REVIEW PAPER

Fluorapatite: A Review of Synthesis, Properties and Medical Applications *vs* Hydroxyapatite

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Abstract: *Recently, using calcium phosphates and at the top of them, hydroxyapatite (HA) has been considered in medical and dental applications as an artificial biomaterial due to their chemical and structural similarity to the body's skeletal tissues such as bone and tooth. Because of reinforcement of hydroxyapatite's mechanical and biological properties by substitution of OH^- groups by F^- ions to produce fluorapaptite (FA) has been proven, in this article synthesis methods, properties and medical applications of fluorapatite and its pros and cons in comparison with hydroxyapatite have been reviewed.*

Keywords: *Fluorapatite, Hydroxyapaptite, Tissue engineering, Biomaterial, Biomedical application, Synthesis and characterization, Calcium phosphates.*

1. INTRODUCTION

Calcium phosphates due to their similarity to the structure of mineralized tissue, biocompatibility, high bioactivity and lack of toxic and allergic properties, have been considered as bone substitutes in various fields of medicine, especially orthopedics and reconstructive medicine, metal implant coatings, composite components, bone cements in maxillofacial and orthopedic surgery and supportive applications in toothpastes and mouthwashes [1-3].

Synthetic calcium phosphate was first time used by Albee in 1920 to regenerate bone tissue defect created in rabbit bones [1]. In the late 1960s, it was found that bioceramic based calcium phosphates could be used as biomaterial in reconstruction of bone defects [2]. Synthetic calcium phosphates is a relatively large group of amorphous and crystalline compounds which hydroxyapatite (HA) and fluorapatite (FA) are at top of them (table1) [3].

HA is the main mineral portion of vertebrate bones and teeth. Fluoride is an important constituent element in the human diet and is essential for the teeth and bone growth [5] and its recommended daily intake is 0.7 mg for small children and 3– 4 mg for adults [6]. According to WHO guidelines, F^- is essential to promote healthy bone growth and prevent dental cavities [7]. But fluoride ions can show an extraordinary chemical and biological activity and easily penetrate many types of cells in living organisms causing disturbances of their metabolism. Excessive exposition on this element may damage various tissues including the liver, kidney and brain. Therefore, the use of fluoride must be controlled and adapted to individual needs [6].

The mineral phase of hard tissue contains low but significant amounts of fluorine ions, some of which have replaced with OH^- groups in the apatite structure [8]. Studies have shown that replacement of OH^- with F^- could enhance mechanical strength, reduce dissolution rates and raise its stability in biological environments [9, 10]. Fluorapatite which is characterized by many attractive properties including bioactivity, biocompatibility, antibacterial behavior, high stability and good hardness values widely used in bone repair purposes [11-14].

It is known that the natural bone collects fluoride ions from the blood to form a fluoride-containing HA. [15]. FHA and FA are found in bone tissue and tooth enamel, respectively [16]. The presence of fluoride in saliva and plasma has been demonstrated and its necessity for dental and skeletal development has been confirmed. Fluoride plays a great role in suppressing tooth decay.

The osteoblastic response in term of adhesion, differentiation, and proliferation enhances by the incorporation of fluoride into HA [17, 18] and accelerates the process of mineralization and bone growth [19, 20].

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Table 1. Calcium phosphates in biomedical applications [3, 4]

Calcium phosphates (CaPs)		
Name (Abbreviation)	**Formula**	**Ca/P**
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Fluorapatite (FA)	$Ca_{10}(PO_4)_6F_2$	1.67
Chlorapatite (ClA)	$Ca_{10}(PO_4)_6Cl_2$	1.67
Carbonated apatite (CO_3A)	$Ca_{10}(PO_4)_6CO_3$	1.67
Amorphous calcium phosphates (ACP)	$Ca_xH_y(PO_4)_2.nH_2O$, n= 3- 4.5	
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2{\cdot}H_2O$	0.50
Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	0.50
Dicalcium phosphate dihyrate (DCPD, brushite)	$CaHPO_4{\cdot}2H_2O$	1.00
Dicalcium phosphate anhydrous (DCPA, monetite)	$CaHPO_4$	1.00
Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4{\cdot}5H_2O$	1.33
Tetracalcium phosphate (TTCP, hilgenstockite)	$Ca_4(PO_4)_2O$	2.00
α-Tricalcium phosphate (α-TCP)	α-$Ca_3(PO_4)_2$	1.50
β-Tricalcium phosphate (β-TCP)	β-$Ca_3(PO_4)_2$	1.50
Whitlockite (mineral)	$Ca_{18}(Mg,Fe)_2H_2(PO_4)_{14}$ $Ca_{18}(Mg,Fe)_2(Ca,)(PO_4)_{14}$	1.29 1.36

2. SYNTHESIS METHODS AND PROPERTIES

There are several ways to make HA: sedimentation method, hydrothermal synthesis, bacterial synthesis, in situ synthesis, hydrolysis, solid state reaction, mechanochemical activation and sol-gel [18, 21-30]. Among these methods, sol-gel has attracted a lot of attention due to its many benefits including high product purity, homogeneous composition, low synthesis temperature, low cost, ease of use and incorporation of ions [31, 32].

Incorporating fluorine into HA is done in different ways. FHA can be obtained by reacting between solid HA and NaF solution [10]. In an alternative method, the solid state reaction between HA and ammonium fluoride at high temperatures could produce FHA [33]. In the same method, HA can produce FHA in reaction with calcium fluoride [26]. FHA can also be obtained by the hydrolysis of tetracalcium phosphate in potassium hydrogen phosphate solution in the presence of potassium fluoride [34]. In addition mechanochemical synthesis method allows the obtaining of FHA or FA by replacing OH^- groups with F^- partially or completely [35]. All of these methods have advantages and disadvantages such as difference in initial chemicals, required devices, cost, time, byproducts, and composition of final product which could be varied in each method. In a simple way to prepare HA-FA granules, it is described that based on mixing fine HA particles with FA and spreading the powder/gelatin mixture in a diffuser medium, spherical raw granules would obtain which then should heated to exit gelatin and harden the granules [36].

In a study, HA and FA were prepared by isothermal mechanical blending method. The increase in sintering temperature and time was directly related to dramatical increase in density, hardness, elastic modulus. Fracture strength increased with increasing FA content of the sintered composite [37].

Zahrani *et al.* evaluated ball milling parameters on FA nanoparticle synthesis, illustrated that the size and number of balls did not have a significant effect on the time of synthesis and grain size of FA while reduction in rotation speed or balls to powder weight ratio increased the synthesis time and FA grain size [38].

Fluoroapatite (FA) is a bone and tooth non-organic substitution material which is used for repair and replacement. Fluorhydroxyapatite (FHA) is a synthetic compound that contains equal amounts of OH^- and F^- ions [39]. In tooth enamel, replacing some of OH^- with F^- ions leads to augmentation of crystals' hardness and stability and preserve it against low acidic pH of mouth environment [2, 40-42].

FA outperforms HA by having properties such as higher chemical stability, more stable crystal structure [43], lower degradation rate as scaffolds or implant coatings within the body [44, 45]. FA is structurally and chemically similar to HA [46]. In addition, FA could release fluoride at a controlled rate [10] and several studies have

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shown that fluoride stimulates bone formation [47, 48].

Physicochemical studies have shown that the density of FA is higher than HA, and it increases with increasing F^- levels in hydroxyapatite [49, 50]. Hardness, elastic modulus and fracture toughness also increase linearly with increasing F^-, except for the flexibility that is at its maximum in F/OH= 0.6 ratio [49].

2.1. Scaffolds

Regeneration of bone tissue requires the osteogenic signal, the response of host cells to the signal and a three-dimensional scaffold to support the growth of host cells, extracellular matrix formation, and a bed for vascularization [51].

Synthetic polymeric scaffolds for bone substitution are easily produced by 3D printing or other technologies, however, they lack the necessary minerals and trace elements to form bone. Even if these essential substances can be incorporated into the scaffold, only a limited range of compounds could have sufficient solubility and biodegradability [52]. Several natural and synthetic polymers have been used for synthesis of polymeric composite scaffolds such as agar, agarose, silicate gel, poly lactic-co-glycolic acid, poly caprolactone, polyvinyl alcohol (PVA), polyurethane or methyl methacrylate [52-58]

Borkowski *et al.* who studied on synthesis of FA for bone tissue regeneration by modified sol-gel method concluded that FA sintered at 800°C showed slow release of fluoride at a safe level for osteoblast cell line which supported osteoblast proliferation and could be considered as a suitable biomaterial for bone tissue defects [59].

Optimal porosity has a direct effect on bone regeneration. If the scaffold contains pores with 100- 400 micrometers diameter, it allows cells to migration and vascularization [60]. Nowadays, there are several ways to make high-porosity bioceramic scaffolds [61]. In addition to 3D printing, the gel casting method is a good method to make scaffolds with appropriate rigidity in various shapes and dimensions [62]. Natural proteins such as collagen and polysaccharides such as agarose are often used as a gelling agent in this method [61].

Seyedmajidi *et al.* have made nanocomposite foams using the casting method with combination of equal amounts of hydroxyapatite and bioactive glass (HA/BG) and fluorapatite and bioactive glass (FA/BG) with an average particle size of 78 and 42 nm, respectively and an average porosity size of 100- 400 μm. The maximum compressive strength and elastic modulus for the two composites were 0.22 and 17.8 for HA/BG and 0.13 and 22 for FA/BG, respectively. The results of the simulated body fluid (SBF) soaking test demonstrated good bioactivity, making it a good choice for use in bone tissue engineering at non load-bearing areas [63].

Chaari *et al.* while constructing porous fluorapatite ceramics using polyvinyl butyral as a porous agent investigated the effect of conditioning, including polyvinyl butyral concentration, sintering time and pressure in die-pressing techniques on the size and structure of the cavity. Results showed that the FA ceramics can be achieved with controlled hole features such as size, volume and structure [64].

Electrospun polycaprolactone (PCL) nanofiber is also appropriate for making three-dimensional scaffold for bone tissue [65], which are biocompatible and biodegradable and have a high surface/volume ratio to increase cell adhesion [66].

Yoon *et al.* succeeded in constructing collagen/FA composite with reinforced structure and similar to biomimetic appearance and appropriate cellular response using different amounts of ammonium fluoride as a source of fluoride through the co-precipitation method [67].

2.2. Cements

Calcium phosphate cements (CPC) have been considered as bone graft biomaterials in medicine and dentistry due to their setting, biocompatibility and bioconductivity properties [68, 69] which can be replaced with new bone without reducing volume [68]. Based on the CPC's compound, they set by mixing with distilled water, phosphate aqueous solution, or H_3PO_4 solutions [70-73]. The matter is that CPC with different compounds leads to produce end products such as dicalcium phosphate dihydrate [71], octa calcium phosphate [72] or fluoroapatite [73] in addition to HA. Since CPC's end products could control the absorption rate, it can be developed for different clinical applications with different degradation rates [74]. In a study was found that addition of NaF to these CPCs formed fluoridated hydroxyapatite or FA and CaF_2 [75], which significantly shows less

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solubility in acidic environments [74]. Another study was conducted by Kazuz *et al.* revealed that composite cements based on α-tricalcium phosphate (α-TCP) and nanostructured FA is appropriate as root canal filling material with the improved mechanical properties and no adverse effect on biocompatibility [70].

2.3. Implant's coating

Many studies have shown that long-term success of implants depends mainly on its osteointegration [76]. Many factors, such as surface chemistry, affect the rate of osteointegration [77]. Alteration and change of surface chemistry can be achieved by using a osteoconductive coatings to cause optimal osteointegration [45, 78].

Bone formation on implant surface is a prerequisite for osteointegration [77]. HA is the most widely used substance as a osteoconductive coating due to its high resemblance to the mineral phase of natural bone and tooth [79]. FA have been considered as osteoconductive coatings due to improved mechanical properties, cell adhesion, and compressive strength [80] and more desirable properties and than HA [81]. Substitution of Ca for Mg in FA could decrease coating corrosion rate, too [80].

Because of the altered properties of HA structure that can be obtained by replacing hydroxyl ions with fluoride to produce FHA or FA, these compounds are used as alternatives to HA coatings [82, 83].

The sol-gel method has benefits for implant coatings such as phase homogeneity and structure due to low temperature in its process. In addition, it is simple, affordable and useful for creating complex forms [84]. Study of Tredwin *et al.* has shown that it is possible to prepare HA, FA, FHA by this method effectively [83].

Coating absorption weakens and reduces bond strength between implant and substrate, which can lead to layering and thus implant failure [85]. FA has beneficial properties such as a more crystallinity, dense network and less potential in ion releasing than HA throughout dissolving [86-88]. FA coating has also more rigidity [89]. As a result, bonding of FA coating with bone can provide greater and long-term reliability and confidence [90].

In a comparative study of decomposition rate of coating with HA-FA-FHA compounds with thicknesses of 50 and 100 μm on implants were placed in dog jaws and retrieving them at 3, 6 and 12 months after implantation, it was concluded that there was no difference between groups with different thicknesses. FA and HA coatings were completely decomposed during the implantation period, while the FHA did not show a significant decomposition [10].

In another study, which the absorption rate of HA and FA ceramic coatings on load-bearing implants were placed within the dogs' internal femural condyl, after 25 weeks of implantation were investigated quantitatively and morphologically, HA coating replaced by 36% bone in compare with 29% for FA and were in direct contact with the implant surface, which suggests that despite the loss of ceramic coating the implant is firmly fixed [44].

In the study of bone growth on coated implants, it was significantly higher on HA-coated implants than FA-coated implants. FA was more stable than HA in coatings degradation. The results showed that HA is more osteoconductive than FA. The absorption rate for both coatings was about 20% of the initial thickness per year [84].

Of the various implant materials, titanium is a particularly suitable metal for orthopaedic and endosseous dental implants on account of its good mechanical properties and biocompatibility. Post-surgical and long-term mechanical stability is the fundamental requirement for the osteointegration of orthopaedic and endosseous dental implants. [91]. In a study HA, FHA and FA were coated on pure titanium disks using sol-gel rotary coating technique and were crystallized at different temperatures. Coating process velocity inversely affected coating thickness. Increasing in fluoride amount increased strength and thickness of coatings, and raising temperature increased strength [92].

In another study, after 25 weeks of TiAlV alloy implantation with HA and FA plasma spray coatings in goats, it seemed to FA coating remain intact, while the HA coating almost completely disappeared. In addition, FA-coated implants showed more mineralized bone than HA [86, 93]. Dhert *et al.* while using TiAlV alloy implants with FA coating on rabbits' inflamed knee joints, bone formation without any damage shows the beneficial properties of using FA coating on the implant to use in inflamed areas [94].

To enhance the biological properties of stainless

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steel 316L, plasma sprayed bioceramic coatings has been extensively investigated. Ghorbel *et al.* entered a little amount of FA into the alumina to increase bioactivity. The raw materials were successfully sprayed on 316L by atmospheric plasma spray technique[90].
In a study conducted by Kim *et al.* to create a HA/FA coating layer on ZrO_2, a coating with a thickness of about 30 micrometers was made with a strength bond of 22 MPa to the ZrO_2 body. The FA layer inhibits the reaction between HA and ZrO_2 and suppresses HA degradation [95].

3. BIOLOGICAL PROPERTIES

The presence of trace elements such as CO_3^-, Na^+, K^+, Mg^{2+}, Cl^-, F^- in the biological structure of apatite rise its bioactivity more than pure HA [19, 96]. Overall, HA's properties such as bioactivity, biocompatibility, biodegradability and osteoblastic adhesion and differentiation can be altered by structural changes due to ion replacement [97]. The physiological importance of fluorine ions in stimulating the mineralization and crystallization of calcium phosphates in bone formation has been proved [1, 2, 98]. The osteoblastic response in terms of adhesion, differentiation, proliferation and mineralization process is enhanced by importing fluorine into HA compared to pure HA [17]. In several studies, it has been proved that the amount of the released fluoride ions affects directly the cell attachment, proliferation, morphology and differentiation of osteoblast cells [99-101]. In addition, FA has better protein absorption and cell attachment than HA [102]. The first study which expressed that FA might have better biological properties than HA, was implemented by Kim *et al.* who concluded FA has a higher biocompatibility than HA [82]. Other studies have suggested that FA has similar biocompatibility to HA in condition of implantation in bone and internal bone growth [103-105]. These studies investigated the effect of different amount of F^- on the behavior of osteoblastic cells, and showed that fluoride levels affect on cellular attachment, proliferation, morphology, and differentiation, which are directly related to F^- release [99]. Fluorapatite crystal surfaces were shown to be biocompatible and biodegradable. Importantly, they are capable of inducing the mineralization of dental pulp stem cells [106].
In another study, addition of FA (10 to 25 percent by weight) to the diopside system (CaO, MgO, $2SiO_2$) dramatically increased the sintering ability of glass ceramic and the ability of apatite formation along with biodegradable behavior. In cell culture, cellular response was good cell survival and stimulation of osteoblastic differentiation [107].

3.1. Scaffolds

Nanocomposite membranes with polymeric matrix containing ceramic nanoparticles have been used in bone tissue regeneration and osteoconduction. Generally, these membranes are divided into two categories: non-absorbable and bio-absorbable. The principle challenge in absorbable matrixes is their rapid degradation before complete tissue regeneration [108].
Human osteoblast-like cells showed significant more proliferation and differentiation on the typical biomimetic freeze-dried composite scaffold containing collagen fibers and FA nanoparticle crystals than those on the composite scaffold containing HA nanoparticles (according to alkaline phosphatase activity) [67].
In an *in vitro* comparative study of dental pulp stem cells (DPSCs) culturing on polycaprolactone (PCL) scaffolds without and with FA, the expression of pre-osteogenic/odontogenic molecules upregulated on day 7 of culture on scaffolds containing FA, which indicates that this scaffold induces DPSCs differentiation. After 14 and 21 days of culture, increased alkaline phosphatase (ALP) activity in FA containing scaffolds was significantly higher than FA-free scaffold. This scaffold could be used as an odontogenic and osteogenic inducer to treat bony defects of the jaw, face, and alveoli and likely be helpful to survive dental pulp [66].
In an investigation on scaffolds were made of HA/BG and FA/BG nanocomposites by gel casting method which were implanted in rat's tibia defects, the scaffold containing FA reduced inflammation over time. Bone trabecular thickness and rate of new bone formation increased significantly over time. The FA containing scaffold after 60 days of implantation, moreover completely degradation, showed faster and more mineralization in contrast to HA containing one, indicating coordination in the rate of biodegradation and tissue regeneration (figure 1) [60]. The results of bioactivity test of

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machinable glass ceramic containing mica and FA made by high temperature melting method after 3 days of immersion in SBF showed the deposition of the apatite layer on the samples' surface, which indicates its good bioactivity [109].

3.2. Implant's coatings

Calcium phosphate coating is used on medical implants to enhance the alloy bioactivity and implant-tissue osteointegration [110] . Due to the differences between physical and chemical properties of coating and substrate, many defects such as cracks and fissures can occur in the interface, resulting in early destruction and failure in surgery. It has been demonstrated that Ti/HA composite made by powder metallurgy method is biocompatible and bioactive in *in vivo* and *in vitro* tests [111]. Due to the similarity in physical and chemical properties of FA with HA, Ti/FA composite is expected to be bioactive and biocompatible with an additional potential of fluoride ion advantages [112]. In a study with aim of determination of optimal HA/FA ratio in substitute compound made by thermal-sprayed mechanical method, the activity of osteoblastic cells and osteoclastic response on titanium disks coated by various amount of HA and FA were evaluated and was concluded that osteoblasts more attached and proliferated at a higher rate in compound with 40% FA.

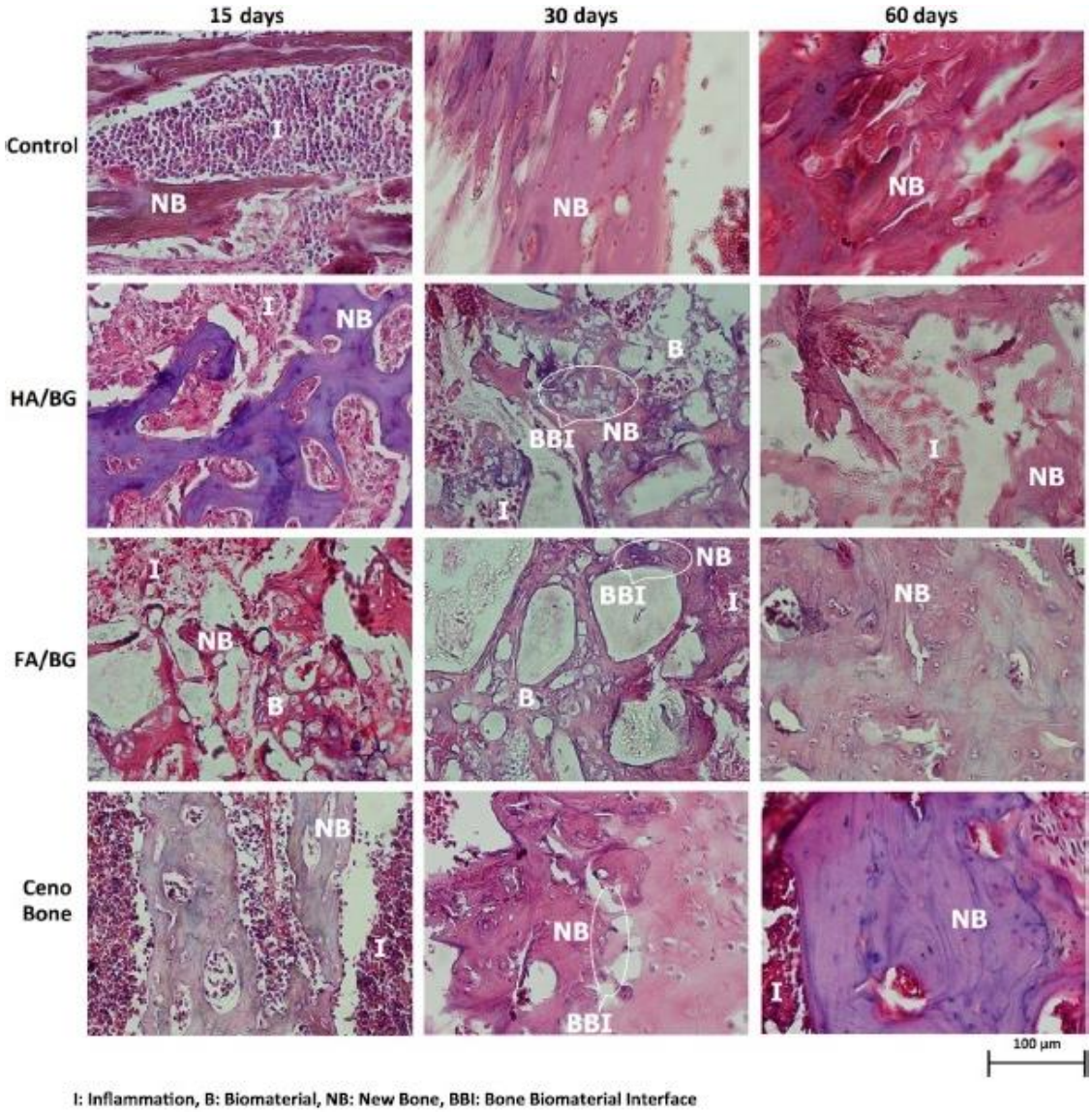

Fig. 1. Histopathologic features of implantation of biomaterials in Rats' tibia bone defect after 15, 30 and 60 days (400X magnification) [60].

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Also, cultivated cells on this compound for 7 days showed a higher level of mRNA expression for different types of proteins which involved in bone metabolism (figure 2) [113].
In another study, the FA glass ceramic coating on alumina showed a higher osteoblasts proliferation in compare with uncoated alumina [114].
In a comparison study between HA and HA/FA coatings on ZrO_2, osteoblast-like cells well spread and attached on the coating layer of porous scaffold. The alkaline phosphatase activity in proliferated cells on HA/FA coating was comparable to that of HA coating and greater than that of pure ZrO_2 [95].

3.3. Stem cells

Recently, adipose derived stem cells (ASCs) have received much attention for their application in tissue engineering [115, 116]. In study of ASCs cultivation on FA crystals surface, the expression of osteocalcin and the formation of minerals on the FA surface after 4 weeks was obvious than that of the metal surface [117]. FA-containing surfaces in contrast to the metal surfaces, stimulate osteogenic differentiation, cell mineralization and higher-density intracellular bone formation, which express important role of FA topography in biological processes [117-119]. When ASCs were cultured for 7 days on FA surfaces without any osteogenic induction, the expression of ALPL, bone morphogenic proteins including BMP3, 4, 5, GDFIO, EGF, PHEX, MSX1 and dentin sialophosphoprotein (DSPP) were stimulated. FA crystals surface also stimulated the expression of extracellular matrix associated genes such as type V, X, and II, and most notably collagen type IV, which were upregulated more than 500 times [117].
Among the various mesenchymal stem cells (MSCs), dental pulp stem cells (DPSCs) showed high self-regulating capacity, high proliferative rate and multi-linear differentiation potential. Because of these features they could collect cells easily and can be a good candidate for tissue engineering and bone and tooth regeneration [120, 121].

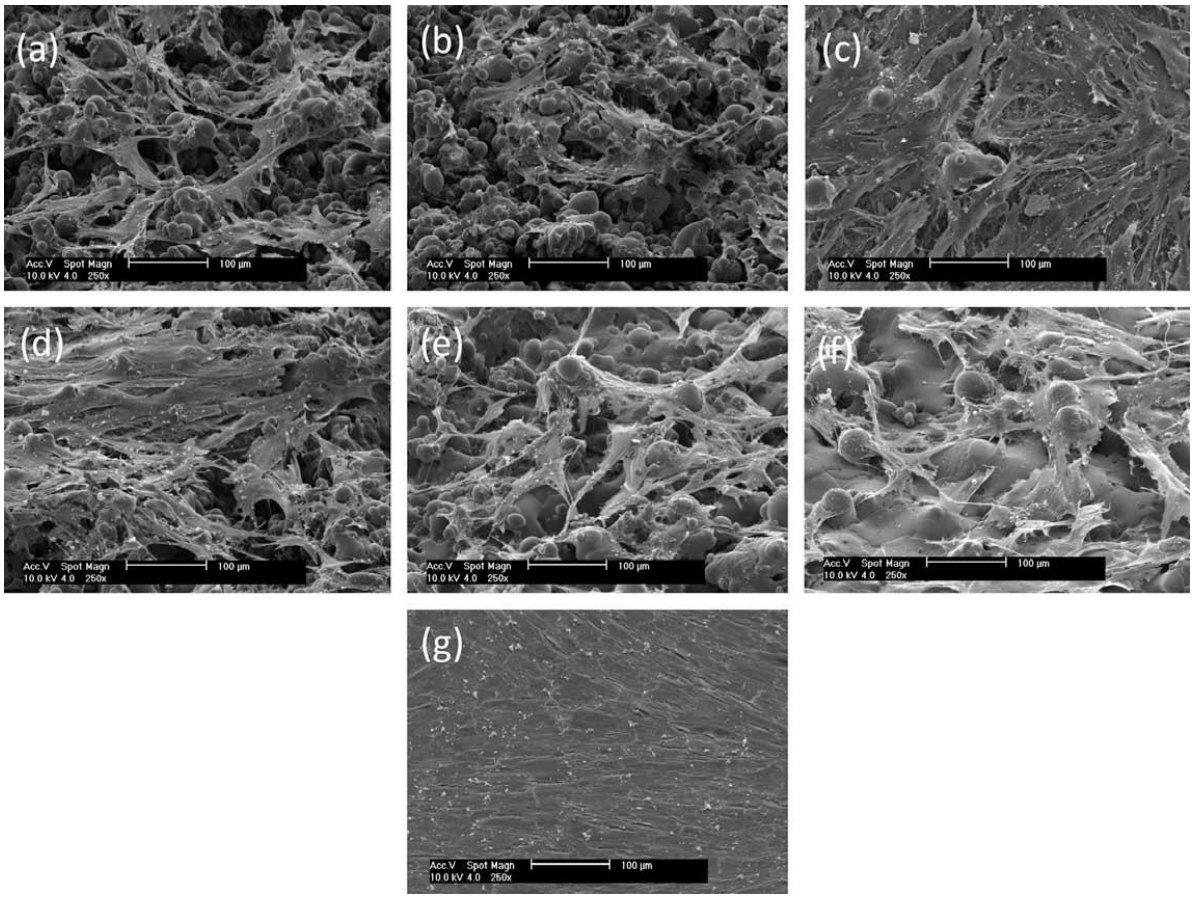

Fig. 2. Osteoblasts cultured on (a) hydroxyapatite, (b) 20% fluorapatite, (c) 40% fluorapatite, (d) 60% fluorapatite, (e) 80% fluorapatite, (f) 100% fluorapatite and (g) dentine after 7 days [113].

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It is suggested that the inherent properties of FA crystals can provide the requirements of pre-osteogenic growth factors from the culture medium to create a osteoinductive microenvironment for DPSCs. However, FA can interact with DPSCs by producing potent inductive factors that cause the cells osteogenic differentiation [66].

Studies have shown that FA has the capacity of attachment and mineralization of DPSCs, MG-63 osteoblast-like cells, and adiposed-drived stem cells without the addition of inductive supplements [66].

In a comparative study of polybutylene succinate with and without nano-FA, it was found that the FA containing compound had higher hydrophilic, compressive strength and elastic modulus. In addition, hMSCs showed good attachment and proliferation in contact with the composite surface and the cells had better growth and diffusion compared to the FA-free surface with a significantly higher alkaline phosphatase activity [122].

3.4. Cytotoxicity and genotoxicity properties

The possibility of cell toxicity by disrupting cell or chromosome activities is a challenge for biomaterials which are made for use in the body. Therefore, due to the possibility of cytotoxicity and genotoxicity of implanted biomaterials for patients and clinicians, we should be concerned about their safety [123].

In the study of cytotoxicity and antiproliferative properties of HA, FA, FHA on morphology, proliferation and cycle of NIH-3T3 cells, the results demonstrated that these properties were depended on time and concentration with HA< FHA< FA pattern. None of the tested materials caused necrotic / apoptotic death [39].

In another study in order to investigate the cytoxicity of HA, FA, FHA on colony formation, DNA damage and mutagenicity of Chinese V79 cells by direct cell counting methods in each colony, SCGE and Hprt gene-mutation and bacterial mutagenicity with *Salmonella typhimurim* TA 100, respectively. The results showed that the highest biomaterial concentrations (75% and 100%) caused poor colony growth inhibition (approximately 10%). On the other hand, the decrease in the number of cells per colony induced by mentioned above concentrations was 18.8 to 42.9%. The results of comet assay showed that biometrials caused DNA rupture by increasing the concentration with the HA< FHA< FA pattern. None of the biometrials induced a mutagenic effect compared with positive control (N-methyl-N′-nitro-N-nitrosoguanidine), and DNA rupture was probably the cause of inhibition of cells division in the V79 cell colony [124].

Manafi *et al.* studied on synthesis and evaluation of toxicity of fluorapatite-bioactive glass nanocomposite with 10%, 20% and 30% bioactive glass S53P4 and showed no toxicity resulted by MTT assay [125]. In a study, while selecting leukemia cells as a cell line model, the effect of biomaterial extracts on survival rate, cell proliferation and anti-proliferative activity mechanism were evaluated and the results showed that FA and FHA extracts inhibit leukemia cells growth and induce cell death programming through mitochondrial/ caspase-9/ caspase-3-dependent rout [126].

3.5. Antimicrobial properties

The problem with implantation of biomaterials in the bone is after surgery infection [127], which if not treated properly could lead to failure and resurgery. Antimicrobial properties are very important to prevent infection of biomedical devices. In recent years, mineral antimicrobial agents have attracted much attention in research for their sustainability and safety [128]. Most of these minerals are copper, silver, and zinc ions [129, 130]. On the other hand, it have been proven that fluoride ions in apatite suppress tooth and bone decay [131].

Stanic *et al.* reported that the antibacterial activity of fluorapatite is due to the fluoride which it releases at a low pH environment. Fluoroapatite is as an excellent reservoir and storage biomaterial for the delivery of unreacted fluoride at the site of bacterial infection. Fluoride ions in the FA crystal lattice inhibit the growth of existing bacteria in an acidic environment [11].

In the study of Shanmugam, HA and FA containing copper made by co-precipitation method and their antibiotic properties on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were investigated quantitatively. 100% reduction in the number of colonies against *Escherichia coli* was seen in the biomaterial with composition of $Ca_{10-x}Cu_x(PO_4)F_2$ (X= 0.15- 0.5) [132]. The

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presence of small amounts of copper is necessary for various activities in living organisms. FA-Cu has been reported as a catalyst in a number of organic transformations such as N-arylation of heterocycles with bromo and iodoarenes [133] and N-arylation of imidazoles [134]. The antimicrobial properties of HA containing fluoride and silver against *Escherichia coli* and HA containing fluoride against *Streptococcus mutans* have been reported [11, 135].

Unlike copper-containing HA, copper-containing FA showed increased antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Copper-containing FA also improved antifungal properties against *Candida albicans* [132] and showed better antibacterial activity in compare with copper-free FA, too [134, 135].

Guida *et al.* also reported that antibacterial activity of glass inomer cement is due to the release of fluoride ions. Studies on the release of fluoride ions in phosphate buffered saline (PBS) shed light on its activity mechanism [136].

In a comparative study of number of attached live bacteria to FA cement compared to enamel, it was found that FA cement greatly reduced the adhesion of *E.coli* compared with enamel, which is dependent on the presence of F^- ions [137].

4. TOOTH

Dental caries is one of the most widespread and costly infectious diseases that has been failed to overcome [138]. To prevent this issue, materials which have the ability to regenerate the enamel should be used. In conservative dentistry, gels, toothpastes and mouthwashes are usually used to maintain the natural structure of tooth by preventing changes associated with certain diseases such as dental caries and tooth hypersensitivity [3]. In this way, dental varnishes containing different forms calcium phosphates such as functionalized tricalcium phosphate (fTCP), casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP), amorphous calcium phosphate (ACP), calcium fluoride, and calcium fluoride have been investigated successfully [139, 140]. Furthermore, using of calcium phosphates, especially dicalcium and tetracalcium phosphate-based desensitizer was effective in reducing dentin permeability via a tubule occlusion mechanism [141].

In 2006, the first toothpaste containing HA was introduced in Europe to be used as an alternative to fluoride toothpaste, which was able to repair and remineralize enamel [142]. Similar studies found that toothpaste containing HA was used as the first step in the treatment of dental hypersensitization [143, 144].

In previous studies, acellular regeneration of enamel included soaking in supersaturated solution [145, 146], plasma spray [147], sol–gel [148], electro-deposition [149], and apatite application [150]. Among these methods, the use of apatite has attracted much attention so that the FA has been considered as an ideal material for enamel regeneration due to its excellent biocompatibility and bioactivity [151].

Fluorapatite glass ceramics have been used widely due to their good esthetics, biocompatibility and mechanical features [152]. It has been proved that using appatite additives in dental glass ionomer cements can improve their remineralization potential [153]. Youness *et al.* succeeded in preparing alumina-based carbonated fluorapatite nanobiocomposites with various amount of FA for dental applications by mechanical alloying using high energy ball milling [154].

Yamagishi *et al.* reported that a fluoridated HA paste could be used to repair fresh caries lesions [155]. It has been confirmed that fluoride not only can effectively enhance the acid resistance of apatite crystals, but the release of fluoride at a certain concentration from FA can prevent oral streptococci (*Streptococcus mutans, Streptococcus sanguinis*) metabolism [156, 157].

When calcium phosphates used as ion-releasing fillers in resin-based materials, they could contribute to extend the service life of adhesive restorations, remineralize caries-affected dentin or prevent caries lesions under sealants and orthodontic brackets [158]. In Altaie *et al.* investigation fluorapatite bundles and rods were substitute as filler in dental composite and new composites showed potential as a bioactive smart biomaterial [159].

Nowadays, there is a consensus on that the effect of fluoride is not systemic and is mainly local. Therefore, fluoride must be present in the right place (biofilm or saliva) and at the right time (when the biofilm is exposed to sugar or immediately after the biofilm is harvested) so that it can interfere with demineralization process and

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improve remineralization process. Values less than ppm are sufficient for this effect [160].

As it has been described, enamel dissolves by acid which produces from digestion of carbohydrates by bacteria in dental plaque. In acidic environment (pH less than 5.5), the biofilm liquid is become under-saturated with phosphate ions and the enamel is dissolved to restore balance. With the decrease of pH, if F^- is present in the biofilm fluid and the pH be higher than 4.5, the biofilm fluid is supersaturated with respect to FA and there is reprecipitation of minerals in the enamel [161]. In other word, HA is dissolved at the same time that FA is formed. The end result is reduction in the amount of enamel dissolution due to Ca and P ions return resulting from the dissolution of HA and formation of FA (figure 3) [160, 162]. This mineral has not been considered as remineralization but rather as a decrease in demineralization because the mineral redeposited is different from that lost. Furthermore, FA is deposited on the surface layer of enamel while HA is dissolved from the subsurface.

When presence of sugar decreases, the acid in the biofilm is cleared by saliva and converted to salt and the pH increases. This occurs when the enamel is cleaned by brushing. At this time saliva is able to remineralize it [163], but in the presence of F this effect is enhanced [164]. At the pH 5.5 and above, the biofilm fluid becomes super saturated with HA and FA which contains Ca and P. Therefore, lost Ca and P can be more effectively recovered [165] (figure 4).

In the study of Zhang *et al.* white FA crystalline paste was made using fluoride and apatite cement that can be used directly into the lesion cavity. The TTCP (basic) and DCPA (acidic) mixture powder reacted with water to form a cement paste, which first formed HA and then HA reacted with ammonium fluoride to form FA. FA cement can adhere well to the enamel surface when cement is set and can be repaired due to the similarity of FA cement in chemical composition and crystal structure to the enamel's apatite. Applying phosphoric acid on the enamel surface (acid etching) before using FA cement, causes the enamel to dissolve, leading to the reaction of the FA cement with the enamel's apatite layer, resulting in an integrated bond. According to the results of XRD, EDS and IR, the most rigid end product in applying of FA cement paste was fluoroapatite, which had elementary ratios of Ca/P= 1.67 and Ca/F= 5 [137].

Wang *et al.* also investigated a method to repair the progressive decays on enamel structure using FA/phosphoric acid paste directly. According to the results, $CaHPO_4.\ 2H_2O$ fluorinated paste, which had Ca/P= 1 ratio, showed a higher solubility of apatite, which made it inappropriate for enamel repair [166].

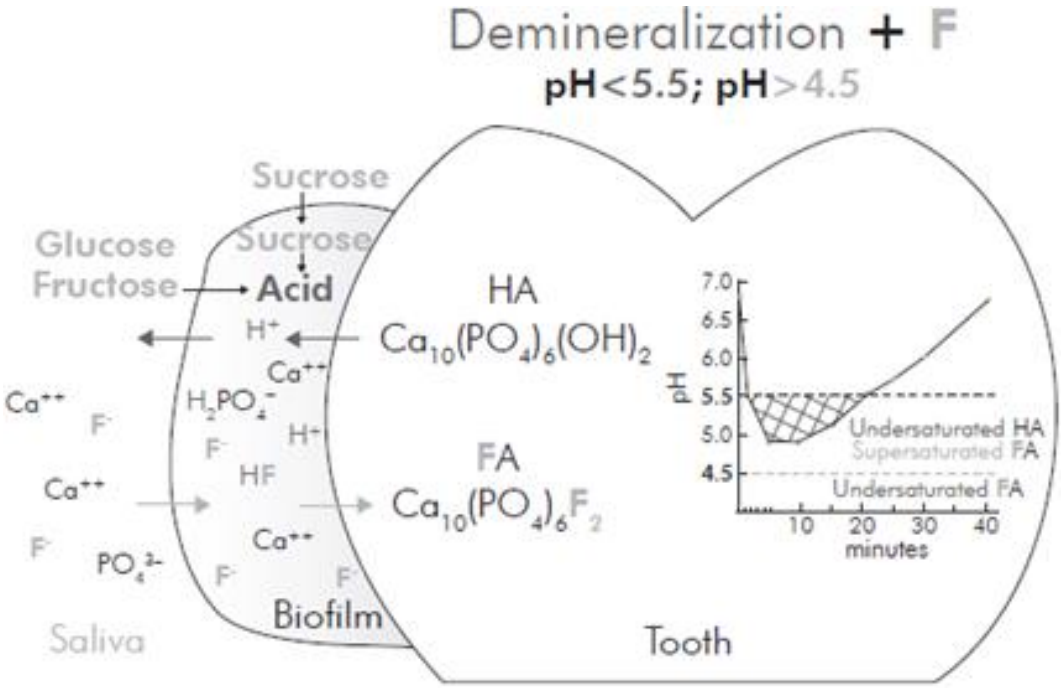

Fig. 3. Enamel demineralization in the presence of F^- in dental biofilm [160].

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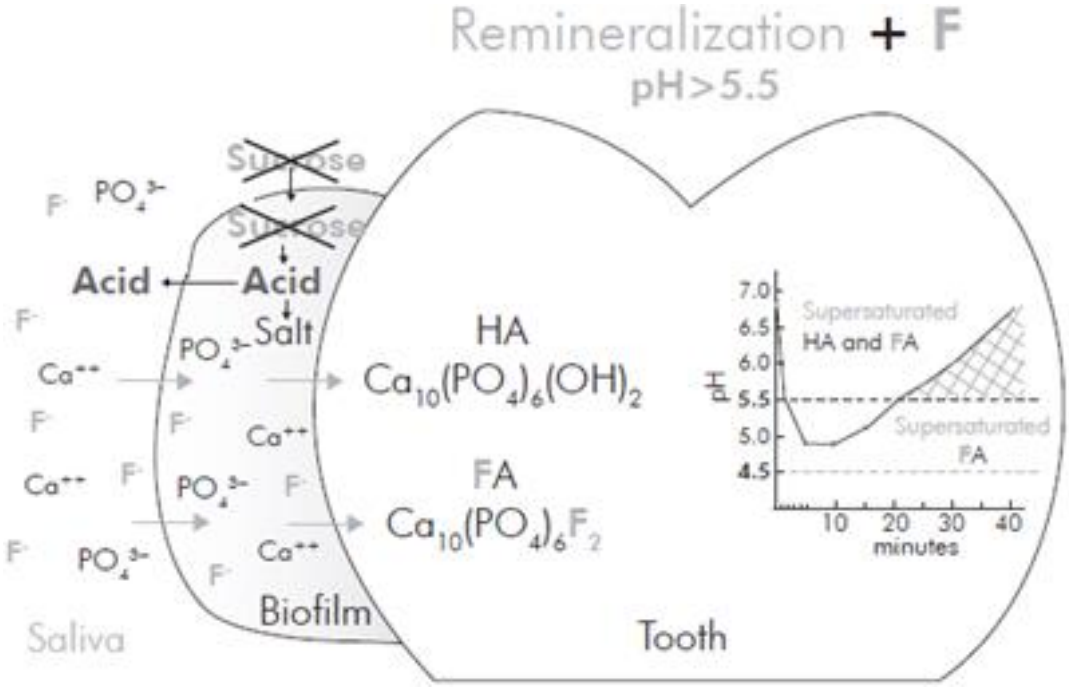

Fig. 4. Enamel remineralization in the presence of F^- in dental biofilm [160].

5. CONCLUSION

In this review article synthesis methods, various properties and applications of fluorapatite as coatings, scaffolds, etc. for medical purposes were discussed. Production of biomaterial with proper composition is very important for filling bone and dental defects that should be have appropriate properties while having biocompatibility and bioactivity. This goal should be analyzed from several aspects, which is needed to the study of biomechanical / biocompatibility properties such as strength, hardness, bioactivity, biodegradibility, cytotoxicity, genotoxicity, etc.

REFERENCES

[1] Vallet-Regi, M., Bio-ceramics with clinical applications, John Wiley & Sons, USA, 2014, 17-22.

[2] Park, J., Bioceramics: properties, characterizations, and applications, Springer Science & Business Media, USA, 2009, 80-112.

[3] Pajor, K., Pajchel, L. and Kolmas, J., "Hydroxyapatite and fluorapatite in conservative dentistry and oral implantology— a review." Materials. 2019, 12, 2683.

[4] Amjad, Z., Mineral scale formation and inhibition, Springer Science & Business Media, USA, 2013, 2-3.

[5] Feroz, S. and Khan, A. S. 7 - Fluoride-substituted hydroxyapatite, In: Khan AS, Chaudhry AA, editors. Handbook of Ionic Substituted Hydroxyapatites, Woodhead Publishing, 2020, 175-196.

[6] Błaszczyk, I., Ratajczak-Kubiak, E. and Birkner, E., "Advantageous and harmfully effect of fluoride." Farm. Pol. 2009, 65, 623-626.

[7] World Health Organization, Guidelines for drinking-water quality. Geneva, 2011, 216, 303-304.

[8] Finkelstein, M. J. and Nancollas, G. H., "Trace fluoride and its role in enamel mineralization." J. Biomed. Mater. Res., 1980, 14, 533-535.

[9] Moreno, E. C., Kresak, M. and Zahradnik, R. T., "Fluoridated hydroxyapatite solubility and caries formation." Nature, 1974, 247, 64-65.

[10] Gineste, L., Gineste, M., Ranz, X., Ellefterion, A., Guilhem, A., Rouquet, N. and Frayssinet, P., "Degradation of hydroxylapatite, fluorapatite, and fluorhydroxyapatite coatings of dental implants in dogs." J. Biomed. Mater. Res., 1999, 48, 224-234.

[DOI: 10.22068/ijmse.2430]

[11] Stanić, V., Dimitrijević, S., Antonović, D. G., Jokić, B. M., Zec, S. P., Tanasković, S. T. and Raičević, S., "Synthesis of fluorine substituted hydroxyapatite nanopowders and application of the central composite design for determination of its antimicrobial effects." Appl. Surf. Sci., 2014, 290, 346-352.

[12] Wang, L., He, S., Wu, X., Liang, S., Mu, Z., Wei, J., Deng, F., Deng, Y. and Wei, S., "Polyetheretherketone/ nano-fluorohydroxyapatite composite with antimicrobial activity and osseointegration properties." Biomaterials, 2014, 35, 6758-6775.

[13] Montazeri, N., Jahandideh, R. and Biazar, E., "Synthesis of fluorapatite–hydroxyapatite nanoparticles and toxicity investigations." Int. J. Nanomedicine., 2011, 6, 197-201.

[14] Youness, R. A., Taha, M. A. and Ibrahim, M., "In vitro bioactivity, physical and mechanical properties of carbonated-fluoroapatite during mechanochemical synthesis." Ceram. Int., 2018, 44(17), 21323-21329.

[15] Wang, X., Jin, T., Chang, S., Zhang, Z., Czajka-Jakubowska, A., Nör, J. E., Clarkson, B. H., Ni, L. and Liu, J., "In vitro differentiation and mineralization of dental pulp stem cells on enamel-like fluorapatite surfaces." Tissue. Eng. Part. C. Methods., 2012, 18, 821-830.

[16] Gross, K. A. and Rodríguez-Lorenzo, L. M., "Sintered hydroxyfluorapatites. Part I: Sintering ability of precipitated solid solution powders." Biomaterials, 2004, 25, 1375-1384.

[17] Basar, B., Tezcaner, A., Keskin, D. and Evis, Z., "Improvements in microstructural, mechanical, and biocompatibility properties of nano-sized hydroxyapatites doped with yttrium and fluoride." Ceram. Int., 2010, 36(5), 1633-1643.

[18] Evis, Z. and Sun, Z., "Structural and mechanical investigations of magnesium and fluoride doped nanosize calcium phosphates." J. Ceram. Process. Res., 2010, 11, 701-715.

[19] Kheradmandfard, M. and Fathi, M., "Preparation and characterization of Mg-doped fluorapatite nanopowders by sol–gel method." J. Alloys. Compd., 2010, 504, 141-145.

[20] Fathi, M. and Zahrani, E. M., "Fabrication and characterization of fluoridated hydroxyapatite nanopowders via mechanical alloying." J. Alloys. Compd., 2009, 475, 408-414.

[21] Sun, Z. P., Ercan, B., Evis, Z. and Webster, T. J., "Microstructural, mechanical, and osteocompatibility properties of Mg^{2+}/F^{-}-doped nanophase hydroxyapatite." J. Biomed. Mater. Res. A., 2010, 94, 806-815.

[22] Montazeri, L., Javadpour, J., Shokrgozar, M. A., Bonakdar, S. and Javadian, S., "Hydrothermal synthesis and characterization of hydroxyapatite and fluorhydroxyapatite nano-size powders." Biomed. Mater., 2010, 5, 045004.

[23] Mostaghaci, B., Fathi, M. H., Sheikh-Zeinoddin, M. and Soleimanian-Zad, S., "Bacterial synthesis of nanostructured hydroxyapatite using Serratia marcescens PTCC 1187." Int. J. Nanotechnol., 2009, 6, 1015-1030.

[24] Mollazadeh, S., Javadpour, J. and Khavandi, A., "In situ synthesis and characterization of nano-size hydroxyapatite in poly (vinyl alcohol) matrix." Ceram. Int., 2007, 33, 1579-1583.

[25] Kurmaev, E., Matsuya, S., Shin, S., Watanabe, M., Eguchi, R., Ishiwata, Y., Takeuchi, T. and Iwami, M., "Observation of fluorapatite formation under hydrolysis of tetracalcium phosphate in the presence of KF by means of soft X-ray emission and absorption spectroscopy." J. Mater. Sci. Mater. Med., 2002, 13, 33-36.

[26] Wei, M., Evans, J., Bostrom, T. and Grøndahl, L., "Synthesis and characterization of hydroxyapatite, fluoride-substituted hydroxyapatite and fluorapatite." J. Mater. Sci. Mater. Med., 2003, 14, 311-320.

[27] Liu, D.-M., Yang, Q. and Troczynski, T., "Sol–gel hydroxyapatite coatings on stainless steel substrates." Biomaterials, 2002, 23, 691-698.

[28] Fathi, M., Hanifi, A. and Mortazavi, V., "Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder." J.

[DOI: 10.22068/ijmse.2430]

Mater. Process. Tech., 2008, 202, 536-542.
[29] Fathi, M. H. and Zahrani, E. M., "The effect of rotation speed and time of milling on synthesis and properties of fluoridated hydroxyapatite biomaterial." Iranian. J. Pharm. Sci., 2008, 4, 201-208.
[30] Fathi, M. and Zahrani, E. M., "Mechanical alloying synthesis and bioactivity evaluation of nanocrystalline fluoridated hydroxyapatite." J. Cryst. Growth., 2009, 311, 1392-1403.
[31] Liu, D.-M., Troczynski, T. and Tseng, W. J., "Water-based sol–gel synthesis of hydroxyapatite: process development." Biomaterials., 2001, 22, 1721-1730.
[32] Zhang, S., Wang, Y., Zeng, X., Khor, K. A., Weng, W. and Sun, D., "Evaluation of adhesion strength and toughness of fluoridated hydroxyapatite coatings." Thin. Solid. Films., 2008, 516, 5162-5167.
[33] Krajewski, A., Ravaglioli, A., Roveri, N., Bigi, A. and Foresti, E., "Effect of fluoride, chloride and carbonate ions introduced by cyclic pH fluctuation on the physico-chemical properties of apatite-based ceramics." J. Mater. Sci., 1990, 25, 3203-3207.
[34] Jha, L., Best, S., Knowles, J., Rehman, I., D SANTOS, J. and Bonfield, W., "Preparation and characterization of fluoride-substituted apatites." J. Mater. Sci. Mater. Med., 1997, 8, 185-191.
[35] Bulina, N. V., Makarova, S. V., Prosanov, I. Y., Vinokurova, O. B. and Lyakhov, N. Z., "Structure and thermal stability of fluorhydroxyapatite and fluorapatite obtained by mechanochemical method." J. Solid. State. Chem., 2020, 282, 121076.
[36] Komlev, V., Barinov, S., Girardin, E., Oscarsson, S., Rosengren, Å., Rustichelli, F. and Orlovskii, V., "Porous spherical hydroxyapatite and fluorhydroxyapatite granules: processing and characterization." Sci. Technol. Adv. Mater., 2003, 4, 503.
[37] Bhadang, K. and Gross, K., "Mechanical property development in isothermally sintered mechanical blends of hydroxyapatite and fluorapatite." J. Australas. Ceram. Soc., 2005, 41, 56-67.
[38] Zahrani, E. M. and Fathi, M., "The effect of high-energy ball milling parameters on the preparation and characterization of fluorapatite nanocrystalline powder." Ceram. Int., 2009, 35, 2311-2323.
[39] Jantová, S., Letašiová, S., Theiszová, M. and Palou, M., "Comparison of murine fibroblast cell response to fluor-hydroxyapatite composite, fluorapatite and hydroxyapatite by eluate assay." Acta. Biol. Hung., 2009, 60, 89-107.
[40] Al-Noaman, A., Karpukhina, N., Rawlinson, S. C. and Hill, R. G., "Effect of FA on bioactivity of bioactive glass coating for titanium dental implant. Part I: Composite powder." J. Non. Cryst. Solids., 2013, 364, 92-98.
[41] Mojumdar, S., Kozánková, J., Chocholoušek, J., Majling, J. and Fábryová, D., "Fluoroapatite-material for medicine, Growth, morphology and thermoanalytical properties." J. Therm. Anal. Calorim., 2004, 78, 73-82.
[42] Nabiyouni, M., Zhou, H., Luchini, T. J. and Bhaduri, S. B., "Formation of nanostructured fluorapatite via microwave assisted solution combustion synthesis." Mater. Sci. Eng. C. Mater. Biol. Appl., 2014, 37, 363-368.
[43] Haverty, D., Tofail, S. A., Stanton, K. T. and McMonagle, J. B., "Structure and stability of hydroxyapatite: density functional calculation and Rietveld analysis." Phys. Rev. B., 2005, 71, 094103.
[44] Overgaard, S., Lind, M., Josephsen, K., Maunsbach, A. B., Bünger, C. and Søballe, K., "Resorption of hydroxyapatite and fluorapatite ceramic coatings on weight-bearing implants: A quantitative and morphological study in dogs." J. Biomed. Mater. Res., 1998, 39, 141-152.
[45] Dunne, C. F., Twomey, B., Kelly, C., Simpson, J. C. and Stanton, K. T., "Hydroxyapatite and fluorapatite coatings on dental screws: effects of blast coating process and biological response." J. Mater. Sci. Mater. Med., 2015, 26, 22.
[46] Driessens, F., "Relation between apatite solubility and anti-cariogenic effect of fluoride." Nature., 1973, 243, 420-421.
[47] Gross, K. A., Hart, J. and Rodríguez-Lorenzo, L. M., Fluor-hydroxyapatite solid solutions as alternative bioceramics, In: Key Engineering Materials, Trans Tech Pub, Switzerland, 2002, 165-170.

[DOI: 10.22068/ijmse.2430]

[48] Lau, K. H. W. and Baylink, D. J., "Molecular mechanism of action of fluoride on bone cells." J. Bone. Miner. Res., 1998, 13, 1660-1667.
[49] Gross, K. A. and Rodríguez-Lorenzo, L. M., "Sintered hydroxyfluorapatites. Part II: Mechanical properties of solid solutions determined by microindentation." Biomaterials., 2004, 25, 1385-1394.
[50] Gao, Y., Karpukhina, N. and Law, R. V., "Phase segregation in hydroxyfluorapatite solid solution at high temperatures studied by combined XRD/solid state NMR." RSC. adv., 2016, 6, 103782-103790.
[51] Burg, K. J., Porter, S. and Kellam, J. F., "Biomaterial developments for bone tissue engineering." Biomaterials., 2000, 21, 2347-2359.
[52] Denry, I., Goudouri, O.-M., Fredericks, D. C., Akkouch, A., Acevedo, M. R. and Holloway, J. A., "Strontium-releasing fluorapatite glass-ceramic scaffolds: Structural characterization and in vivo performance." Acta. Biomater., 2018, 75, 463-471.
[53] Kawata, M., Uchida, H., Itatani, K., Okada, I., Koda, S. and Aizawa, M., "Development of porous ceramics with well-controlled porosities and pore sizes from apatite fibers and their evaluations." J. Mater. Sci. Mater. Med., 2004, 15, 817-823.
[54] Oliveira, A. L., Malafaya, P. B. and Reis, R. L., "Sodium silicate gel as a precursor for the in vitro nucleation and growth of a bone-like apatite coating in compact and porous polymeric structures." Biomaterials., 2003, 24, 2575-2584.
[55] Kim, S.-S., Park, M. S., Gwak, S.-J., Choi, C. Y. and Kim, B.-S., "Accelerated Bonelike Apatite Growth on Porous Polymer/Ceramic Composite Scaffolds in Vitro." Tissue. Eng., 2006, 12(10), 2997-3006.
[56] Walsh, D., Furuzono, T. and Tanaka, J., "Preparation of porous composite implant materials by in situ polymerization of porous apatite containing ε-caprolactone or methyl methacrylate." Biomaterials., 2001, 22, 1205-1212.
[57] Zhang, R. and Ma, P. X., "Porous poly (L-lactic acid)/apatite composites created by biomimetic process." J. Biomed. Mater. Res., 1999, 45, 285-293.
[58] Andersson, J., Areva, S., Spliethoff, B. and Lindén, M., "Sol–gel synthesis of a multifunctional, hierarchically porous silica/apatite composite." Biomaterials., 2005, 26, 6827-6835.
[59] Borkowski, L., Przekora, A., Belcarz, A., Palka, K., Jozefaciuk, G., Lübek, T., Jojczuk, M., Nogalski, A. and Ginalska, G., "Fluorapatite ceramics for bone tissue regeneration: Synthesis, characterization and assessment of biomedical potential." Mater. Sci. Eng. C. Mater. Biol. Appl., 2020, 116, 111211.
[60] Seyedmajidi, M., Haghanifar, S., Hajian-Tilaki, K. and Seyedmajidi, S., "Histopathological, histomorphometrical, and radiological evaluations of hydroxyapatite/bioactive glass and fluorapatite/bioactive glass nanocomposite foams as cell scaffolds in rat tibia: an in vivo study." Biomed. Mater., 2018, 13, 025015.
[61] Ghomi, H., Fathi, M. and Edris, H., "Effect of the composition of hydroxyapatite/bioactive glass nanocomposite foams on their bioactivity and mechanical properties." Mater. Res. Bull., 2012, 47, 3523-3532.
[62] Chen, B., Zhang, Z., Zhang, J., Dong, M. and Jiang, D., "Aqueous gel-casting of hydroxyapatite." Mater. Sci. Eng. A. Struct. Mater., 2006, 435, 198-203.
[63] Seyedmajidi, S., Seyedmajidi, S., Alaghehmand, H., Hajian-Tilaki, K., Haghanifar, S., Zabihi, E., Rajabnia, R. and Seyedmajidi, M., "Synthesis and characterization of hydroxyapatite/ bioactive glass nanocomposite foam and fluorapatite/ bioactive glass nanocomposite foam by gel casting method as cell scaffold for bone tissue." Eurasian. J. Anal. Chem., 2018, 13, em17.
[64] Chaari, K., Ayed, F. B., Bouaziz, J. and Bouzouita, K., "Elaboration and characterization of fluorapatite ceramic with controlled porosity." Mater. Chem. Phys., 2009, 113, 219-226.
[65] Dong, Y., Liao, S., Ngiam, M., Chan, C. K. and Ramakrishna, S., "Degradation behaviors of electrospun resorbable polyester nanofibers." Tissue. Eng. Part. B.

[DOI: 10.22068/ijmse.2430]

Rev., 2009, 15, 333-351.
[66] Guo, T., Li, Y., Cao, G., Zhang, Z., Chang, S., Czajka-Jakubowska, A., Nör, J., Clarkson, B. and Liu, J., "Fluorapatite-modified scaffold on dental pulp stem cell mineralization." J. Dent. Res., 2014, 93, 1290-1295.
[67] Yoon, B.-H., Kim, H.-W., Lee, S.-H., Bae, C.-J., Koh, Y.-H., Kong, Y.-M. and Kim, H.-E., "Stability and cellular responses to fluorapatite– collagen composites." Biomaterials., 2005, 26, 2957-2963.
[68] Sugawara, A., Fujikawa, K., Takagi, S. and Chow, L. C., "Histological analysis of calcium phosphate bone grafts for surgically created periodontal bone defects in dogs." Dent. Mater. J., 2008, 27, 787-794.
[69] Sugawara, A., Nishiyama, M., Kusama, K., Moro, I., Nishimura, S., Kudo, I., Chow, L. C. and Takagi, S., "Histopathological reactions of calcium phosphate cement." Dent. Mater. J., 1992, 11, 11-16.
[70] Kazuz, A., Radovanović, Ž., Veljović, D., Kojić, V., Miletić, V., Petrović, R. and Janaćković, D., "α-Tricalcium phosphate/ fluorapatite based composite cements: Synthesis, mechanical properties, and biocompatibility." Ceram. Int., 2020, 46, 25149-25154.
[71] Mirtchi, A. A., Lemaitre, J. and Terao, N., "Calcium phosphate cements: study of the β-tricalcium phosphate— monocalcium phosphate system." Biomaterials., 1989, 10, 475-480.
[72] Markovic, M. and Chow, L. C., "An Octacalcium Phosphate Forming Cement." J. Res. Natl. Inst. Stand. Technol., 2010, 115, 257-265.
[73] Takagi, S., Frukhtbeyn, S., Chow, L. C., Sugawara, A., Fujikawa, K., Ogata, H., Hayashi, M. and Ogiso, B., "In Vitro and in Vivo Characteristics of Fluorapatite-Forming Calcium Phosphate Cements." J. Res. Natl. Inst. Stand. Technol., 2010, 115, 267-276.
[74] Suzuki, Y., Hayashi, M., Yasukawa, T., Kobayashi, H., Makino, K., Hirano, Y., Takagi, S., Chow, L. C. and Ogiso, B., "Development of a novel fluorapatite-forming calcium phosphate cement with calcium silicate: in vitro and in vivo characteristics." Dent. Mater. J., 2015, 2014-2255.
[75] Takagi, S., Ogata, H. and Chow, L., "Properties of DCPA+ CaO and DCPA+ $CaCO_3$ fluorapatite-forming calcium phosphate cements." J. Dent. Res., 2011, 90, Spec Iss B 2476.
[76] Cho, S.-A. and Park, K.-T., "The removal torque of titanium screw inserted in rabbit tibia treated by dual acid etching." Biomaterials., 2003, 24, 3611-3617.
[77] Albrektsson, T. and Johansson, C., "Osteoinduction, osteoconduction and osseointegration." Eur. Spine. J., 2001, 10(2), S96-S101.
[78] Kim, H.-W., Noh, Y.-J., Koh, Y.-H. and Kim, H.-E., "Enhanced performance of fluorine substituted hydroxyapatite composites for hard tissue engineering." J. Mater. Sci. Mater. Med., 2003, 14(10), 899-904.
[79] Hench, L., "Bioceramics: From Concept to Clinic" J. Amer. Ceram. Sot., 1991, 74, 1487-1510.
[80] Ferreira, J. M., Rajendran, V., Simonelli, G., Silva, A. C. M., Santos, L. C. L., Mattedi, S., Pontes, L. A. M., Costa, I., Rossi, J. L. and Baker, M. A., "Deposition and characterization of a sol-gel Mg-substituted fluorapatite coating with new stoichiometries." Appl. Surf. Sci., 2020, 505, 144393.
[81] O'Flynn, K. P. and Stanton, K. T., "Optimisation of the enamelling of an apatite– mullite glass–ceramic coating on Ti 6 Al 4 V." J. Mater. Sci. Mater. Med., 2011, 22, 2035.
[82] Kim, H.-W., Kim, H.-E. and Knowles, J. C., "Fluor-hydroxyapatite sol–gel coating on titanium substrate for hard tissue implants." Biomaterials., 2004, 25, 3351-3358.
[83] Tredwin, C. J., Young, A. M., Georgiou, G., Shin, S.-H., Kim, H.-W. and Knowles, J. C., "Hydroxyapatite, fluor-hydroxyapatite and fluorapatite produced via the sol–gel method. Optimisation, characterisation and rheology." Dent. Mater., 2013, 29, 166-173.
[84] Overgaard, S., Søballe, K., Lind, M. and Bünger, C., "Resorption of hydroxyapatite and fluorapatite coatings in man: an

[DOI: 10.22068/ijmse.2430]

experimental study in trabecular bone." J. Bone. Joint. Surg. Br., 1997, 79, 654-659.
[85] Bauer, T. W., "Hydroxyapatite: coating controversies." Orthopedics., 1995, 18, 885-888.
[86] Dhert, W., Klein, C., Jansen, J., Van der Velde, E., Vriesde, R., Rozing, P. and De Groot, K., "A histological and histomorphometrical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats." J. Biomed. Mater. Res., 1993, 27, 127-138.
[87] Lugscheider, E. and Weber, T. F., "Production of biocompatible coatings of hydroxyapatite and fluorapatite" Thermal Spray Technology, Proceedings of the National Thermal Spray Conference, USA, 1989, 337-343.
[88] Posner, A. S., "The mineral of bone." Clin. Orthop. Relat. Res., 1985, 200, 87-99.
[89] Bhadang, K. A. and Gross, K. A., "Influence of fluorapatite on the properties of thermally sprayed hydroxyapatite coatings." Biomaterials., 2004, 25, 4935-4945.
[90] Ghorbel, H. F., Guidara, A., Danlos, Y., Bouaziz, J. and Coddet, C., "Alumina-fluorapatite composite coating deposited by atmospheric plasma spraying: An agent of cohesion between bone and prostheses." Mater. Sci. Eng. C. Mater. Biol. Appl., 2017, 71, 1090-1098.
[91] Martini, D., Fini, M., Franchi, M., Pasquale, V. D., Bacchelli, B., Gamberini, M., Tinti, A., Taddei, P., Giavaresi, G., Ottani, V., Raspanti, M., Guizzardi, S. and Ruggeri, A., "Detachment of titanium and fluorohydroxyapatite particles in unloaded endosseous implants." Biomaterials., 2003, 24, 1309-1316.
[92] Tredwin, C. J., Georgiou, G., Kim, H.-W. and Knowles, J. C., "Hydroxyapatite, fluor-hydroxyapatite and fluorapatite produced via the sol–gel method: bonding to titanium and scanning electron microscopy." Dent. Mater., 2013, 29, 521-529.
[93] Dhert, W., Klein, C., Wolke, J., van der Velde, E., de Groot, K. and Rozing, P., "A mechanical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats." J. Biomed. Mater. Res., 1991, 25, 1183-1200.
[94] Dhert, W., Thomsen, P., Klein, C., De Groot, K., Rozing, P. and Ericson, L., "Fluorapatite-coated implants in experimental arthritis: the response of rabbit trabecular bone." J. Mater. Sci. Mater. Med., 1994, 5, 59-66.
[95] Kim, H.-W., Lee, S.-Y., Bae, C.-J., Noh, Y.-J., Kim, H.-E., Kim, H.-M. and Ko, J. S., "Porous ZrO2 bone scaffold coated with hydroxyapatite with fluorapatite intermediate layer." Biomaterials., 2003, 24, 3277-3284.
[96] Cai, Y., Zhang, S., Zeng, X., Wang, Y., Qian, M. and Weng, W., "Improvement of bioactivity with magnesium and fluorine ions incorporated hydroxyapatite coatings via sol–gel deposition on Ti6Al4V alloys." Thin. Solid. Films., 2009, 517, 5347-5351.
[97] Eslami, H., Solati-Hashjin, M. and Tahriri, M., "The comparison of powder characteristics and physicochemical, mechanical and biological properties between nanostructure ceramics of hydroxyapatite and fluoridated hydroxyapatite." Mater. Sci. Eng. C. Mater. Biol. Appl., 2009, 29, 1387-1398.
[98] LeGeros, R. Z., "Calcium phosphate-based osteoinductive materials." Chem. Rev., 2008, 108, 4742-4753.
[99] Tredwin, C. J., Young, A. M., Abou Neel, E. A., Georgiou, G. and Knowles, J. C., "Hydroxyapatite, fluor-hydroxyapatite and fluorapatite produced via the sol–gel method: dissolution behaviour and biological properties after crystallisation." J. Mater. Sci. Mater. Med., 2014, 25, 47-53.
[100] Joseph Nathanael, A., Mangalaraj, D., Hong, S. I., Masuda, Y., Rhee, Y. H. and Kim, H. W., "Influence of fluorine substitution on the morphology and structure of hydroxyapatite nanocrystals prepared by hydrothermal method." Mater. Chem. Phys., 2013, 137, 967-976.
[101] Taktak, R., Elghazel, A., Bouaziz, J., Charfi, S. and Keskes, H., "Tricalcium phosphate-Fluorapatite as bone tissue engineering: Evaluation of bioactivity and biocompatibility." Mater. Sci. Eng. C. Mater. Biol. Appl., 2018, 86, 121-128.

[DOI: 10.22068/ijmse.2430]

[102] Cheng, K., Shen, G., Weng, W., Han, G., Ferreira, J. M. and Yang, J., "Synthesis of hydroxyapatite/fluoroapatite solid solution by a sol–gel method." Mater. Lett., 2001, 51, 37-41.
[103] Heling, I., Heindel, R. and Merin, B., "Calcium-fluorapatite. A new material for bone implants." J. Oral. Implantol., 1981, 9, 548-555.
[104] Overgaard, S., Lind, M., Glerup, H., Grundvig, S., Bünger, C. and Søballe, K., "Hydroxyapatite and fluorapatite coatings for fixation of weight loaded implants." Clin. Orthop. Relat. Res., 1997, 336, 286-296.
[105] Qu, H. and Wei, M., "The effect of fluoride contents in fluoridated hydroxyapatite on osteoblast behavior." Acta. biomater., 2006, 2, 113-119.
[106] Wang, X., Jin, T., Chang, S., Zhang, Z., Czajka-Jakubowska, A., Nör, J. E., Clarkson, B. H., Ni, L. and Liu, J., "In vitro differentiation and mineralization of dental pulp stem cells on enamel-like fluorapatite surfaces." Tissue. Eng. Part. C. Methods., 2012, 18, 821-830.
[107] Kansal, I., Goel, A., Tulyaganov, D. U., Pascual, M. J., Lee, H.-Y., Kim, H.-W. and Ferreira, J. M., "Diopside (CaO MgO $2SiO_2$)–fluorapatite ($9CaO\ 3P_2O_5\ CaF_2$) glass-ceramics: potential materials for bone tissue engineering." J. Mater. Chem., 2011, 21, 16247-16256.
[108] Pihlstrom, B. L., Michalowicz, B. S. and Johnson, N. W., "Periodontal diseases." The lancet., 2005, 366, 1809-1820.
[109] Xiang, Q.-j., Liu, Y., Huang, B.-y. and Sheng, X.-x., "Preparation and bioactivity of machinable bioactive mica/fluorapatite glass-ceramics [J]." J. Cent. South. Univ., 2006, 37, 1025-1030.
[110] Dyshlovenko, S., Pierlot, C., Pawlowski, L., Tomaszek, R. and Chagnon, P., "Experimental design of plasma spraying and laser treatment of hydroxyapatite coatings." Surf. Coat. Tech., 2006, 201, 2054-2060.
[111] Ning, C. and Zhou, Y., "Correlations between the in vitro and in vivo bioactivity of the Ti/HA composites fabricated by a powder metallurgy method." Acta. biomater., 2008, 4, 1944-1952.
[112] Ye, H., Liu, X. Y. and Hong, H. P., "Cladding of titanium/ fluorapatite composites onto Ti6Al4V substrate and the in vitro behaviour in the simulated body fluid." Appl. Surf. Sci., 2009, 255, 8126-8134.
[113] Bhadang, K., Holding, C., Thissen, H., McLean, K., Forsythe, J. and Haynes, D. R., "Biological responses of human osteoblasts and osteoclasts to flame-sprayed coatings of hydroxyapatite and fluorapatite blends." Acta. biomater., 2010, 6, 1575-1583.
[114] Verne, E., Bosetti, M., Brovarone, C. V., Moisescu, C., Lupo, F., Spriano, S. and Cannas, M., "Fluoroapatite glass-ceramic coatings on alumina: structural, mechanical and biological characterisation." Biomaterials., 2002, 23, 3395-3403.
[115] Lindroos, B., Aho, K.-L., Kuokkanen, H., Räty, S., Huhtala, H., Lemponen, R., Yli-Harja, O., Suuronen, R. and Miettinen, S., "Differential gene expression in adipose stem cells cultured in allogeneic human serum versus fetal bovine serum." Tissue. Eng. Part A., 2010, 16, 2281-2294.
[116] Bieback, K., Ha, V. A.-T., Hecker, A., Grassl, M., Kinzebach, S., Solz, H., Sticht, C., Klüter, H. and Bugert, P., "Altered gene expression in human adipose stem cells cultured with fetal bovine serum compared to human supplements." Tissue. Eng. Part A., 2010, 16, 3467-3484.
[117] Liu, J., Wang, X., Jin, Q., Jin, T., Chang, S., Zhang, Z., Czajka-Jakubowska, A., Giannobile, W. V., Nör, J. E. and Clarkson, B. H., "The stimulation of adipose-derived stem cell differentiation and mineralization by ordered rod-like fluorapatite coatings." Biomaterials., 2012, 33, 5036-5046.
[118] Liu, J., Jin, T., Chang, S., Czajka-Jakubowska, A., Zhang, Z., Nör, J. E. and Clarkson, B. H., "The effect of novel fluorapatite surfaces on osteoblast-like cell adhesion, growth, and mineralization." Tissue. Eng. Part A., 2010, 16, 2977-2986.
[119] Liu, J., Jin, T., Chang, S., Czajka-Jakubowska, A. and Clarkson, B., "Adhesion and growth of dental pulp stem cells on enamel-like fluorapatite surfaces." J. Biomed. Mater. Res. A., 2011, 96, 528-

[DOI: 10.22068/ijmse.2430]

534.
[120] Gronthos, S., Mankani, M., Brahim, J., Robey, P. G. and Shi, S., "Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo." Proc. Natl. Acad. Sci., 2000, 97, 13625-13630.
[121] Gronthos, S., Brahim, J., Li, W., Fisher, L., Cherman, N., Boyde, A., DenBesten, P., Robey, P. G. and Shi, S., "Stem cell properties of human dental pulp stem cells." J. Dent. Res., 2002, 81, 531-535.
[122] Niu, Y., Cao, L., Wei, J., Ma, Y., Song, S., Weng, W., Li, H., Liu, C. and Su, J., "Development of a bioactive composite of nano fluorapatite and poly (butylene succinate) for bone tissue regeneration." J Mater. Chem. B., 2014, 2, 1174-1181.
[123] Seyedmajidi, S., Seyedmajidi, M., Zabihi, E. and Hajian-Tilaki, K., "A comparative study on cytotoxicity and genotoxicity of the hydroxyapatite-bioactive glass and fluorapatite-bioactive glass nanocomposite foams as tissue scaffold for bone repair." J. Biomed. Mater. Res. A., 2018, 106, 2605-2612.
[124] Jantová, S., Theiszová, M., Letašiová, S., Birošová, L. and Palou, T. M., "In vitro effects of fluor-hydroxyapatite, fluorapatite and hydroxyapatite on colony formation, DNA damage and mutagenicity." Mutat. Res. Genet. Toxicol. Environ. Mutagen., 2008, 652, 139-144.
[125] Manafi, S., Mirjalili, F., Hajisafari, M. and Orand, F., "Preparation and characterization of fluorapatite-bioactive glass S53P4 nanocomposite." J. Nanoanalysis., 2019, 6, 145-156.
[126] Theiszova, M., Jantova, S., Letasiova, S., Palou, M. and Cipak, L., "Cytotoxicity of hydroxyapatite, fluorapatite and fluor-hydroxyapatite: a comparative in vitro study." Neoplasma., 2008, 55, 312-316.
[127] Lidwell, O., Lowbury, E., Whyte, W., Blowers, R., Stanley, S. and Lowe, D., "Infection and sepsis after operations for total hip or knee-joint replacement: influence of ultraclean air, prophylactic antibiotics and other factors." Epidemiol. Infect., 1984, 93, 505-529.
[128] Korai, H., "Current situation and future of inorganic antimicrobial agent." J. Inorg. Mater. Jpn., 1999, 6, 428-436.
[129] Stanić, V., Dimitrijević, S., Antić-Stanković, J., Mitrić, M., Jokić, B., Plećaš, I. B. and Raičević, S., "Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders." Appl. Surf. Sci., 2010, 256, 6083-6089.
[130] Rameshbabu, N., Sampath Kumar, T., Prabhakar, T., Sastry, V., Murty, K. and Prasad Rao, K., "Antibacterial nanosized silver substituted hydroxyapatite: synthesis and characterization." J. Biomed. Mater. Res. A. 2007, 80, 581-591.
[131] Best, S., Porter, A., Thian, E. and Huang, J., "Bioceramics: past, present and for the future." J. Eur. Ceram. Soc., 2008, 28, 1319-1327.
[132] Shanmugam, S. and Gopal, B., "Copper substituted hydroxyapatite and fluorapatite: synthesis, characterization and antimicrobial properties." Ceram. Int., 2014, 40, 15655-15662.
[133] Kantam, M. L., Venkanna, G., Sridhar, C. and Kumar, K. S., "Copper fluorapatite catalyzed N-arylation of heterocycles with bromo and iodoarenes." Tetrahedron. lett., 2006, 47, 3897-3899.
[134] Kantam, M. L., Venkanna, G. T., Sridhar, C., Sreedhar, B. and Choudary, B. M., "An efficient base-free N-arylation of imidazoles and amines with arylboronic acids using copper-exchanged fluorapatite." J. Org. Chem., 2006, 71, 9522-9524.
[135] Turkoz, M., Atilla, A. O. and Evis, Z., "Silver and fluoride doped hydroxyapatites: investigation by microstructure, mechanical and antibacterial properties." Ceram. Int., 2013, 39, 8925-8931.
[136] Guida, A., Towler, M., Wall, J., Hill, R. and Eramo, S., "Preliminary work on the antibacterial effect of strontium in glass ionomer cements." J. Mater. Sci. Lett., 2003, 22, 1401-1403.
[137] Wei, J., Wang, J., Shan, W., Liu, X., Ma, J., Liu, C., Fang, J. and Wei, S., "Development of fluorapatite cement for dental enamel defects repair." J. Mater. Sci. Mater. Med., 2011, 22, 1607.
[138] Onuma, K., Yamagishi, K. and Oyane, A., "Nucleation and growth of hydroxyapatite

[DOI: 10.22068/ijmse.2430]

nanocrystals for nondestructive repair of early caries lesions." J. Cryst. Growth., 2005, 282, 199-207.
[139] Shen, P., Bagheri, R., Walker, G., Yuan, Y., Stanton, D., Reynolds, C. and Reynolds, E., "Effect of calcium phosphate addition to fluoride containing dental varnishes on enamel demineralization." Aust. Dent. J., 2016, 61, 357-365.
[140] Cochrane, N., Shen, P., Yuan, Y. and Reynolds, E., "Ion release from calcium and fluoride containing dental varnishes." Aust. Dent. J., 2014, 59, 100-105.
[141] Zhou, J., Chiba, A., Scheffel, D. L., Hebling, J., Agee, K., Niu, L.-n., Tay, F. R. and Pashley, D. H., "Effects of a dicalcium and tetracalcium phosphate-based desensitizer on in vitro dentin permeability." PloS. one., 2016, 11, e0158400.
[142] Pepla, E., Besharat, L. K., Palaia, G., Tenore, G. and Migliau, G., "Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature." Ann. Stomatol. (Roma)., 2014, 5, 108-114.
[143] Shaffiey, S. R. and Shaffiey, S. F., "Surface enamel remineralization by biomimetic nano hydroxyapatite crystals and fluoride ions effects." J. Ceram. Process. Res., 2016, 17, 109-112.
[144] Vano, M., Derchi, G., Barone, A. and Covani, U., "Effectiveness of nano-hydroxyapatite toothpaste in reducing dentin hypersensitivity: A double-blind randomized controlled trial." Quintessence. int., 2014, 45, 703-711.
[145] Li, H., Huang, W., Zhang, Y. and Zhong, M., "Biomimetic synthesis of enamel-like hydroxyapatite on self-assembled monolayers." Mater. Sci. Eng. C. Mater. Biol. Appl., 2007, 27, 756-761.
[146] Fan, Y., Sun, Z. and Moradian-Oldak, J., "Effect of fluoride on the morphology of calcium phosphate crystals grown on acid-etched human enamel." Caries. Res., 2009, 43, 132-136.
[147] Fogarassy, P., Gerday, D. and Lodini, A., "Agglomerated nanostructured particles disintegration during the plasma thermal spraying process." Mech. Res. Commun., 2005, 32, 221-239.
[148] Busch, S., Schwarz, U. and Kniep, R., "Morphogenesis and structure of human teeth in relation to biomimetically grown fluorapatite− gelatine composites." Chem. Mater., 2001, 13, 3260-3271.
[149] Liao, Y.-M., Feng, Z.-D. and Li, S.-W., "Preparation and characterization of hydroxyapatite coatings on human enamel by electrodeposition." Thin. Solid. Films. 2008, 516, 6145-6150.
[150] Chen, H., Clarkson, B. H., Sun, K. and Mansfield, J. F., "Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure." J. Colloid. Interface. Sci., 2005, 288, 97-103.
[151] Agathopoulos, S., Tulyaganov, D., Marques, P., Ferro, M., Fernandes, M. and Correia, R., "The fluorapatite–anorthite system in biomedicine." Biomaterials., 2003, 24, 1317-1331.
[152] Wang, G., Wang, S., Bian, C., Li, Y. and Shao, J., "Tribological behavior evaluation of dental fluorapatite glass ceramic." J. Aust. Ceram. Society., 2019, 55, 363-370.
[153] Duminis, T. "Development and characterisation of novel fluorapatite glass-ceramics for use in glass ionomer cements" Doctoral dissertation, Queen Mary University, London, 2019.
[154] Youness, R. A., Taha, M. A. and Ibrahim, M., "Dense alumina-based carbonated fluorapatite nanobiocomposites for dental applications." Mater. Chem. Phys., 2021, 257, 123264.
[155] Yamagishi, K., Onuma, K., Suzuki, T., Okada, F., Tagami, J., Otsuki, M. and Senawangse, P., "A synthetic enamel for rapid tooth repair." Nature., 2005, 433, 819-819.
[156] Wegehaupt, F. J., Solt, B., Sener, B., Wiegand, A., Schmidlin, P. R. and Attin, T., "Influence of fluoride concentration and ethanol pre-treatment on the reduction of the acid susceptibility of enamel." Arch. Oral. Biol., 2009, 54, 823-829.
[157] Nakajo, K., Imazato, S., Takahashi, Y., Kiba, W., Ebisu, S. and Takahashi, N., "Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci." Dent. Mater., 2009, 25, 703-708.

[DOI: 10.22068/ijmse.2430]

[158] Braga, R. R., "Calcium phosphates as ion-releasing fillers in restorative resin-based materials." Dent. Mater., 2019, 35, 3-14.
[159] Altaie, A., Bubb, N., Franklin, P., German, M. J., Marie, A. and Wood, D. J., "Development and characterisation of dental composites containing anisotropic fluorapatite bundles and rods." Dent. Mater., 2020, 36, 1071-1085.
[160] Cury, J. A. and Tenuta, L. M. A., "Enamel remineralization: controlling the caries disease or treating early caries lesions?" Braz. Oral. Res., 2009, 23, 23-30.
[161] Fejerskov, O. and Kidd, E., Dental caries: the disease and its clinical management, John Wiley & Sons, 2009.
[162] Mazyad, O., Elmarakby, A., Souror, Y., Abo-Ghannam, M., Salem, M., Salamah, M., Hawrani, A. and Showail, A., "Topical Application of Fluoride and Its Anti-Cariogenic Effect." Int. J. Adv. Res., 2017, 5, 1483-1488.
[163] Edgar, W. and Higham, S., "Role of saliva in caries models." Adv. Dent. Res., 1995, 9, 235-238.
[164] Dijkman, A., Huizinga, E., Ruben, J. and Arends, J., "Remineralization of human enamel in situ after 3 months: the effect of not brushing versus the effect of an F dentifrice and an F-free dentifrice." Caries. Res., 1990, 24, 263-266.
[165] Cury, J. and Tenuta, L., "How to maintain a cariostatic fluoride concentration in the oral environment." Adv. Dent. Res., 2008, 20, 13-16.
[166] Wang, X., Xia, C., Zhang, Z., Deng, X., Wei, S., Zheng, G. and Chen, H., "Direct growth of human enamel-like calcium phosphate microstructures on human tooth." J. Nanosci. Nanotechnol., 2009, 9, 1361-1364.

[DOI: 10.22068/ijmse.2430]