Recent Advancements in Preventing Teeth Health Hazard: The Daily Use of Hydroxyapatite Instead of Fluoride

Norberto Roveri*, Elisabetta Foresti, Marco Lelli and Isidoro G. Lesci

Dipartimento di Chimica “G. Ciamician”, Alma Mater Studiorum Università di Bologna, Via Selmi 2, 40126 Bologna, Italy

Received: May 15, 2009; Accepted: July 7, 2009; Revised: July 16, 2009

Abstract: Hydroxyapatite (HA) is commonly considered the most promising synthetic biomaterial for biomedical applications in orthopaedic, dental and maxillofacial surgery for its biocompatibility, bioresorption and bioactivity. Only recently a chemical-physical experimental approach has been utilized to investigate the capability of synthetic carbonated hydroxyapatite nanocrystals (CHA) to produce in vivo biomimetic mineral deposition on enamel and dentine surface through a daily use. Demineralised enamel and dentine slabs have been treated in vitro with synthetic biomimetic hydroxyapatite nanocrystals for a few minutes. This induced a surface remineralisation, forming a biomimetic apatite coating on enamel and dentine surface. In fact, enamel remineralisation quickly occurs thanks to the specific chemical-physical characteristics of innovative nanostructured hydroxyapatite particles which closely resemble mineral enamel constituents. Therefore the experimental results suggest the possibility to perform teeth wear-deterioration prevention.

Carbonated hydroxyapatite nanocrystals synthesized with tailored biomimetic characteristics for composition, structure, size and morphology can chemically bind themselves on the surfaces of teeth hard tissues, filling the scratches, producing a bound biomimetic apatitic coating, protecting the enamel surface structure. Over the past few decades many products for dental damage prevention (toothpastes, rinses and gels) have been commercialized and patented expressly for the fluoride remineralisation effect. Only recently the numerous applications of hydroxyapatite as bone filler biomaterial for implantation surgery have evidenced innovative and important opportunities for decay prevention through a daily use of oral care products containing hydroxyapatite.

The aim of this paper is to review these recent patents and classify them according to their actual possibilities to safeguard teeth health.

Keywords: Hydroxyapatite, biomimetism, nanocrystals, enamel, dentine, teeth remineralization, fluoride.

INTRODUCTION

Teeth

Enamel is the hardest material in vertebrates and is the most highly mineralized skeletal tissue present in the body [1]. Mature enamel is composed of CHA (95-97% wt) and less than 1% wt of organic material. Unlike other biomineralized tissues, such as bone and dentine, mature enamel has no cells and therefore cannot be biologically remodelled. Consequently, enamel regeneration cannot take place in vivo. The mammalian tooth is made up of four distinct structures: enamel, dentine, pulp and cementum. Fig. (1) [2].

Enamel makes up the uppermost 1-2mm of the tooth crown and contains a high mineral content, giving it a high modulus, but also making it susceptible to cracking. Dentine lies below the enamel and is tougher, forming the bulk of the tooth and absorbing stresses from enamel, preventing its fracture [3]. The cementum is the mineralized layer that surrounds the root of the tooth covering the dentine layer and anchoring the tooth to the alveolar bone (jawbone) through the periodontal ligament. The primary function of the teeth is for mastication of food and it is very important to maintain them healthy keeping their mechanical properties long life lasting in a bacteria-filled environment.

Enamel prismatic HA crystals consist of a weaving of prisms ranging from 3 to 5μm in diameter. A single prism reveals a highly organized array of fastened needle like HA crystallites (approximately 30nm thick, 60nm wide, and several millimetres long) Fig. (2A). They are preferentially aligned along the HA crystallographic c-axis organized in interweaving bundles of aligned crystallites that are “woven” into intricate architectures approximately 3-5μm in diameter Fig. (2B) [4].

The composition and morphology of dentine resembles that of bone and is characterized by exhibition of numerous tubules containing nervous tails Fig. (3).

Enamel and dentine are tough, crack-tolerant and abrasion-resistant tissues for their unique architectures and mineral compositions [5].

Teeth Health Care

Dental erosion is the chemical wear of the dental hard tissue without the involvement of bacteria [6]. Its clinical relevance is becoming wider and wider [7-11], and it is...
Recent Patents on Biomedical Engineering, 2009, Vol. 2, No. 3

Considered one of the main tooth pathologies able to cause patient discomfort, after periodontal diseases and caries.

Erosion aetiology appears related to the enormous increase in consumption of soft drinks, fruit juices and sport drinks consumption [12]. However, other acid sources, such as drugs containing syrups, analgesics and vitamin C intake and environmental acid exposure in working condition are claimed to be related to enamel erosion development [13-17].

The mechanisms involved in the damage of dental hard tissue are related to the acid attacks on the outer few micrometers of the enamel, with the consequent demineralization and dissolution of the minerals [18-24].

Dentine hypersensitivity, defined from Holland et al. [25] as “the short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of defect, pathology or disease” [26], has been explained in term of hydro-dynamic mechanism [27]. Essentially, if dentine is exposed and the dentine tubules are open, the fluid movement provokes stimulation [28]. Even if much remains unknown or unproven about the aetiology of dentine hypersensitivity, according to many authors it can be considered a tooth wear phenomenon [29]. The smear layer, which is an artificial surface about one micron thick, consisting of collagen and hydroxyapatite from the native dentine, is formed when dentine is abraded [30]. It covers the

---

**Fig. (1).** Tooth anatomic draft: enamel (E), dentine (D), pulp (P) and cementum (C) Reproduced by permission from Ref. [2]).

**Fig. (2).** Scanning Electron Microscopy enamel images of highly organized array of fastened needle like HA crystallites (A) Reproduced by permission from Ref. [76]) organized in interweaving bundles into intricate architectures (B) (Reproduced by permission from Ref. [4]).
underlying dentine occluding the tubules, but can be removed by attrition, acid erosion and tooth brushing with toothpaste [31, 32]. To prevent dental erosion progression, the reduction or elimination of exposure to acidic soft drinks and juices can be recommended to patients. Frequent application of a high concentration of topical fluoride may be of some benefit in preventing further demineralization and increasing the abrasion resistance of erosion lesions [33].

The demineralised area and micrometric sized scratches normally occur on enamel surface as a consequence of micro wear and acid attack [34] and cannot be repaired biologically, nor prosthetically. In fact, unlike in bone, when apatitic crystals are dissolved or abraded, they cannot be spontaneously re-deposited in enamel and dentine because enamel contains no cells able to secrete extra-cellular matrix. The regeneration and dentine apposition occurs only through the pulp tissue. Therefore, both enamel and dentine can be reconstructed only by the application of alloplastic materials which provide a sort of prosthetic restoration.

Remineralization by Fluoride

In vitro, fluoride (0.02-0.10mg/L) addition to a supersaturated solution of calcium phosphate induces the crystallization of hydroxyapatite, $\text{Ca}_{10}\left(\text{PO}_4\right)_{6}\text{(OH)}_2$ which is the mineral phase of bone and teeth. Increasing fluoride concentration fluoroapatite $\text{Ca}_{10}\left(\text{PO}_4\right)_6\text{F}_2$ is formed and appears in more ordered and bigger apatite crystals which are less acid soluble [44-46]. In the body, in vivo fluoride is mainly associated with calcified tissue, bone and teeth due to its high affinity to calcium. Fluoride modifies the bone mineral phase by replacing the hydroxyl groups in hydroxyapatite phase producing its partial conversion into fluoroapatite. The more electrostatic stability and crystallinity of fluoride substituted hydroxyapatite increases the bone density and hardness reducing the mechanical strength [47,48]. Fluoride in high doses was found to be mutagenic in osteoblasts and inhibitory of osteoclasts [47,49-51].

![Fig. (3). Scanning Electron Microscopy image dentine showing tubules in a bone like morphology.](image)
In spite of the observed negative physicochemical effects on the bone, fluoride exhibits both a cariostatic effect on children and adults erupted teeth and a pre-eruptive effect through increasing fluoridation of the developing enamel [52,53]. Fluoridated enamel is less acid soluble [54]. The reduction of caries in both deciduous and permanent teeth was more marked where the children were earlier exposed to fluoridated tap water [52,55]. The fluoride cariostatic effect on erupted teeth can be ascribed to an inhibition of the demineralization of sound enamel due to ingested acid foods and drinks and cariogenic bacteria in the dental plaque. Sound enamel results more acid resistant containing more fluoride [56,57]. However, caries is not a fluoride deficiency disease and no specific fluoride deficiency syndrome has been found.

Adsorbed fluoride is rapidly distributed by circulation to the intracellular and extracellular fluid is retained prevalently in calcified tissues and the fluoride plasma concentration is dependent on the ingested fluoride dose. Fluoride concentrations in oral environment and glandular saliva closely follow the plasma concentration, but at a lower concentration than two-thirds of the plasma level [58]. Children with no caries experience were found to have higher salivary fluoride concentrations than children highly affected by caries [59]. Contrary to skeletal bone and dentine which accumulate fluoride in proportion to the fluoride adsorbed during life, enamel reflects the biologically available fluoride during teeth formation. Enamel maturation of deciduous teeth is completed between the age of 2 to 12 months while in permanent teeth enamel maturation is completed at the age of 7-8 years except for the third molars whose maturation continues until the age of 12-16 years. Post-eruptive fluoride uptake of enamel is expressed only in the outer layer and depends on fluoride in saliva, food, drinks, dental plaque and prevalently in dental products. In order to prevent enamel demineralization by acid food and drinks and cariogenic bacteria in the dental plaque, many dental products as toothpaste, rinses and gels containing fluoride have been recommended by medical societies in many countries for caries prevention. On the contrary several studies conducted in fluoridated and non fluoridated communities have suggested that water fluoridation may be unnecessary for caries prevention, especially in the industrialized countries where the caries presence has become low [60]. The use of fluoridated oral care products are recommended especially in countries where the fluoride concentration from drinking water is low. However fluoride from toothpaste swallowed by a four year old child was found to contribute up to one third to one half of total daily fluoride intakes of 3.6 and 2.3mg respectively [61]. In the European communities about 90% of all toothpastes are fluoridated in the range from 1000mg/kg to 1500mg/kg with a maximum level of 1500mg/kg. The Scientific Committee on Cosmetic products and non-Food Products Intended for Consumers (SCCNFP, 2003) states that the amount of toothpaste applied to the toothbrush for a child below the age of 6 years can vary between 0.05 and 0.8g. The recommended “pea size” amount is considered to be 0.25g. According SCCNFP simulation model the fluoride ingestion from such toothpastes could amount up to 50% of the fluoride intake of children at that age. In the model calculation for 3-5 year old children in the USA the fluoride intake from ingested toothpaste was estimated to be 30-60% of the dietary [62].

**Fluoride Tolerable Intake**

Fluoride is not essential for human growth and development and its content in the body is not under physiological control. Adsorbed fluoride is rapidly distributed by circulation to the intracellular and extra cellular fluid, but is retained only in calcified tissues. In adults, adsorbed fluoride is only partially less than 50% retained in skeleton and the remainder excreted prevalently via the kidney. On the contrary, in infants fluoride retention in bone can be as high as 90% and appears also incorporated into dental enamel during teeth formation. Excessive intake of fluoride during enamel maturation from birth to eight years of age, when enamel formation is complete, can lead to reduced mineral phase content of enamel and to dental fluorosis of deciduous, but prevalently of permanent teeth. Dental fluorosis has been usually associated with increased resistance to caries, but it is increasing the consideration of appreciable fluorosis as an adverse effect [63]. While mild dental fluorosis is not readily apparent, moderate dental fluorosis can be easily appreciated and is characterized by white spots and opaque striations staining and minute pitting of teeth Fig. (4) [64]. Fluorides in toothpaste, whilst well known for their anti-caries benefits, are toxic if ingested at high levels, in particular in children because of an adverse dose to weight ratio [65,66].

![Photographic images of teeth affected by dental fluorosis.](Reproduced by permission from Ref. [64]).

The International Standards Organization Toothpaste minimizes this safety matter by fixing the maximum fluoride dose allowed in oral care products. The recently produced toothpastes containing a very high fluoride concentration exceeding the International Standards Organization limits are exclusively prescribed by professionals and are not recommended for children [67]. The European Food Safety Authority (EFSA) Scientific Panel considers that the maximum fluoride intake is 0.1mg fluoride/kg/day in children.
Fluoride accumulation in skeleton changes bone mechanical behaviour reducing bone strength and increasing its density and stiffness, causing skeletal deformities and risk of fractures. Therapeutic studies with fluoride in post-menopausal osteoporosis suggest an increasing risk for skeletal fractures at or above fluoride intakes of 0.6mg/kg body weight per day. Fluoride increases with age in bone, more rapidly in women than in men and preferably in cancellous bone [68,69]. Fluoride is not irreversibly bound to bone and can be released during remodelling of bone [70].

Excluding the fluoride exposure via inhalation and the skin which in normal circumstances is really negligible, fluoride intake is due to oral ingestion by drinking water, beverages and foodstuffs, including fluoridated salt, dental health products and fluoride tablets for caries prevention.

Fluoride concentration in drinking water (0.3-1.5mg/L) differs according to the countries’ natural circumstances and to water fluoridation (U.K., Ireland, Spain and Switzerland). This has been recently reduced or terminated. In fact drinking water containing more than 2mg/L of fluoride may develop cosmetic discoloration of their teeth (dental fluorosis). Dental fluorosis, in its moderate or severe forms, may result in a brown staining and/or pitting of the permanent teeth Fig. (4).

This problem occurs only in developing teeth, before they erupt from the gums. Drinking water containing more than 4 mg/L of fluoride (the U.S. Environmental Protection Agency’s drinking water standard) can increase your risk of developing bone disease. Some home water treatment units are also available to remove fluoride from your drinking water [64].

Vegetables and fruit containing from 0.02 to 0.20mg/kg fresh weight, milk and milk products 0.05-0.15mg/kg, meat and meat products 0.15-0.29mg/kg, eggs 0.18mg/kg, fish 0.48-1.91mg/kg. Exceptions are tea which can contain considerable amounts of fluoride (0.34-5.2mg/L), and some brands of instant teas were reported to contain significant amounts of fluoride even 6.5mg/L. On the contrary, in human milk the fluoride concentration is about 0.2mg/L.

Children aged 1-8 years get fluoride intakes from food and water usually far below the UL. The clear increase of mild dental fluorosis occurred in some countries has been ascribed to the inappropriate use of fluoridated dental care products in particular fluoridated toothpastes. Dental products like toothpastes, rinses and gels containing fluoride can increase the total intake of fluoride especially when inappropriately used [71]. This happens especially in children younger than 7 years ingesting high amounts of toothpaste [61, 72-74]. In the model calculation for 3-5 year old children in the USA the fluoride intake from ingested toothpaste is estimated to be 30-60% of the dietary CTE For these reasons, in the European Communities all toothpastes are fluoridated with a maximum level of 1500mg/kg. The need to discover and develop an alternative to fluoride for teeth health care is clearly opportune.

Biogenic Hydroxyapatite

Vertebrate bones and teeth are biological hybrid materials where a calcium phosphate, in the form of hydroxyapatite (HA), represents the inorganic component intimately inter grown with the organic matter prevalently constituted of proteins and polysaccharides [75,76]. Biological HA is not stoichiometric according to the ideal formula Ca10(PO4)6(OH)2, but at low extent Ca2+ is replaced by other ions like Zn2+, Sr2+, Mg2+, Na+, K+, while PO43− and OH− can be partially substituted by other anions like CO3−, HPO42−, P2O7−, SiO44−, F−.

The bone mineral phase is more correctly called carbonate hydroxyapatite. Carbonate is the prevalent foreign anion and represents about 4-8wt % [77,78]. The substitution of CO3− groups into the PO43− sites (type B carbonate apatite) is prevalent in young humans, while the carbonate replacement to OH− groups (type A carbonate apatite) increases with the age of the individual [79].

The bone carbonate hydroxyapatite nanocrystals, which can represent a typical example of an “organic matrix-mediated” biogenic material, have a blade shape of approximately 25nm width, 2-5nm thickness and about 60nm length. Biogenic hydroxyapatite crystals exhibit non-stoichiometric composition, structured carbonate ions in the crystal lattice, low degree of crystallinity, plate acicular morphology and a nano size which give a large surface area of about 120m2/g Fig. (5) [76, 80, 81].

Dentine resides within the central region of the tooth and is similar to bone in composition and structure [82-91]. Enamel, the tooth external surface coating, has a much larger inorganic content than bone and dentine, close to 95% wt, which is mainly constituted of long thin ribbon-like prismatic crystals of hydroxyapatite, that exhibit a higher degree of cristallinity and a lower carbonate content than bone and dentine apatite crystals. Amelogenins, present in relatively large amount in the early stages of enamel formation, are enzymatically degraded and removed up to 5% wt as the hydroxyapatite crystals grow [92]. There is no biological process that can repair degraded or damaged...
enamel, evidencing the need for synthetic enamel biocompatible materials able to repair teeth decay [93-95].

**Biomimetic Synthetic Hydroxyapatite**

Biomimetism of synthetic materials for biomedical applications can be carried out at different levels in view of composition, structure, morphology, bulk and surface chemical-physical properties. Biomaterials can be turned biomimetic imprinting all these characteristics in order not only to optimize their interaction with biological tissues, but even to mimic biogenic materials in their functionalities. Chemists, biologists, physicists and engineers interested in material science are amazed by the high degree of sophistication, miniaturization, hierarchical organization, hybridising, reliability, efficiency, resistance and adaptability characterizing natural materials. These properties which biogenic materials have achieved through specific building principles selected by evolution, can be only partially obtained in manmade materials by present synthetic processes. For this reason Nature is a school for material science. Biomimetism and bioinspiration represent important tools for the design and the synthesis of innovative materials and devices [96-100].

The highly elaborated performances of biologically occurring materials are the results of an evolved convergence on limited constituents, which occur at a precise moment, and are available at that time. Nature produces soft and hard materials exhibiting remarkable functional properties by controlling the hierarchical assembly of simple molecular building blocks from the nano to the macroscale [101]. Biomineral morphogenesis is related to specific strategies for the long-range chemical construction of well organized architectures from preformed nano or micro crystalline inorganic building blocks. In fact, many biologic complex structures are obtained by promoting specific links induced by the conformation variability at the nanometre scale of biological macromolecules. Biosystems reveal a high level of integration of three fundamental aspects: the nano-micro “spatial confinement” of biochemical reactions, the inorganic and organic “hybridization” compounds and the “hierarchy” from nano to macro scale, in order to produce a biomaterial able to exhibit the appropriate chemical-physical properties at any different scale level [102-105]. Biogenic materials are nucleated in defined nanomicro dimensioned sites inside the biological environments in which chemistry can be spatially controlled. The spatial delimitation is essential to biological mechanisms to control size, shape and structural organization of biomaterials. With the development of nanotechnology, this strategy employing natural material genesis, has attracted a lot of attention in designing bioinspired materials such as polymeric micelles, nanoparticles, dendrimers and nanocrystals synthesized in nanoscale dimensions [106-111].

Porous HA, simulating spongy bone morphology has been prepared using various technologies to control pore dimension, shape, distribution and interconnections. HA ceramics processed by high-temperature treatment [112] present a significant reduction of bioreactivity and growth kinetics of new bone due to the not resorbability. New synthetic methods at lower temperatures have been developed, allowing one to obtain porous bioceramics with a low degree of crystallinity. Colloidal processing [113], starch consolidation [114], gel casting and foam out [115] have yielded excellent results, producing bioceramics with a bimodal distribution of the pore size that can be modified as a function of the sintering conditions.

Synthetic bioresorbable biomimetic hydroxyapatite nano and micro crystals exhibit excellent properties as bone filler biomaterial, such as biocompatibility, bioactivity, osteoconductivity, direct bonding to bone, etc., exciting new applications of HA in the fields of bone tissue engineering and orthopaedic therapies [116,117]. There are many synthetic strategies to produce HA nanocrystals, including wet producing, hydrothermal, electrochemical and ultrasonic mobilization methods, sol-gel and solid state synthesis. HA nano-microcrystals with different stoichiometry and morphology have been prepared and the effects of varying synthesis conditions on stoichiometry, crystallinity, morphology, surface properties, reactivity and bioactivity has been investigated [118-122]. In order to optimize its specific biomedical applications, especially new bone formation and drug delivery function, the physical-chemical features which should be tailored in synthetic biomimetic HA nanocrystals are dimensions, porosity, morphology and surface properties [123-126].

The chemical and biological properties of HA crystals are strictly linked to their dimensions, the regulation of which requires a high level of biological and chemical control at the nano scale. Thus, the recent trend in biomaterials research is focused on overcoming the limitations of calcium phosphates, precisely hydroxyapatite ceramics, and in improving their biological properties via exploring the unique advantages of nanotechnology [127]. The trend is shifting towards nanotechnology to improve the biological responses of HA, because nano-HA is a constituent of bone improving the biomaterial-bone interface. It has been established that biomimetism offers a unique approach to overcome many traditional materials shortcomings. Nanostructured biomimetic materials offer much higher performances than their larger particle sized counterparts, due to their large surface to volume ratio and unusual chemical/electronic synergistic effects. In addition, the surface adsorption properties of these materials led to interesting applications in drug delivery systems [122,128,129]. The surface functionalization of HA nano-crystals with bioactive molecules makes them able to transfer information to and to act selectively on the biological environment, and this represents a main challenge for innovative bone substitute materials. In this way HA nanocrystals will not only guarantee, for instance, either osteointegration or osteoinduction enhanced properties, but they will also perform at the molecular level, by stimulating specific cellular responses.

Only in recent years scientists have begun to use molecules for the synergistic coupling of crystals synthesis and functionalization. In fact, previous studies have limited the use of biomolecules as simple growth inhibitors of HA crystallization, rather than considering their use as a strategy to fine-tune the bioactivity of the nanoparticles [130,131]. Studies of the effect of biological molecules onto hydroxyapatite crystal growth have been related directly to
physiological or pathological calcification processes. The exposure of biomaterials to plasma proteins, blood or biological fluids normally leads to the adsorption of blood proteins onto the biomaterial surface. The adsorbed protein layer can further mediate additional biological responses, such as cell attachment and activation, and can create unpredicted perturbations to device operation [132-139].

SYNTHESIS AND CHARACTERISATION OF BIOMIMETIC CARBONATE-HYDROXYAPATITE NANOCRYSTALS

Biomimetic carbonated hydroxyapatite nanocrystals have been synthesized with a nearly stoichiometric in bulk Ca/P molar ratio of about 1.6-1.7 and containing 4±1 wt% of carbonate ions replacing prevalently phosphate groups. CHA nanocrystals have been synthesized both about 100nm and 20nm sized with an acicular and plate morphology respectively. TEM images of synthetic CHA nanocrystals 20nm sized showing the plate shaped morphology and synthetic CHA nanocrystals 100nm sized showing the acicular morphology are reported in Fig. (6A, B) respectively.

CHA nanocrystals can aggregate in micro sized crystal clusters, whose dimensions increase prolonging maturation time in mother solution at constant temperature and stirring [140]. Powder X-ray diffraction patterns of plate shaped about 20nm sized CHA nanocrystals and acicular shaped about 100nm sized CHA nanocrystals Fig. (7b, c) respectively show characteristic diffraction maxima of an apatite single phase (JCPDS 9-432). These X-ray diffraction patterns are compared with those collected for natural carbonate hydroxyapatite from deproteinized dentine and enamel reported in Fig. (7a, d) respectively. The broadening of the diffraction maxima present in the X-ray diffraction patterns reported in Fig. (7a, b, c) indicate a relatively low degree of crystallinity. The degree of crystallinity of synthesized about 20nm sized CHA nanocrystals with plate morphology and synthesized about 100nm sized CHA nanocrystals with acicular morphology is 30% and 50% respectively.

The crystallinity degree of about 20nm sized CHA nanocrystals is very close to that one determined from the X-ray diffraction pattern of deproteinized dentine natural carbonate-hydroxyapatite (28%). Furthermore the crystallinity degree of natural hydroxyapatite of deproteinized enamel is 70%. X-ray diffraction investigation reveal that the crystal structures of the synthesized CHA nanocrystals are very close to those observed for natural dentine.

The same similarity can be observed from the comparison of the FTIR spectra of synthesized CHA nanocrystals and natural apatite of deproteinized dentine as reported in Roveri et al. [141]. In these spectra the characteristic absorption bands of phosphate and carbonate groups are clearly resolved. The absorption band at 1468cm\(^{-1}\) is related to the carbonate group substitution to the phosphate one, while the shoulder at 1545cm\(^{-1}\) can be considered the contribution of the carbonate group substituting the hydroxyl group in the apatite structure. This finding reveals that synthesized CHA nanocrystals not only contain a similar carbonate amount, but also, underline that the carbonate substitution to the phosphate and/or hydroxyl group is very similar in the synthetic and biological crystals revealing that it can be considered a type B carbonate apatite.

A surface characterization of the synthetic carbonated hydroxyapatite nanocrystals has been carried out in order

Fig. (6). TEM images of synthetic CHA nanocrystals 20 nm sized showing the plate-shaped morphology (A), synthetic CHA nanocrystals 100nm sized showing the plate-acicular morphology (B), (scale bar = 200nm).

Fig. (7). X-ray diffraction pattern of synthetic CHA about 20nm sized plate shaped nanocrystals (a), natural carbonate-hydroxyapatite from deproteinated dentine (b), synthetic CHA about 100nm sized plate-acicular shaped nanocrystals (e), and natural carbonate-hydroxyapatite from enamel (d) (Reproduced by permission from Ref. [141]).

The same similarity can be observed from the comparison of the FTIR spectra of synthesized CHA nanocrystals and natural apatite of deproteinized dentine as reported in Roveri et al. [141]. In these spectra the characteristic absorption bands of phosphate and carbonate groups are clearly resolved. The absorption band at 1468cm\(^{-1}\) is related to the carbonate group substitution to the phosphate one, while the shoulder at 1545cm\(^{-1}\) can be considered the contribution of the carbonate group substituting the hydroxyl group in the apatite structure. This finding reveals that synthesized CHA nanocrystals not only contain a similar carbonate amount, but also, underline that the carbonate substitution to the phosphate and/or hydroxyl group is very similar in the synthetic and biological crystals revealing that it can be considered a type B carbonate apatite.
highlight their surface chemical-physical characteristic which directly interfaces and reacts with exposed dental tissues. The ATR spectra of the synthetic about 20nm and 100nm sized CHA nanocrystals reveal a 4% and 3% wt surface carbonate respectively. The consistent amount of surface % wt of carbonate present in synthetic CHA is appreciably higher than the surface % wt of carbonate present in enamel and dentine about 2% wt.

Specific surface area of $100m^2g^{-1}$ and $80m^2g^{-1}$ has been determined for 20nm sized CHA nanocrystals with plate morphology and synthesized 100nm sized CHA nanocrystals with acicular morphology respectively. These specific surface area values obtained for synthetic nanocrystals are only slightly lower than the $110m^2g^{-1}$ obtained for biological nanocrystals.

The surface Ca/P molar ratio determined by X-ray Photoemission Spectroscopy (XPS) analysis for CHA nanocrystals and CHA crystals micro-clusters do not reveal appreciable differences and result significantly lower than Ca/P molar ratio determined by ICP analysis in bulk indicating a surface calcium deficiency probably due to surface disorder. In fact the Ca/P molar ratios of 1.7 determined in bulk for synthetic CHA nanocrystals reduces to a value of 1.4-1.5 when determined on the crystals surface by XPS analysis [141].

TEETH REMINERALIZATION BY BIOMIMETIC CARBONATE HYDROXYAPATITE

The dentine remineralizing effect of sintetic biomimetic CHA nanometric crystal has been studied with a Scanning Electron Microscopy putting a CHA nano crystals slurry solution onto slices of dentine previously demineralized with orthophosphoric acid. Biomimetic hydroxyapatite nanocrystals were demonstrated able to remineralize the surfaces of the dentine etched by orthophosphoric acid application and able to progressively occlude dentine tubules in few minutes till a regeneration of a layer of mineralized tissue within few hours Fig. (8A-D). This remineralization rate seems to be compatible with the development of toothpastes with remineralizing effect and able to contrast dentine hyper sensibility [142].

Scanning Electron Microscopic analysis allows investigating the morphology of both demineralized enamel and the features observed after remineralization procedures induced by biomimetic HA nanocrystals in vitro application. In fact after treatment for 10 minutes by aqueous slurry of synthetic 100nm sized CHA nanocrystals, the surface of the demineralized enamel by orthophosphoric acid 37% for 1 minute appears partially covered by the CHA phase and the interprismatic and prismatic enamel structure become not completely hidden. In contrast, on the surface treated for 10 minutes by aqueous slurry of synthetic 20nm sized CHA nanocrystals, the interprismatic and prismatic enamel structures appear to be covered by a thicker and more homogeneous apatitic layer [143]. This finding reveals an advantage of the 20nm sized synthetic building block in respect to the 100nm sized in producing an apatitic coating on the enamel surface. XPS analysis of spectral features of the O 1s region of the enamel demineralized by orto-

Fig. (8). Scanning Electron Microscopy images showing the features of the dentine surface demineralised (A), to remineralized specimens after application of HA nanocrystals slurry solution for 10min (B), 1h (C) and 6h (D). The pictures show a progressive crystals formation and consequent obliteration of dentine tubules (Reproduced by permission from Ref. [142]).
phosphoric acid 37% for 1 minute compared with that of the enamel remineralized by synthetic 20 and 100nm sized CHA nanocrystals for 10 minutes unequivocally confirm the presence of synthetic CHA at the surface of the treated enamel and the consequent validation of the enamel remineralization. The same finding is pointed out by the ATR spectrum of enamel treated for 10 minutes by synthetic 20 and 100nm sized CHA nanocrystals, showing appreciable higher intensity of the characteristic absorption bands of carbonate ions (at 1420-1460 and 1680cm\(^{-1}\)) in respect of the same absorption bands present in the demineralized enamel ATR spectrum, revealing that the surface of remineralized enamel is richer in carbonate than the natural one, like synthetic 20 and 100nm sized CHA nanocrystals [143].

Despite the complicated hierarchical structures, it has been revealed that the basic enamel building blocks are generally 20–40nm HA nanoparticles [144]. In fact during in vitro experiments of acid–induced enamel demineralization, nanosized crystallites have been observed attached to the enamel surfaces or escaped into the bulk solution. These nanosized crystallites were kinetically protected against further dissolution, even though the solutions remained undersaturated [145]. It has been suggested that the enamel repairing effect of HA can be greatly improved if their dimensions can be reduced to the nano scale of the natural building blocks. Compared with conventional HA and nano amorphous calcium phosphate (ACP), in vitro experimental results demonstrate the advantages of 20nm HA in enamel repairs. Scanning Electron Microscopy, confocal laser scanning microscopy, quantitative measurement of the adsorption, dissolution kinetics and nanoindentation show the strong affinity, excellent biocompatibility, mechanical improvement and the enhancement of erosion-free by using 20nm particles as the repairing agent. However, these excellent in vitro repairing effects cannot be observed when conventional HA and ACP are applied. Clearly, 20nm nano HA share similar characteristics of the natural enamel building blocks, so that they may be used as an effective repairing material and anticaries agent. Our current study highlights the analogies of nano building blocks of biominerals during biomedical applications, which provide a novel pathway for biomimetic repair [146].

**DIFFERENT ENAMEL REMINERALIZATION IN VITRO BY TOOTHPASTES CONTAINING FLUORIDE OR BIOMIMETIC CHA NANOCRYSTAL MICRO-CLUSTERS**

SEM analysis has been used to investigate the morphology of demineralized enamel and the features observed after a remineralization process which utilises in vitro application of toothpastes containing fluoride or CHA micro-clusters constituted of nanocrystals 100 nm sized [141].

The surfaces of the teeth treated with fluoride Fig. (9A) were not consistently changed in respect to those demineralized by orthophosphoric acid Fig. (9B). Actually both interprismatic and prismatic enamel structures still appear evident. On the contrary, after treatment of the enamel slabs with a toothpaste containing synthesized CHA micro-clusters constituted of nanocrystals 100 nm sized the interprismatic and prismatic enamel structures appear to be completely hidden by a thick homogeneous apatitic layer Fig. (9C).

The XRD patterns collected on the surface of enamel slabs after treatment with CHA or fluoride toothpastes and water are reported in Fig. (10b, c, d) respectively and compared with the XRD pattern Fig. (10a) of CHA micro-clusters constituted of 100nm sized nanocrystals utilized to prepare the used CHA toothpaste. The XRD diffraction maxima recorded on the surface of enamel slabs treated with fluoride containing toothpastes appear slightly more sharpened than those obtained on the enamel etched slabs brushed only with water. This observation reveals an increased crystallinity degree probably due to a partial structural conversion of hydroxyapatite into fluoride substituted...
hydroxyapatite. On the contrary, the XRD pattern obtained on the surface of enamel slabs brushed with CHA containing toothpaste shows the broadened diffraction maxima characteristic of the synthetic biomimetic CHA, revealing its presence on the enamel surface. The CHA not removed by brushing procedures suggests the formation of chemical bonds between the synthetic CHA micro-clusters constituted of 100nm sized nanocrystals and natural enamel apatite crystals. These bonds allow the formation of a persistent CHA coating on the enamel surface whose morphology was detected by SEM analysis.

The surface Ca/P molar ratio determined by XPS analysis for demineralized enamel slabs before and after in vitro remineralization by brushing with toothpastes containing fluoride or CHA have been compared with the Ca/P molar ratio of CHA micro-clusters constituted of 100nm sized nanocrystals. The enamel surface Ca/P molar ratio practically does not change before and after the brushing treatment with toothpastes containing fluoride. This finding reveals how the only structural modification of enamel hydroxyapatite induced by fluoride is restricted to a partial hydroxyl group replacement by fluoride ions without affecting appreciably the Ca and phosphate structural network. On the contrary, enamel slabs after the brushing treatment with the toothpaste containing synthesised CHA micro-clusters of 100nm sized nanocrystals the enamel slabs exhibit a surface Ca/P molar ratio very close to that of the synthetic CHA micro-clusters of 100nm sized nanocrystals. The results highlight that biomimetic nano sized CHA crystals produce an apatite coating deposition on the enamel surface. An advantage of the 100nm sized synthetic building block respect the 20nm sized in binding on to the enamel surface has been appreciated, but it can be ascribed to a different and more suitable micro-cluster aggregation.

This coating is much less crystalline than native enamel apatite and consists of a new apatitic mineral deposition which progressively fills the scratches and pits. On the contrary, the surface remineralisation observed on the specimens treated with fluoride contained in toothpaste is mainly based on chemical-physical enamel apatite surface modifications rather than on the formation of a new mineral deposition.

The documented CHA biomimetic coating formation is the first remineralization process corresponding to a real new mineral apatitic deposition in the demineralized area of enamel surface.

ANALYZING PATENTS

For our patent search, the United States Patent office search engine (available through www.uspto.gov) has been used.

Numerous patents were detected concerning fluoridated products for daily teeth damage prevention through the formation of fluoride salts on the teeth surface, in this way contrasting caries. Only a few patents of these concern the precipitation of fluoridated hydroxyapatite on the teeth surface in order to promote enamel and dentine remineralization and repairing. A compound similar to hydroxyapatite likely to be partially converted into fluorine apatite on the tooth surface is named only in few patents; caused by the reaction of the fluoride present in the product with the oral environment. Very few patents move towards the daily use of products containing hydroxyapatite without the contemporay presence of fluoridated components in repairing and remineralizing teeth.

PATENTS OF ORAL CARE PRODUCTS INDUCING FORMATION OF FLUORIDE SALTS ON TEETH SURFACE

Patents which utilize fluoride anions in toothpastes, rinses and gels for a daily teeth health care through the formation of fluoridated salts on the teeth differ prevalently
for the used fluoridated compound present in the product and for the different utilization of a single or two components delivery system.

Toothpastes containing insoluble calcium compounds in a single component delivery system, utilized as abrasive dentifrices with a lower amount of EDTA or its sodium salts and also including a fluoride compound, preferably sodium monofluorophosphate (MFP), has been described to induce the calcium fluoride precipitation on teeth surface [147].

An aqueous dental preparation includes a fluoride component in a solution having a pH lower than 2. The compound can be applied to teeth either before or after the calcium treatment. This provides the precipitation of CaF$_2$ as a thin homogeneous layer on the teeth enamel [148].

The sustained release on teeth surface of calcium fluoride is expected by a toothpaste composition including MFP and an ionizable calcium source, while sodium fluoride may be added to the composition as desired [149].

Some single component delivery systems are characterized by the contemporary presence in the product of fluorine, calcium and phosphorous. In fact Pearce [150] describes a dental rinse which includes water soluble salts of fluorine, calcium and phosphorous. The composition additionally includes a substance metabolized into an alkali, such as urea, which raises the solution pH causing calcium precipitation.

A clear, stable aqueous mouthwash free of calcium phosphate crystals and containing calcium ions, phosphate ions and fluoride ions has been described by Rudy et al. [151]. The mouthwash includes a chelating agent combined with a calcium ion and phosphate ion sources. The calcium ion source consists of a component capable of providing also fluoride ions.

Intradal [152] describes a remineralizing dentifrice composition including a calcium component, two fluoride components, an alkali or alkaline earth metal fluoride and an alkali metal fluorophosphate, two phosphate components, a soluble cyclic alkali metal phosphate and a soluble linear phosphate.

Stable solutions for dental remineralization including a source of calcium ions, a source of phosphate ions and a source of fluoride are described by Gaffar et al. [153]. The solutions also include an anti-nucleating agent consisting of diamine tetramethylene phosphonic acids having a specific formula. The anti-nucleating agent stabilizes the calcium ions and phosphorous ions and prevents them from precipitating as large, insoluble apatite crystals by absorbing onto spherical nucleated particles as they form and blocking crystal growth. PBTA as anti-nucleating agent has been proposed by Gaffar et al. [154].

In order to increase the amounts of fluoride to precipitate out of the delivery medium and deposit on or into dental tissues, [155] a novel system consisting of two components delivery system has been developed. When the two components come to contact, a rapid, but controlled reaction precipitates calcium fluoride continuously in about a 1-minute time. One significant disadvantage of the two-component F$_2$ system described above is that it requires the use of a complex F that has a specific hydrolytic property and none of the suitable complex F salts are currently approved by the Federal Food and Drug Administration for use in rinses, dentifrices and other oral health care products [156].

Another novel two-component system: component A of the system contains a soluble calcium source and a soluble Ca-complexing anion such as ethylene diaminetetraacetic acid (EDTA). The calcium in this phase is largely bound to the Ca-complexing agent. Component B contains an FDA approved F compound such as sodium fluoride or stannous fluoride. When the two components are combined, precipitation of calcium fluoride (CaF$_2$) removes free Ca$^{2+}$ from the solution. Raaf et al. [157] describe a two-phase dental composition in which the two phases are combined when applied to teeth. The first phase includes a calcium component, while the second phase includes a water soluble phosphate component and a water soluble fluoride component.

In conventional fluoride systems, any agent, such as phosphate, that interferes with the formation of calcium fluoride, is considered undesirable because it would reduce the fluoride deposition [158]. In contrast to the conventional concept, calcium fluoride inhibitors, when present in either or both of the components in the two-component system, can, under controlled conditions, produce the desired delay in the formation of calcium fluoride on or into dental tissues. “Control of calcium fluoride formation in mouth rinses, dentifrices and gels” has been reported in the patents [159].

**PATENTS OF ORAL CARE PRODUCTS INDUCING FORMATION OF FLUOROAPATITE ON TEETH SURFACE**

Not abrasive oral hygienic composition containing alkali metal fluoride and monofluorophosphate and orthophosphate anions [160] has strong effects against caries. In fact, the fluoride agents cause formation of fluorohydroxyapatite in the enamel, hardening the teeth, contrasting caries and reducing plaque growth.

Brown et al. [161] describe solutions, gels and substantially non aqueous suspensions that are useful in topical or direct mineralizing dental tissue, such as enamel, dentine and exposed root surfaces. The incorporated fluoride is in the form of $Ca_5(PO_4)_3F$ and is more permanently retained than CaF$_2$ and other fluoridation products.

Tomlinson et al. [162] disclose a single dental preparation with calcium/phosphorous/fluoride components including fluorapatite, fluorohydroxyapatite, apatite, calcium deficient apatite and hydroxyapatite substituted by a fluoranion.

Digiulio et al. disclose a method for the remineralization of tooth enamel using a two solution system [163]. The first solution is a cationic solution containing a calcium salt and optionally a heavy metal cation. The second solution is an anionic one containing a phosphate salt and optionally non-phosphate anions including fluoride ions. The pH of the solutions ranges from 2 to 4 and the ratio of calcium to phosphorous ranges from 0.01 to 100. The solution, obtained by mixing the two-components, is described as a...
"metastable" solution and requires a stay in the mouth from 10 seconds to about 3 minutes to raise the pH of the solution, so that the components of the solution precipitate in the teeth turn in an enamel remineralization.

A two-step process for remineralizing dental enamel is described in [164]. In this process, two solutions, one comprising a calcium salt and the other comprising a phosphorous salt along with an optional fluoride salt, are sequentially contacted with dental enamel. The sequential solution contact results on the surface of the enamel being remineralized.

A process for applying fluoride to teeth with a material having calcium and phosphate components is reported in [165]. The dental material includes a salt which ionizes to produce fluoride ions and the formation of fluoroapatite is expected.

PATENTS OF ORAL CARE PRODUCTS CONTAINING FLUOROAPATITE

Many dental filling materials containing Ca phosphate compounds, preferably hydroxyapatite, fluoroapatite and fluorohydroxyapatite, have been patented in porous [166], cement [167], glass-ceramic [168], plates in collagen [169] and coating forms [170-172]. Agents for the treatment of dental hypersensitivity contain hydroxyapatite and fluoroapatite together with other inorganic salts, hydrophobic polymers and ethyl cellulose [173,174].

Very few patents move towards the use of hydroxy ad fluoro apatite in oral care products for daily use. Mixtures of rod shaped hydroxy ad fluoro apatite crystals are dispersed in toothpastes or gels, mouthwashes or chewing-gums to induce or promote bone tissue formation.

Freunsch describes [175] toothpaste containing rod shaped hydroxyapatite, fluoroapatite and fluorohydroxyapatite with length-to-width ratio significantly greater than 5, preferably 9 to 12. The thickness and the width are 0.01 to 0.02μm respectively, while the crystal length is about 0.1-0.2μm. These apatitic crystals are synthesised according to the process reported in [176].

Carbonate-fluoride-hydroxyapatite is synthesised by a neutralization process, at inert atmosphere, of calcium hydroxide water solution by a mixture of orthophosphoric and hydrofluoric acids at 8-10°C and continuous introduction of Ca(HCO₃)₂ into reaction solution. This amorphous carbonized and fluorinated hydroxyapatite has been patented for toothpaste and method for its obtaining [177].

A kit for performing use in a process of dental anti caries prophylaxis by supplying fluoride to the teeth, inducing the growth of apatite crystals so that some remineralization may occur on pits and fissures in the enamel. The kit is constituted of two containers, the first containing aqueous solution of orthophosphoric acid with dissolved sodium-flouride at pH 3 and the second containing a solution of calcium and orthophosphate ions at pH about 7. The first solution is applied topically to teeth and after five minutes the second liquid is then subsequently applied for about five minutes [178].

Sugiyama et al. describe a mouthwash with hydroxyapatite or fluorooapatite or carbonate apatite and magnesium salt which has been patented to prevent sticking of plaque [179].

Coulson [180] and Bristow et al. [181] describe a dentifrice formulated comprising hydroxyapatite particulate and fluoride-containing anti caries agent, particularly sodium fluoride or sodium mono fluorophosphates. The authors aim to reduce upon storage the interaction between hydroxyapatite and fluoride which takes place at pH within the range 7.0-10.0 and authors can achieve the goal buffering the dentifrice pH. In this way, the loss of fluoride is reduced and fluoride remains available in a sufficient suitable amount against dental caries. Synthetic hydroxyapatite which is present in the toothpaste, usually from 2% to 20%, but preferably less 15% by weight, has an average particle size ranging preferably from 3 to 10 microns. This inorganic phase is constituted of at least 92% of high purity hydroxyapatite and a minor amount (2% wt) of calcium carbonate. Hydroxyapatite available commercially is sold under the trade name CAPITAL by British Charcoals & Macdonals of Greenock in Scotland and produced as described by Bristow et al. [182]. Hydroxyapatite is utilised as innovative abrasive agent and the teeth health care has been ascribed to a fluoride containing anti-caries agent. In fact hydroxyapatite acts like abrasive agent, but also produces a synergistic effect with other fluoride containing components for the prevention of teeth decay [183]. According to authors, in the dentifrice compositions delivering simultaneous hydroxyapatite and a fluoride, HA efficiently absorbs dental plaque and is adsorbed onto the surface layer or enamel in an extremely remarkable manner. The adsorbed hydroxyapatite is recalcified on teeth surface and the recalcified hydroxyapatite is efficiently transformed into fluoride apatite by coexisting in the toothpaste.

Scheller et al. describe toothpaste containing hydroxyapatite and soluble fluoride having the suspicion that soluble fluoride converts hydroxyapatite into fluoro apatite [184].

In order to avoid the reaction, although partial, of hydroxyapatite and fluoride inside the toothpaste container, Aoki et al. [185] describe a composite toothpaste product comprising a toothpaste containing hydroxyapatite as a main active ingredient and another toothpaste containing a fluoride compound as a main active ingredient, which are enclosed with a container made of a flexible tube but separated from each other by a partition under the container. In the composite toothpaste product, the two single different toothpastes are separated from each other with no contact when it is out of use. They are squeezed out of the container, when in use, in such a manner that the latter toothpaste is enclosed with the former. The aim is to prevent dental caries than toothpaste products containing only either a hydroxyapatite or fluoro compound. However, it is difficult to suppose that, after the contact of hydroxyapatite with fluoride in oral environment, the hydroxyapatite does not convert in fluorohydroxyapatite.

Biocompatible nanocrystalline materials based on commercial apatite having by milling average size ranging between 0.5 and 200nm, which have possible lattice deformations, defects and ions substitution have been proposed.
for use in the fields of dentistry and dental hygiene in order to induce enamel and dentine remineralization [186].

Tomlinson et al. [187] report dental preparations containing fluoroapatite, fluorohydroxyapatite and hydroxyapatite. In this patent is reported the synthetic method to obtain fluorohydroxyapatite by alternately changing the pH from 7 to 4 to 7 several times in order to obtain incorporation of different ions (carbonate, monofluorophosphate, $\text{ZnF}_4^{2-}$) in a apatitic structure.

Hydroxyapatite is expected to deposit on dental enamel by a two components product which put in contact a carrier of a soluble calcium phosphate salt at pH less than 7 and a second carrier containing an alkaline material and a fluoride ion source to achieve a pH greater than 7.5 [188].

Pierce et al. [189] propose dental preparations containing materials having calcium and phosphate components in the range from 10 to 90%wt. Different 18 groups of mineral and synthetic inorganic materials have been reported and among these fluoroapatite, fluorohydroxyapatite and hydroxyapatite are contained. Alternatively fluoroapatite, fluorohydroxyapatite and hydroxyapatite growth and crystallization is expected during the pH variation within the physiological wide limits 4, 5 and 7, 5 for effect of the previous mentioned containing calcium and phosphate components. Their deposition in the vulnerable sites as pits and fissures on teeth has expected to repair the tissue and prevent or delay further carious attacks. The patent reports a lot of synthetic methods to prepare the mentioned components containing calcium and phosphate materials components, but any in vitro or in vivo evidences of the claims veracity has been reported. This ancient patent puts in evidence how old is the idea to utilise hydroxyapatite in repairing enamel and dentine. A so forerunning idea anticipating the needed technologies to synthesise biomimetic hydroxyapatite able to realize the repairing expected functions. Recently nanotechnology has been utilized to obtain nanoaggregates of compounds containing calcium fluoride and amorphous calcium phosphate. In the mouth environment the nanocomposites can release calcium, phosphate and fluoride which can convert into fluoroapatite on the teeth surface [190].

**PATENTS OF NON FLUORIDATED ORAL CARE PRODUCTS CONTAINING HYDROXYAPATITE**

The use of pure hydroxyapatite without the presence of fluorine has been patented [191] in a dentifrice for hypsensitve teeth. The toothpaste contains at least 15%wt of hydroxyapatite having an average particle size less than 8μm as the sole crystalline and polishing substance, no further soluble mineral salts being present and optionally local anaesthetic may be added. The proposed dentifrice exhibits an abrasion value of less than 30 measured as RDA.

After 20 applications in vitro, there was already a reduction in diameter of the dentine channels of about 50%. Author’s investigations have shown that an increased remineralization occurs, more specially with apatite particles sizes lower than 4μm [192].

Koeleermann [193] discloses an oral or dental preparation containing 5 to 90% by weight of finely divided hydroxyapatite having an average particle size of less than 10μm, in particular in the region of about 6 to 8μm. According to this patent, if this finely divided hydroxyapatite is repeatedly brought into contact with sensitive teeth over a prolonged period of time, for example when cleaning the teeth or when chewing a chewing gum, a long lasting remineralisation of the teeth may be achieved thanks to the diffusion of the hydroxyapatite into the exposed dentinal canaliculi by virtue of its very slight solubility in water and saliva due to hydrolysis. Accordingly, author’s opinions, this sparingly soluble hydroxyapatite becomes deposited in the microscopically fine cavities left in the natural hydroxyapatite structure, so that the dentinal canaliculi are gradually sealed and permanent relief from pain is obtained. The importance of using hydroxyapatite particles with the smallest possible dimensions has been acquired two decades ago at the beginning of nanotechnologies. In fact, particles with smaller dimension exhibit a larger surface area and appear higher reactive. Hydroxyapatite micronized in order to obtain microcrystals about the 50% of which having a diameter ranging between 0.20 and 1.00μm has been associated to KHCO₃ in a toothpaste able to induce remineralization and contrast dentine hypersensitivity and plaque formation [194]. Nanocrystals of commercial hydroxyapatite has been suggested as active component in toothpaste for enamel remineralization [186]. The micro-nano dimensions of the hydroxyapatite particles proposed in the above mentioned patents are obtained by milling larger particles of commercial synthetic hydroxyapatite according to the well known “top down” process. By this process nano-micro particles appear surface smoothed as river stones without high bioreactivity.

A composition characterized also by the presence of surfactants as glycerin, sorbitol and propilenglicole for use in dental tissues which includes 0.1% wt hydroxyapatite having a particle size in a range from about 0.05μm to about 10μm has been patented in order to repair pits present on tooth surface and protect the tooth afterward, preventing tooth decay [195].

The method for producing an improved hydroxyapatite composition in the form of a suspension or paste which may have a homogenous concentration in the range of from 7% to 96% and their industrial applications has been patented [196].

Sakuma et al. [197] provide compositions for enhancing transdermal and/or transmucosal absorption, comprising a hydroxyapatite and essential ingredients. The hydroxyapatite has a maximum particle size of 1μm or less, preferably 0.1μm or less, and the content of the hydroxyapatite relative to a drug to be formulated ranges from 0.1 to 1000 weight percent.

Hydroxyapatite is expected to crystallise into saliva upon exposure of both calcium and phosphate ions from soluble phosphate salts present in a one component system patented for providing remineralization [198]. Nevertheless, a dental paste constituted of silicate containing hydroxyapatite powder in a aqueous solution of hydrogen peroxide and phosphoric acid has been patented to prevent dental caries filling voids in enamel surface [199]. According to this patent, silicate containing hydroxyapatite powder is
synthesised mixing an aqueous solution of phosphoric acid-sodium silicate and a calcium phosphate.

A toothpaste composition containing calcium compound and carbamide peroxide has been patented [200] in order to associate the whitening and the recalcification of the enamel simultaneously. Hydroxyapatite crystallization is expected on teeth surface by mixing more than two different calcium phosphates among: tricalcium phosphate, calcium hydrogen phosphate, calcium dehydrogen phosphate, octacalcium phosphate and calcium pyrophosphate present in the product. Hydroxyapatite formation on the teeth surface is expected by several oral care products containing a source of calcium and a source of phosphate ions which are physically separate prior to the use of the product [201-203]. Recently a dentifrice composition has been patented [204] containing hydroxyapatite which can grow according to a typical template process upon an amino acidic substrate (synthetic polypeptides, gelatine and collagen) which produces also encapsulation of the neo formed apatite phase from dissociable calcium and phosphate sources. A mineralization benefit on the teeth is expected upon use.

Some patents do not describe properly toothpastes for daily use, but bioactive endodontic materials for filling the teeth and bone cavities or defects which are particularly suitable being used as vital pulp therapy, root-end filling, perforation repair, root canal filling and sealing up to reparative bone surgery. At exemplum Asgary [205] has patented a product containing as essential constituents calcium silicate, calcium phosphate and calcium salts which mixing with water solution of cationic and anionic components produces bioactive calcium and phosphate enriched material. These mixed compounds form hydroxyapatite during and after setting to form an effective seal against re-entrance of microorganisms into the filled tooth cavities stimulating hard tissue healing. Analogous applications are suggested by the medical material described in the patent [206] which describes a hydroxyapatite complex with a polymer-based material containing at least one functional group as isocyanate and/or alkoxysilyl group which chemically bond hydroxyapatite previously sintered. Hydroxyapatite is referred as a ceramics material obtained by sintering an amorphous phase at a temperature ranging between 800°C and 1300°C. A composite of synthetic ceramic hydroxyapatite with alumina and silica for implant fixture has been patented [207], while for the same bone repairation use a bioceramic including hydroxyapatite nanocrystals and gelatine has been described [208]. Nanotechnology has been successfully utilised to synthesise hydroxyapatite nanocrystals which can utilise delivery of nucleic acids [209]. These nanocrystalline hydroxyapatite particles can be utilized in tissue engineering, but also in teeth repairing and gene delivery. In fact, the above described hydroxyapatite particles can be complexed with biomolecules forming mono-dispersed CaP-pDNA nano particles. The hydroxyapatite nano complex can be used to transform cells in vitro or in vivo by contacting them with cells. In dental applications the hydroxyapatite complexes might be incorporated in dental implants to deliver an osteogenic gene to enhance implant osteointegration, pulp gene delivery, treat caries and induce dentine formation. If gene delivery requires specific implant conditions, in daily use products hydroxyapatite has been patented not only as agent for enamel and dentine remineralization, but also as deliver of antibacterial ions like Ag, Zn, which can explicate a strong anti plaque action. Sakuma et al. [210] disclose a dentifrice in which an antibacterial metal-ion agent is stably and firmly carried or supported by a calcium compound such as hydroxyapatite. Antibacterial metal carried by hydroxyapatite is linked in a highly stable way so as to prevent the toxicity due to the ion form of the metal. In recent times and based on the fact that the teeth and bone tissues are primary constituted by non-stechiometric hydroxyapatite containing specific substituting ions at both the cations and anionic reticular sites, the use of ions substituted hydroxyapatite has been proposed for the treatment of bone defects in the fields of reconstructive bone surgery, surgical stomatology, traumatology, orthopaedics and dentistry. At exemplum an international patent application [211] discloses magnesium-containing carbonate hydroxyapatite suitable for creating a freeze-dried granulate suitable for bone implants, particularly in the field of dentistry. According to this reference, an organic polymer, preferably an alginate, including such a modified hydroxyapatite is capable of efficiently repairing bone defects following application and interaction with bone tissue for an extended period of time of the necessary amount of product at the level of the existing cavity in the bone.

The preparation of carbonate-substituted hydroxyapatite ceramic materials must be easy and reproducible in order to achieve commercial exploitation. Different methods have been reported to prepare carbonate-substituted hydroxyapatite. A process is consistent with the heating of stoichiometric hydroxyapatite ceramic composition in CO2 atmosphere at about 900 °C for several days [212] resulting in low levels of carbonate substitution, with poor control over the extent and homogeneity of carbonate substitution and providing the substitution in to the hydroxyl site, i.e. type A, in spite of type B as in bone hydroxyapatite. A wet precipitation method using Na2CO3, NaHCO3 or (NH4)2CO3 as a source of carbonate ions results in the substitution of additional ions (Na+, NH4+) into hydroxyapatite structure [213] while the synthesis of an A-type carbonate substituted Hydroxyapatite has been reported [214,215]. Nakaso et al. describe a carbonate substituted Hydroxyapatite synthesis which is characterized by the adding Ca(OH)2 and CaCO3 to a slurry of CaHPO4. After sintering at 1000 °C the carbonate content is less than 0.1% [216]. A process for the preparation of hydroxyapatite with carbonate substituted in both the A and B sites without containing Na+ and NH4+ has been provided [217]. Bonfield et al. describe a process consistent with the preparation of a single phase carbonate-stabilized hydroxyapatite composition, which process comprises the steps of (i) preparing an aqueous solution containing CO3²⁻ and PO4³⁻ ions in the substantial absence of cations other than H⁺ ions; (ii) mixing the solution from step (i) with an aqueous solution or suspension of a calcium compound; and (iii) collecting and drying the precipitate formed in step (ii); the ratio of Ca/P in the calcium-containing solution or suspension and the phosphorus-containing solution, when mixed together, being maintained above 1.67. The product of the process is novel with a Ca/P molar ratio of greater than 1.67 and comprises up to 5% by weight of CO3²⁻ ions.
substituted in the B site or the B and A sites of the hydroxyapatite structure, with at least 50% of the CO$_3^{2-}$ ions being substituted on the B site [218].

Apatite whiskers with length along c-axis/length along a-axis not less than 10 and containing carboxylic acid groups not less than 0.01 % wt, but preferably wt 5%, have been prepared according to a method which involves precipitating and growing an apatite from a liquid containing phosphoric acid and calcium. This carbonate substituted hydroxyapatite has been proposed as reinforcing component in composite structural materials not devoted to biomedical applications [219].

Sakuma et al. [220] disclose a toothpaste having an antibacterial effect to prevent production of carious tooth and generation of periodontal diseases such as alveolar blemorhrea. The dentifrice contains hydroxyapatite powder. The hydroxyapatite powder carries therein an antibacterial metal such as Ag, Cu, and Zn in the metallic oxidation state. Only recently carbonate hydroxyapatite, Zn substituted has been synthesised in nanostructured microparticles 0.5-1.0μm sized in order to fill and seal enamel surface scratches contrasting enamel demineralization and dentine hypersensitivity. Nanostructured microparticles having a large surface area are strongly reactive and easily bound to enamel and dentine natural apatite. At neutral pH hydroxyapatite is practically insoluble, but when plaque attacks and covers the enamel surface apatite microparticles start to dissolve at the plaque acid pH and deliver Zn$^{2+}$ ions contrasting plaque through their antibacterial effect. This delivery mechanism is particularly sophisticated. In fact it appears physiologically stimuli responsive. Hydroxyapatite micro-nano particles utilised as active components in oral care products more recently patented are synthesized according to new nanotechnological processes which utilize the "bottom up" method. According to this method, nanocrystals are previously synthesised and then clustered in microparticles which exhibit a nanostructured particularly biomechanically fanged surface. These biomimetic hydroxyapatite particles are usually poorly crystalline resembling more closely biological apatite for composition, structure and morphology. Furthermore Zn substituted carbonate hydroxyapatite represent a smart drug deliver which releases drug as stimuli responsive in fact hydroxyapatite micro clusters remain in solubilized at buffered pH of mouth, but in situ between tooth and gum under the acid pH of plaque, the crystals are dissolved and Zn$^{2+}$ released. According to this mechanism Zn antibacterial action contrasting plaque is exploited properly under plaque formation [140].

CURRENT & FUTURE DEVELOPMENTS

From some decades the teeth health care has been committed to the fluoride effect on hydroxyapatite. Fluoride interacts with hydroxyapatite promoting the conversion into fluorapatite, which is less soluble and more mechanically resistant, but also more brittle than hydroxyapatite. The action of fluoride on the surface enamel apatite appears ideal to prevent demineralization due to acid food and drinks and to contrast plaque damage. A lot of scientific works support the fluoride positive action for teeth health care and consequently a lot of fluoridated oral care products have been patented till nowadays and in some countries drinking water has been enriched with fluoride. Fluoride is not essential for human growth and its content in the body is not under physiological control. Adsorbed fluoride is rapidly distributed in the body by circulation and is retained only in calcified tissues. In the last decades dangerous effects in human health have been ascribed to the daily fluoride amount ingested and many studies have put in evidence its high risk, especially for children, to get fluorosis and bone diseases in old people. European Food Safety Authority (EFSA) Scientific Panel has advised about the risk of fluoride intake from ingested oral care products and toothpastes can be fluoridated with a maximum level of 1500mg/Kg. In spite of this knowledge, fluoride continues to be widely used in orthodontia and dental applications to prevent enamel surface demineralization and represents the main component in toothpastes claiming remineralization effects. If nowadays fluoride represents the unique agent to contrast caries, plaques and remineralization we may probably ascribe this situation to the expensive cost of odontoiatric products containing hydroxyapatite. In spite of this, hydroxyapatite is commonly considered the main synthetic biomaterial as bone filler and substitute and only few daily use products for teeth health care have been patented. Too expensive is the hydroxyapatite cost to be consistently utilised as an active agent in toothpastes or mouth washes. In fact, almost all the patents previously reported and highlighting the ability of hydroxyapatite to remineralize enamel and dentine propose different compositions containing calcium and phosphate ions which can produce hydroxyapatite on teeth if mouth pH induces this crystallization and frequently are obliged to insert a low amount of fluoride in order to promote the apatite crystalisation. Small amounts of industrial hydroxyapatite are associated to other cheaper components and submitted to milling to improve hydroxyapatite surface area and reactivity. Only recently the development of nanotechnologies has opened new opportunities in obtaining cheap hydroxyapatite micro-nano particles by the "bottom up" methods. These hydroxyapatites are surface nanostructured and have higher surface area and consequently higher reactivity, allowing them to bind to enamel and dentine apatite producing a biomimetic coating on enamel, contrasting plaque formation and sealing dentine tubules, amnnulling hypersensitivity.

ACKNOWLEDGEMENT

Paper has been financially supported by: MIUR (PRIN 2008), University of Bologna (Funds for Selected Research Topics), the Inter-University Consortium for Research on Metal Chemistry in Biological Systems (C.I.R.C.M.S.B.) and Chemical Center S.r.l. Authors thank Mr. Paolo Gualandi for his cheering enthusiasm in suggesting this research work.

CONFLICT OF INTEREST

None.

REFERENCES

Advancements in Preventing Teeth Health Hazard


Advancements in Preventing Teeth Health Hazard

Recent Patents on Biomedical Engineering, 2009, Vol. 2, No. 3


