoral bacteria. This process has lactic acid as result, which decreases the oral pH to critical value and the HA demineralization begins.

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Ca_{10}(PO_4)_{6}(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O
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Enamel crystals are one of the most amazing structures in the nature featuring extremely long and parallel-organized hydroxyapatite crystals organized in bundles, which are called prisms. [1] The basic unit of enamel is called an enamel rod. Measuring 4-8 μm in diameter, an enamel rod, formally called an enamel prism, is a tightly packed mass of hydroxyapatite crystals in an organized pattern. In cross section, it is best compared to a keyhole, with the top/ head, oriented toward the crown of the tooth, and the bottom/tail oriented toward the root of the tooth. [2] The enamel consists of a huge number of rods; for example: 5 million in mandibular central incisor and 12 million in maxillary molars. Enamel rods are commonly accepted as perpendicularly oriented to the tooth surface and this concept has influenced the design of cavity preparations [3].

Due to its properties, HA is one of the synthetic materials that have been developed over the past 30 years to replace the damaged human bone, and most recently it is used also in dentistry. It is considered one of the most biocompatible and bioactive materials and has gained wide acceptance in medicine and dentistry in recent years. [4]

The aim of this study was to evaluate the remineralization capacity of 4 different products containing nano-HA on the invitro demineralized dental enamel.

Experimental part

Materials and methods

A number of 24 (n=24) enamel specimens were prepared from bovine teeth and demineralized with H3PO4 gel - 37% for 100 seconds. Before and after demineralization, specimen surfaces were examined using SEM and then stored by immersion in NaCl 0.9% solution at 37 degrees C. The 24 enamel specimens were randomly assigned to 4 groups (n=6) and brushed twice daily for 2 min using a hand piece (Nsk Ti-max Z25L) and curve hand piece bowl dental brushes on half of their surface. The specimen were brushed with: group 1: HA hydrogel synthesized at 40 degrees C; group 2: nano-HA + nano-calcium-silicate (galvanometrical proportion 9:1); group 3: nano-HA + nano-calcium-silicate (g.p. 8:2); group 4: nano-HA + nano-calcium-silicate + calcium-carbonate (g.p. 8:1:1).

The HA hydrogel product used in this study for the damaged enamel surface remineralisation (for nanofissures sealing) has been obtained through the sintering reaction, at 40 degrees and uniform dispersed in a aqueous carboxymethylcellulose gel. We used the reaction between H3PO4 and Ca(OH), both in aqueous phase, at constant pH of 7,5 units, through a continuous process perfected in a microreactor assembly equipped with static mixers. The reagents were analytical pure: phosphoric acid from MERCK (Germany); calcium hydroxide from AppliChem (Germany); carboxymethylcellulose for alimentary use Wadlocel C/Dowwolff Celusosics (Germany), and bidistilled water.

In the same way we obtained the nano-calcium-silicate, and nano-calcium-carbonate used for the group 2, 3 and 4.

All the laboratory equipment required for the procedure was offered by SETICO SRL (www.setico.ro).

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The brushing procedure took place twice a day, during two weeks. After this period, the specimens were observed using scanning electron microscopy (SEM).

Results and discussions

SEM images showed a characteristic keyhole structure after demineralization, with the rod core extensively demineralized while the interrod remained intact [5]. After two weeks of brushing, a significant mineral deposition was observed at the top of all exposed enamel prisms thanks to the nano-HA remineralization capacity. We observed that the nano-HA hydrogel is very effective to remineralize in vitro superficial lesions, artificial induced by demineralization with H3PO4 gel - 37% for 100 seconds; but is also effective in enamel micro-fissure sealing and deep demineralization (fig. 1- a, b, c, d).

Adding nano-calcium-silicate and calcium-carbonate to this composition, seems to increase the remineralization property of HA, by giving rise to different crystalline minerals that form a thin layer at the enamel surface (fig. 2).

Conclusions

Remineralization of enamel represents a useful tool to counteract the loss of mineral tissue due to bacteria metabolism foods and drinks ingestion. The use of nanotechnology as well as the crystallization of hydroxyapatite starting from amorphous compound are effective strategies to gain remineralization even if they fail to reconstruct the histological and crystallographic integrity of enamel tissue [6].

A comparative analysis of the 4 groups using SEM images, led to the conclusion that the combination of: nano-HA + nano-calcium-silicate + calcium-carbonate has the most effective result in the damaged enamel surface remineralization.

The subject was also studied in [7, 8].

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