Microscopic investigations of Synthetic Biomimetic Hydroxyapatite

N. Roveri, E. Foresti, M. Lelli, I. G. Lesci and M. Marchetti
University of Bologna – Department of Chemistry “G. Ciamici” – Via Selmi 2, 40126 – Bologna Italy

In the honour of Professor Alberto Ripamonti in the occasion of his 80th birthday.

A biomimetic approach aims at inducing synthetic materials to transfer information and consequently to act selectively in the biological environment.

Synthetic biomaterials can be turned biomimetic by imprinting them with the morphology of biogenic materials. Biomimetic hydroxyapatite represents an elective material, because it is very similar for composition to the mineral component of bone and moreover its chemical – physical properties and surface reactivity can be managed by modifying synthetic parameters. The morphology of synthetic hydroxyapatite is essential to optimise its interaction with biological tissues and also to mimic biogenic materials in their functionalities. In fact, microscopic characterizations have been successfully utilised to define both the morphology of synthetic apatite biomaterials and the surface nano-structure, making it possible to explain their surface reactivity in the biological environmental. Transmission and scanning electronmicroscopy investigations are the principal tools to explain the functionality of biomimetic synthetic apatite materials like porous scaffolds, acicular and plate-like nanocrystals and nanostructured surface coatings. These biomimetic hydroxyapatite biomaterials offer promising biomedical applications in orthopaedic, odontohiartic and maxillofacial surgery, able to act as bone fillers, coatings of metallic prostheses and controlled deliverers of biologically active molecules both in space and time.

**Keywords** Biomimetic Hydroxyapatite; Morphology; Biomaterials; Synthetic Apatite; Biomedical Applications

**1. Introduction**

Biomimetism of synthetic materials for biomedical applications can be carried out at different levels according to the 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 at the high degree of sophistication, miniaturization, hierarchical organization, hybridizing, reliability, efficiency, resistance and adaptability characterizing natural materials.

These properties can be only partially obtained in manmade materials by present synthetic processes. For this reason, nature is a school for material science; in fact biomimetics and bioinspiration represent important tools for the design and the synthesis of innovative materials and devices.

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 nanometric scale of biological macromolecules. In fact 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.

There are many synthetic strategies to produce biomimetic Hydroxyapatite. Simulating spongy bone morphology, porous HA has been prepared using various technologies to control pore dimension, shape, distribution and interconnections. HA with different nanocrystal morphology is prepared introducing different subastande during the same synthesis or changing the temperature of reaction. Hydroxyapatitic nanostructured coating are prepared by electrochemical method on different types of support. In order to optimize their specific biomedical applications, especially referring to new bone formation and drug delivery function, the physical–chemical features which should be tailored in synthetic biomimetic HA nanocrystals are the dimensions, porosity, morphology and surface properties. 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.

Biogenic materials are nucleated in defined nano–micro-dimensioned sites inside the biological environments in which chemistry can be spatially controlled. The spatial delimitation is essential to biological mechanisms to control the 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. [1-6] These biomaterials can represent the highlighted ‘nano’ drug carriers called ‘nanovehicles’. [7] Inorganic biomimetic nanovehicles are prevalently constituted of phosphates which mimic inorganic components of bone tissue.
Biomimeticism of synthetic materials for biomedical applications can be carried out at different levels according to the 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.

2. Synthetic biomimetic hydroxyapatite

Biocompatibility, bioactivity, osteoconductivity, direct bonding to bone, etc. promote the applications of synthetic hydroxyapatite (HA) as an excellent biomaterial to be used as bone filler and substitute in orthopaedic, maxillofacial and odontoiatric surgery and also as scaffolds in bone tissue engineering. [8] Hydroxyapatite can be prepared by many different synthetic methods, including wet producing, hydrothermal, electrochemical and ultrasonic assisted methods, sol-gel and solid-state synthesis. [9-13] The variation of the reagents concentration, the reaction temperature and time, initial pH, ageing time and the atmosphere within the reaction vessel have been studied in order to synthesize Hydroxyapatite with different stoichiometry, morphology, crystallinity, crystal size dimension, surface area and reactivity. [14, 15]

Hydroxyapatite is also known for its capability to bond a wide variety of molecules [16-18] and most therapeutic agents for bone diseases under physiological conditions. [19] A main challenge for innovative bone substitute biomaterials is their functionalization with bioactive molecules which can transfer information and act specifically on the hydroxyl group in the apatite structure.

Hydroxyapatite with different stoichiometry, morphology, crystallinity, crystal size dimension, surface area and reactivity can be considered the contribution of the carbonate group substituting the bone crystals. Nanostructured biomimetic materials offer much higher performances than their larger particle sized traditional counterparts, due to their large surface to volume ratio and unusual chemical/electronic synergistic effects. In other words, the implanted biomaterial can act as local scaffold for cell invasion and formation of functional tissue and, in the meantime, deliver previously loaded biomolecules. In this way synthetic biomimetic hydroxyapatite can enhance their osteointegration or osteoinduction properties and also stimulate specific cellular responses at a molecular level.

3. Synthetic biomimetic hydroxyapatite nanocrystals

The recent trend in biomaterials research is focused on overcoming the limitations of calcium phosphate ceramics and in improving their bioreactivity exploring the unique advantages of nanotechnology. [20]

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.

The chemical-physical and biological properties of HA is strictly linked to their dimensions, the regulation of which requires a high level of chemical control at the nano-scale. The use of biomimetic HA synthetic crystals in orthopedics is considered to be very promising, owing to its composition, structure, nano dimensional and morphology similarity with the bone crystals. Nanostructured biomimetic materials offer much higher performances than their larger particle sized traditional counterparts, due to their large surface to volume ratio and unusual chemical/electronic synergistic effects. In Fig 1 are reported the TEM high resolution images of synthetic biomimetic HA nano crystals showing (d) the surface crystal disorder which confers them an high chemical reactivity.

Moreover, as the bone mineral phase is constituted of carbonated hydroxyapatite crystals with a length of about 100 nm, width of 20–30 nm and thickness of 3–6 nm, biomimetic need to be synthesized with similar nanoscale dimensions, as well as low crystallinity, non stoichiometric composition, crystalline disorder and the presence of carbonate ions in the crystal lattice. Indeed, the excellent biological properties of HA, such as nontoxicity, lack of inflammatory and immunitory responses and high bio-resorbability can be significantly increased by lowering the crystallinity of synthetic apatite.

Biomimetic carbonate-hydroxyapatite nanocrystals (CHA) 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 at about 100 nm and 20-30 nm size with acicular and plate morphology respectively. TEM images of synthetic CHA nanocrystals 20-30 nm sized, showing the plate shaped morphology and synthetic CHA nanocrystals 100 nm sized showing the acicular morphology are reported in Fig. 1 (a) and (b) respectively.

Powder X-ray diffraction patterns of plate shaped about 20-30 nm sized CHA nanocrystals and acicular shaped about 100 nm sized CHA nanocrystals show characteristic diffraction maxima of an apatite single phase (JCPDS 01-074-0565) characterized by a poor degree of crystallinity.

The X-ray diffraction investigations reveal that the crystal structures of the synthesized biomimetic CHA nanocrystals are very close to those observed in natural bone and dentin.

The same similarity can be observed comparing the FT-IR spectra of synthesized CHA nanocrystals and natural apatite of deproteined dentin or bone. In these spectra the characteristic absorption bands of phosphate and carbonate groups are clearly resolved. The absorption band at 1468 cm⁻¹ is related to the carbonate group substitution to the phosphate one, while the shoulder at 1545 cm⁻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 underlines 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.

Specific surface area of $100 \text{m}^2\text{g}^{-1}$ and $80 \text{m}^2\text{g}^{-1}$ has been determined for 20-30 nm sized CHA nanocrystals with plate morphology and synthesized 100 nm sized CHA nanocrystals with acicular morphology respectively. These specific surface area values obtained for synthetic nanocrystals are only slightly lower than the $110 \text{m}^2\text{g}^{-1}$ obtained for biological nanocrystals.

The surface Ca/P molar ratio determined by XPS analysis for CHA nanocrystals results 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 [21] This finding is coherent with the periphery surface disorder clearly observable in TEM high resolution image of biomimetic CHA nanocrystals reported in Fig.1(d).

Fig. 1 TEM high resolution images of Biomimetic synthetic HA nanocrystals at different enlargements.

Fig. 2 Tunnelling Electron Microscopy images of biomimetic hydroxylapatite nanocrystals synthesized according different methodologies in order to obtain different crystal morphologies and size dimensions.
The non stoichiometric surface Ca/P molar ratio of CHA nanocrystals is responsible for their high reactivity in the physiological environment representing also by this behaviour an excellent biomaterial like bone filler and substitute in orthopaedic, maxillofacial and odontoiastric surgery.

Synthetic biomimetic hydroxyapatite nanocrystals can be prepared by many different synthetic methods, including wet producing, hydrothermal, electrochemical and ultrasonic assisted methods, sol-gel and solid-state synthesis. [9-13]

The chemical-physical and biological properties of apatite powders are strictly linked to their dimensions, the regulation of which requires a high level of chemical control at the nano-scale. The variation of the reagents concentration, the reaction temperature and time, initial pH, ageing time and the atmosphere within the reaction vessel have been studied in order to synthesize hydroxyapatite nanocrystals with different stoichiometry, morphology, crystallinity, crystal size dimension, surface area and reactivity. [14, 15] Dimensions, morphology, crystallinity degree and surface properties represent the physical-chemical features which should be tailored in synthetic HA crystals for optimising their specific biomedical applications.

Fig. 3 TEM images of CHA nanoparticles synthesized in the presence of amino acids.

(a) Alanine. Panel A: general view; panel B: detail of the surface termination of a particle at high resolution.

(b) Arginine. Panels A and B: general view; panel C: view of particles stacked on each other; panel D: high resolution image of a particle oriented with the c-axis perpendicular to the image plane.

(c) Aspartate. Panels A and B: general view; panel C: detail of the surface termination of a particle at high resolution.
TEM images of biomimetic hydroxyapatite nanocrystals synthesized according different methodologies in order to obtain different crystal morphologies and size dimensions, in front of the same stoichiometry, are reported in Fig.2.

The effect of biological molecules on HA crystal growth, and consequently on their chemical-physical properties, have been widely studied and directly related to physiological or pathological calcification processes. [22, 23] At exemplum amino acids bound to the CHA crystals induce crystal growth inhibition predominantly at the Ca-rich surfaces during the initial stages of CHA crystallization. Moreover, high-resolution transmission electron microscopy measurements suggest a model for needle-shaped CHA nanocrystals formation in the presence of either arginine or aspartic acid based on the oriented aggregation of primary crystallite domains specifically along the c-axis direction and the self-assembly of preformed nanoparticles.

The results have significant importance for the control of the shape, morphology and aggregation of the CHA nanocrystals, while the observed surface modifications are of marked importance for the nature, stability and reactivity of the functionalized surfaces produced. [24] In Fig 3 are reported the TEM images of CHA nanocrystals synthesized in the presence of amino acids.

4. Synthetic biomimetic hydroxyapatite coating

In medical devices such as ceramic coated metallic implants or drug-eluting stents, mechanical strength can only be achieved with metals, which lack the required biocompatibility. Surface treatments to improve metal prosthesis biocompatibility have been extensively studied to coat opportuneley their surface. One of the most promising techniques in the production of biomimetic composite coatings is the electrochemically-assisted deposition on a metallic surface. This method not only allows overcoming the difficulty of depositing protein component by plasma spray or physical vapour deposition, but also allows controlling the coating process easily. With a view to reducing the thrombogenic potential of artificial blood-contact devices and natural tissues, we developed a new antithrombogenic coating material, consisting of an hydroxyapatite nanocrystals-heparin conjugate. A biomimetic surface activated coating, made of carbonate hydroxyapatite nanocrystals, functionalized with heparin, has been performed by an electrochemically-assisted deposition on titanium plate (Fig 4).

Fig. 4 TEM image of electrodeposited CHA nanocrystal functionalized by heparin (left) and SEM image of CHA-Heparin functionalized coating on titanium plate (right)

Hydroxyapatite biomimetic nanostructured coatings have been obtained on conductive material surface, like titanium, by an electrochemically assisted deposition in order to improve the surface bioactivity. [25] During the electrochemical process, the pH increases up to about 9.0 – 10.0 around the cathode, leading the precipitation of a mineral apatitic phase on the cathode electrode. [26-28] It is possible to obtain also calcium-phosphate/collagen coating on titanium surface with electrochemical cell containing slightly acidic collagen molecules suspension in a Ca^{2+} and PO_{4}^{3-} ions aqueous solution. In such a process, the collagen/calcium phosphate composite formation involves the self-assembly of collagen molecules into reconstituted fibrils during the contemporary crystallization of calcium phosphate mineral on the electrode surface. [25] In an electrochemical cell composed by two electrodes the nucleation-crystallization of carbonate hydroxyapatite and the collagen molecules self-assembling in to micro-fibres take place simultaneously for effect of the pH raising on the titanium electrode surface, allowing the formation of a nanostructured hydroxyapatite/collagen biomimetic coating. [26]

Following the biomimetic approach, inspiring to Nature, it is possible to realize [29] a nanostructured hydroxyapatite/collagen biomimetic coating. This is an innovative hybrid material to prepare innovative bone substitute and bone tissue engineering scaffold, with the possibility of synergistically join the porous bio inspired morphology and mechanical property of biomorphic silicon carbide.

Natural wood templates have been selected as a starting point to obtain open-pore geometries with wide surface area and microstructure allowing cell in-growth and reorganization and providing the necessary space for vascularization.
In fact the alternation of fibre bundles and channel-like porous areas makes the wood an elective material to be used as template in starting the development of new bone substitute biomaterials by an ideal biomimetic hierarchical structure. Particularly, Si/SiC wood-derived structures have been optimized to be employed as bioinert bone scaffolds. In fact during the last decade, Biomorphic Silicon Carbide (BioSiC) prepared by Si vapour or by Si melted reactive infiltration of carbon templates, previously obtained by pyrolysis of different kinds of wood, have received great attention. The reason of this is due not only to wood good thermo mechanical properties, but also to its large availability, renewability, low cost and very low environmental impact. 

The structural and morphological characteristics of BioSiC can change according to the different kind of wood utilized for its preparation. In fact, the macroscopic organization and structural hierarchy arrangement of cells and channels in coniferous, oak, bamboo and many other woods appear greatly different till the nano/micro size level. Biomorphic Silicon Carbide is a siliconized carbon material produced by reactive infiltration of molten Si into a carbon template. It is obtained from wood pyrolysis and represents a novel kind of porous ceramic constituted of elongated tubular cells with diameter of some hundred micrometers, preferentially aligned with the axis of tree trunk. BioSiC cannot be prepared by traditional technologies in porous ceramics manufacturing, but it represents a new generation of light, tough and strong material for biomedical applications. BioSiC good biocompatibility and biological response allow to consider this porous material a bone filler and substitute in orthopaedic, odontology, dental and maxillofacial implantation. Biomorphic SiC has been used as a support of suspended particles to realize a nickel/silicon carbide nanocomposite by electro deposition and thermal treatment (Fig. 5).

Fig. 5 TEM image of nanocrystal hydroxyapatite/collagen electrodeposited (left) and SEM image of HA/Collagen coating on BioSiC surface (right)

5. Synthetic biomimetic porous hydroxyapatite

Synthetic HA crystals dimensions, porosity, morphology and surface properties can be tailored changing synthetic parameters in order to improve the HA physical–chemical features and obtain an hydroxyapatite optimized for its specific biomedical applications. Since the last few decades calcium phosphate ceramics have been, and still are today, very popular implant materials for diverse orthopaedic clinical applications. Biomimetic porous HA in simulating spongy bone morphology (porosity varying from a microporosity > 1 µm to a macro porosity ranging from 300 to 2000 µm) has been prepared using various technologies to control pore dimension, shape, distribution and interconnections. However, HA ceramics processed by high-temperature treatment present a significant reduction of bio reactivity and growth kinetics of new bone due to the lack of resorbability. Porous bio ceramics with a low degree of crystallinity and an appreciable biore sorbability have been obtained by synthetic methods at lower temperatures. Colloidal processing, starch consolidation, gel casting and foam out have allowed to produce bio ceramics with a bimodal distribution of the pore size that can be tailored as a function of the sintering conditions. However, the low resorbability of sintered HA-ceramics appears useful when they have to be implanted with a defined 3D form.

Porous HA can be synthesised by a hydrothermal method directly from natural sea corals and cuttlefish bones. In fact, HA phase replaces aragonite whilst preserving its natural porous structure. Coralline apatite could be improved by a sol-gel-derived nano-coating layer to cover meso- and nano-pores. This new material can be utilized for bone graft applications where high strength requirements and longevity are required.

The interconnected network of pores promotes bone in-growth, but also allows bio ceramics to be utilized as drug delivery agents, by inserting different bioactive molecules or by filling the macro and micro pores with gelatine, which can act as delivery agent of these molecules. Many studies have demonstrated that hydroxyapatite ceramics can be used to deliver steroids, antibiotics, proteins, hormones, anticancer drugs. Porous ceramics closely mimicking spongy bone morphology have been synthesized by impregnation of cellulosic sponges with poorly crystalline HA water suspension.
6. Biomedical applications

6.1 Drug delivery system

Hydroxyapatite is known for its capability to bind a wide variety of molecules and most therapeutic agents for bone diseases under physiological conditions.[17, 18] A main challenge for innovative bone substitute biomaterials is their functionalization with bioactive molecules which can transfer information and act specifically on the biological environment. In other words, the implanted biomaterial can act as local scaffold for cell invasion and formation of functional tissue and, in the meantime, deliver previously loaded biomolecules. In this way HA nanocrystals can enhance their osteointegration or osteoinduction properties and also stimulate specific cellular responses at a molecular level. The adsorption and release properties of bioactive molecules are strongly influenced not only by the chemical properties of the drug molecules, but also by the chemical and structural characteristics of the HA substrates.[19]

Nowadays cisplatin is one of the five most used drugs in the treatment of solid tumours such as testicular, ovarian, and bladder carcinomas.[18] However, the use of cisplatin is limited by some serious drawbacks such as nausea, vomiting, ototoxicity, myelotoxicity and concentration dependent nephrotoxicity. [22] The systemic side effects of
cisplatin and related Pt-drugs can be lowered by several methods. A promising strategy makes use of carrier ligands able to promote a specific accumulation of the drug in the target tissue. [19] The local treatment of the tumor by the implantation of a matrix in which the drug has been embedded could represent another appealing prospective.

Furthermore, the surface functionalization of HA nanocrystals with bioactive molecules makes them able to transfer information to and act selectively on the biological environment, and this represents a main challenge for innovative bone substitute materials. In this context, the synthesis of apatite nanocrystals loaded with antitumor drugs that can be released by a controlled kinetic process represents an attractive goal. For example, the application of such a material to the chemotherapeutic treatments of osteosarcoma could result in tumour inhibition accompanied by low levels of systemic toxicity. [55]

![Fig. 8 Example of functionalized Hydroxyapatite nanocrystals with antitumoral drug](image)

### 6.2 Coating on enamel surface

CHA nanocrystals can aggregate in micro sized crystal clusters, whose dimensions increase when prolonging maturation time in mother solution at constant temperature and stirring.[56]

Actually biomimetic carbonate Hydroxyapatite nanostructured micro crystals are successfully utilized like active agent in some oral care products as toothpastes and mouthwashes. In fact biomimetic hydroxyapatite nanocrystals demonstrated able to remineralized the surfaces of enamel and exposed dentin. The dentin remineralizing effect of sintetic biomimetic CHA nannometric crystal has been studied in vivo with a scanning electron microscopy putting a CHA nano crystals slurry solution onto slices of dentin. Biomimetic hydroxyapatite nanocrystals demonstrated able to remineralize the surfaces of the dentin previously etched by orthophosphoric acid application and to progressively reduce the number of dentin exposed tubules in few minutes, till the quick regeneration of a new layer of mineralized tissue.

This rates of remineralization seems to be compatible with the development of toothpastes with remineralizing effect and able to contrast dentin hypersensitivity. [57] Scanning Electron Microscopic analysis allows to investigate the morphology of demineralized enamel and the features observed after remineralization procedures induced by biomimetic HA nanocrystals in vitro application. [58]

This study reveals an advantage of the 20 nm sized synthetic building blocks in respect of the 100 nm 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 orthophosphoric acid 37% for 1 minute compared with that of the enamel remineralized by synthetic 20 and 100 nm 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 in synthetic 20 and 100 nm sized CHA nanocrystals, showing appreciable higher intensity of the characteristic absorption bands of carbonate ions (at 1420-1460 and 1680 cm⁻¹) in respect of the same absorption bands present in the demineralised enamel ATR spectrum revealing that the surface of remineralized enamel is richer in carbonate than natural one, like synthetic 20 and 100 nm sized CHA nanocrystals.

It has recently been revealed that the basic building blocks of enamel are 20–40 nm HAP nanoparticles and it has been suggested that the enamel repairing effect of HAP can be greatly improved if its dimensions can be reduced to the scale of the natural building blocks. Compared with conventional HAP and nano amorphous calcium phosphate (ACP), in vitro experimental results demonstrate the advantages of 20 nm HAP in enamel repair by using hydroxyapatite nanoparticles as the building blocks. The results of 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 protection from erosion by using 20 nm particles as the repairing agent. However, these excellent in vitro repair effects cannot be observed when conventional HAP and ACP are applied. Clearly, nano HAP with a size of 20 nm shares similar characteristics to the natural building blocks of enamel so that it may be used as an effective repair material and anti-caries agent. This current study highlights the analogues of nano building blocks of biominerals during biomedical applications, providing a novel pathway for biomimetic repair. [59] SEM analysis has been used to investigate the morphology of both demineralized enamel and the features observed after a remineralisation process which utilises in vitro application of toothpastes containing both fluoride or CHA micro-clusters constituted of nanocrystals 100 nm size. The XRD diffraction maxima recorded on the
surface of human enamel slabs treated with fluoride containing toothpaste 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 removable by brushing procedures suggests the formation of chemical bonds between the synthetic CHA micro-clusters constituted of 100 nm 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. 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, the results highlight that biomimetic nano sized CHA crystals produce an apatite coating deposition on the enamel surface, which is much less crystalline than native enamel apatite, but consists of a new apatitic mineral deposition which progressively fills the scratches and pits (Fig 9). [21, 60]

Fig. 9: SEM image of enamel surface before (left) and after treatment with Hydroxyapatite nanocrystals based toothpaste (right)

6.3 Coating on biomedical implants

One of the most important problems in implantology is the osteo-integration of the prosthesis. With the electrodeposition method previously described, it is possible to improve the biomimetism of implant surface with the realization of a very thin coating of hydroxyapatite nanocrystals on the implant surface. The chemical-physical property of this HA and the morphology are very close to the bone hydroxyapatite and, to improve this property, it is possible to obtain a carbonate-hydroxyapatite nanocrystal coating on the implants surface (Fig. 10).

The composition of coating is not always the same; in fact it is possible to realize not only the HA coating, but also the HA/Collagen coating for innovative long bone substitutes [61] In this case the composition of the coating isn’t the bone composition, but it mimics the cortical bone composition in which there are collagen and HA nanocrystals together. With the electrodeposition method it is possible to realize this innovative hybrid-material with thickness that is function of electrochemistry parameters used during electrodeposition, the experimental conditions and the time of electrodeposition used.

Fig. 10: SEM image of titanium dental implant covered by biomimetic Carbonate- Hydroxyapatite nanocrystals coating obtained by electrodeposition method
7. Conclusions

The high degree of sophistication, miniaturization, hierarchical organization, hybridizing, reliablility, efficiency, resistance and adaptability characterizing natural materials which biogenic hydroxyapatites 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 representing important tools for the design and the synthesis of innovative materials and devices.

 Synthetic biomimetic hydroxyapatite nano and micro crystals exhibit excellent properties as bone filling biomaterials, such as biocompatibility, bioactivity, osteoconductivity, direct bonding to bone etc., exciting new applications of biomimetic HA in the fields of bone tissue engineering and orthopaedic therapies. 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. 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. This aim induces to surface functionalize HA nanocrystals with different linking agents, such as bisphosphonates, to anchor biologically active molecules which can be released by breaking the linkage as a consequence of external stimuli or internal chemical factors, such as pH and ionic force variation due to physiological or pathological biological processes.

Acknowledgements We thank the Universities of Bologna, (funds for selected research topics), the Chemical Center S.r.l. (for technical support and instrumental facilities) and the Inter University Consortium for Research on Chemistry of Metals in Biological Systems (C.I.R.C.M.S.B)

References


©FORMATEX 2010


