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Progress in Hydroxyapatite-Starch Based Sustainable Biomaterials for Biomedical Bone Substitution Applications

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Abstract

Hydroxyapatite is a calcium phosphate intensively proposed as a bone substitution material because of its resemblance to the constituents of minerals present in natural bone. Since hydroxyapatite's properties are mainly adequate for non-load bearing applications, different solutions are being tested for improving these properties and upgrading them near the target-values of natural bone. On the other hand, starch (a natural and biodegradable polymer) and its blends with other polymers have been proposed as constituents in hydroxyapatite mixtures due to the adhesive, gelling and swelling abilities of starch particles, useful in preparing well dispersed suspensions and consolidated ceramic bodies. This article presents the perspectives of

incorporating starch and starch blends in hydroxyapatite materials. Based on the role of starch within the materials, the review covers its use as i) polymeric matrix in hydroxyapatite composites used as adhesives, bone cements, bone waxes, drug-delivery devices or scaffolds, and ii) sacrificial binder for fabrication of porous hydroxyapatite scaffolds. The suitability of these materials for bone reconstruction has becomes a reachable aim considering the recent advancements in ceramic fabrication and the current possibilities of controlling the processing parameters.

Keywords: *Hydroxyapatite; starch; gelatin; chitosan; silk fibroin; cellulose acetate; starch blends; starch composites; porous ceramics; scaffolds; drug-delivery systems; bone cements.*

Introduction

The increasing prevalence of bone diseases and the higher life expectancy has led to an acute need for materials that can substitute bones. For this reason, the research and development of synthetic materials which can be further guaranteed as one of the potential viable options for replacing bone tissues is a major objective of biomaterials science research[1,2]. Unlike the bone grafts used in the current practice, the synthetic materials are available in large batches. Also, these materials can be easily processed and modified for fulfilling the requirements of various medical applications related to bone substitution. Moreover, the risks of biological contamination and immunologic incompatibility are lower if appropriate fabrication procedure are applied[3,4]. Various synthetic materials are currently being tested as potential bone substitution solutions[5–7]. One of the development strategies aims to produce materials that highly resemble the natural bone tissue, which is considered a composite material with a ceramic phase dispersed in an organic matrix[8]. Given the fact that bone is able to regenerate, the synthetic material used in substitution should possess appropriate bioactive and resorbable properties that enhances host tissue's regeneration and help in replacing the implanted material with newly-formed bone tissue. The bio-ceramics based on calcium phosphates are similar to bone's mineral component and have osteoconductive properties that allow the bone regeneration if the material is designed with a porosity appropriate for vascularization [9]. But despite their capacity to interact advantageously with the bone, calcium

phosphates have a brittle nature and are difficult to process in complex shapes. The use of calcium phosphates in composite materials with natural polymers is aligned with the strategy of mimicking the bone's organization[10]. Natural polymers are safe biomaterials currently used in various biomedical research as independent biomaterial[11–13]. Due to their availability within the nature, both vegetal and animal-derived polymers are biocompatible (some are also biodegradable) and may enhance ceramics' bioactivity[14–16].

Seeing the current biomedical issues and the huge potential of biomaterials, in this study, we aim to present the possibilities of using hydroxyapatite and starch mixtures for developing sustainable materials intended for medical applications. Both materials have proved their biocompatibility, and may be extracted from natural sources by simple, safe and economical procedure leading to materials with sustainability aspects[17–19]. The comparatively sustainability of starch based materials especially nanocrystals has been recently summarized[20]. This study reviews the methods for preparing hydroxyapatite-starch composites and the use of starch as porogen agent in sintering of hydroxyapatite. Both research directions are presented in this study through the results of material characterization techniques. In the final part, the study aims to identify the main perspectives for processing the hydroxyapatite-starch based materials through modern procedures of additive manufacturing.

Precursors

Hydroxyapatite

Hydroxyapatite is a calcium phosphate, stable in aqueous media, frequently characterized by its Ca/P ratio (Ca/P = 1.67 for stoichiometric hydroxyapatite) [21,22,4,23]. Due to its bioactive and osteoconductive abilities[24] and its capacity to promote cellular adhesion and differentiation[25], hydroxyapatite is frequently suggested for bone substitutions in bulk form [26], as coating on metallic substrates[27] [28] or as a component in composite materials[29–32]. Mehdi et al have reported their studies on the 3-Dimensional cell-laden nano-hydroxyapatite/protein hydrogels for bone regeneration applications[31]. **Fig.1** shows the schematic representation for fabrication of 3D cell-

laden HAp/hydrogel nanocomposites. It was reported in this study that a definite quantity of HAp nanoparticles (0.5 mg/mL) is optimal enough to get the desired strength and bioactivity for the developed gelatin hydrogel (**Fig. 2**). Furthermore through the encapsulation of MC3T3-E1 cells, it was established that procedure of composite formation is highly compatible with bone cells (**Fig. 3**).

As an alternative to classic chemical synthesis, various procedures aim to prepare hydroxyapatite starting from natural precursors such as coral, eggshell or different types of bone have been reported [33,34]. Some of these procedures have been found to be able to produce nonstoichiometric hydroxyapatite which incorporates in its lattice, carbonate groups and ions like Na and Mg. Hydroxyapatite proved its bioactive and osteoconductive nature in clinical studies[35,36]. The bioactive properties are primarily attributed to its capacity of absorbing serous proteins (such as fibronectin and vitronectin) and further promoting cells attachment on its surface through integrin's[37]. Instead of the great advantages of Hydroxyapatite, some major disadvantages are related to its poor mechanical properties, namely fragility and low tensile strength[10]. Besides the mechanical properties, hydroxyapatite is associated with higher risks of post-implantation infections[38]. However, constant developments in preparation and processing of this material, and the fact that approximately 70% of the human bone is built from a nonstoichiometric form of hydroxyapatite keep this highly researched bio ceramic on the list of potential solutions for bone substitution[3,4,21,23,39].

Starch

Starch is one of the most imperative natural polymer used in medical applications due to its low cost, renewable nature and ability to degrade into various environments without releasing toxic products[40]. Some of its physical properties, such as thickening, swelling and gelling abilities, are attributed to starch's composition and to the material alterations in aqueous media at higher temperatures[41]. Starch incorporates two macromolecules: amylose and amylopectin. The latter one is responsible for material's crystallinity. The molecular chains are organized to form alternative amorphous and crystalline regions which are further organized into growing rings. All these

functional units are grouped in micrometric units named "starch grains" which are available in plants such as potato, rice or wheat[42–44]. Various starch characteristics such as composition, grain interaction or swelling ability, depend on the extraction source[45]. It has been reported in the literature that starch with higher amylose content improved the different properties such as tensile strength, elongation, impact strength and tear resistance of polymeric films[46]. The starch gelatinization/melting is primarily determined by the mechanism of interaction with water in aqueous media. As the water molecules penetrate within the starch granules, the material is organized in an amylose gel with amylopectin-rich granules. Gelatinization depends on the quantity of water available in the solution: smaller water quantities will not ensure an adequate swelling for gel forming while excess water may lead to a separation between the amylose gel and the amylopectin crystallites. Starch gelatinization is also influenced by temperature and shear forces which increase molecules mobility and allows faster destruction of crystalline regions[47].

Despite its advantages, the use of starch is limited by the processing difficulties, poor mechanical properties and water-sensitivity to name a few. These are currently overcome by the adequate choice of additives and/or chemical modifications that shall maintain its long-term stability. Also, a better property control is ensured by combining starch with other materials for preparing *starch blends* (with other polymers) or starch composites (among which the most studies are the ones based on starch and phyllosilicates, clays and other polysaccharides).

Other materials used in hydroxyapatite-starch mixtures

- *Gelatin* – is a denatured form of collagen (which is the most abundant protein in the body). Gelatin is usually prepared by hydrolysis from animal-derived collagen and its properties depends both on collagen's origin and the conversion method. The material may be used in medical applications after cross-linking because it embodies multiple functional units available in biological environments (ex. various amino acids). Due to its biocompatibility and biodegradability, gelatin was tested as bone filler and wound dressing[48–52].

- *Chitosan* – is a biocompatible and biodegradable polymer susceptible to enzymatic degradation in the biological environment without releasing toxic products[53]. Chitosan is a deacetylated form of chitin, which is a natural polymer found in crustacean shells, insects' exoskeleton, in diatoms, in protozoa and in some fungi cell walls[14]. Chitosan has been tested in bone, nervous, retinal and blood vessel substitutions and as drug-delivery system due to its biocompatibility and antimicrobial properties[54–58] . **Fig. 4 (a,b)** shows the different modification of chitosan as well as biomedical applications[58,59].
- *Silk fibroin* – is a natural polymer derived from silk which is produced by silkworms. Silk fibroin is non-toxic and hemocompatible, and was used in sutures, biosensors, nanofibers and in bone reconstruction products[60,61].
- *Starch blends* – starch is frequently mixed with other polymers, especially biodegradable ones such as polycaprolactone (PCL), polylactic acid (PLA), and polyvinyl alcohol (PVA), for improving its moisture resistance, mechanical properties and long-term stability. Several studies describe these starch blends[62,63–67].

Additives used in hydroxyapatite-starch mixtures

Sometimes, additives are incorporated in hydroxyapatite-starch materials for enhancing various materials or product properties. Some of these additives are the ones used in processing the brittle starch-based materials. The mechanical properties of starch may be improved by adding other organic substances such as sorbitol, glycerol or citric acid, but their use may induce detrimental effects in final product appearance. Citric acid is given as an example in ref. [68]: although its addition enhances the granules fragmentation, the chemical bonds between starch and citric acid leads to formation of a large quantity of amorphous polymer, thus resulting in a smaller crystallization degree.

In hydroxyapatite-starch mixtures, the influence of various cross-linking and coupling agents in enhancing the mechanical properties was tested[62,63–67], [69,70]. Although their goal is the same, the action mechanism of the two agent types is different up to a point:

- *Cross-linking agents* - break down the polymeric chains improving the starch's processability. Divinylsulfone was considered an efficient cross-linking agent in organic-inorganic hybrids based on starch and hydroxyapatite. After its incorporation, both tensile strength and elastic modulus were improved without affecting other properties of green parts or the bioactivity of the final composite materials[69,70].
- *Coupling agents* may act as cross-linking agents but their main role is to fortify the interface between the mineral and organic component of the material for hydroxyapatite/SEVA-C composites. Various zirconates and titanates as well as silanes were tested as coupling agents. For these substances, the improvements in between-phases interface were found to be dependent on the chemical nature, pH and the metallic center of the additive[71].

Hydroxyapatite-starch composites

Composite materials are prepared from at least two components with different physical and chemical properties, separated at a macroscopic level[72–75]. These materials possess new and enhanced properties in comparison to their individual counterparts[76–79]. A biocompatible composite material is commonly named as "biocomposite"[70,71,80 81–87, 69]. Two types of components or phases may be distinguished in a composite material: a continuous phase, named matrix, and a dispersed phase, or reinforcing material, which enhances matrix properties[85,88–90]. Properties design may be achieved by carefully controlling the amount and the local of global phase repartition (a high volume of dispersed phase usually enhances the mechanical properties while its shape may determine an anisotropic behavior)[4,87]. Besides usual factors, like phase selection or the synthesis technology and processing methods, designing a composite material should take into account the biological response of the future medical devices. This is influenced mainly by the material characteristics, but also by the internal and external device appearance and by the mechanical loads from within its biological environment.

Biocomposites based on hydroxyapatite and starch are derived from biocompatible precursors which are currently tested and used as independent biomaterials. The organization of

these materials resemble the organization of bone tissues, which embodies a calcium phosphate dispersed in an organic matrix. In general, the addition of a ceramic phase in an organic matrix leads to an increase of elastic modulus while diminishing material's ductility, which is expected to happen in composites based on hydroxyapatite and starch. At the same time, some polymeric matrices may facilitate the processing and reduce the fragility of ceramic materials while enhancing their bioactivity. Using a biodegradable polymer also contributes to a better control of material resorption in biological environments and possibly to correlate the resorption with bone regeneration rate[56,91].

Preparation methods

Chemical synthesis of hydroxyapatite in aqueous starch solutions

The biomimetic synthesis of hydroxyapatite from starch solutions implies introducing Ca^{2+} and PO_4^{3-} ions in a gel that contains 5-20%wt. starch[92,93]. In fact, the gel diffusion method as biomimetic approach for generation of calcium phosphate/starch is of utmost importance in the synthesis of these materials. The advantages and limitations of different bio-based materials such as agar, agarose, gelatin for studying and analyzing the mechanisms of biomineralization process has been reported in detail[94]. A detailed study on the growth and characterization of calcium hydrogen phosphate dihydrate crystals from single diffusion gel technique was also carried out[95]. From this study, the authors have suggested a library for identifying and characterizing various kinds of kidney stones and their chemical characteristics based upon different analysis. Bone-like micro-porous calcium phosphate/iota-carrageenan composites were also synthesized by gel diffusion method[96]. In this study, for calcium phosphate/iota-carrageenan biocomposite crystals, the effect of different reaction parameters such as the molarity of ions, carrageenan concentration in the gel, and the type of diffusion technique was investigated using numerous analytical techniques. It was concluded from the study that iota-carrageenan has the high potential to produce novel crystal morphologies that depends upon the ion / gel concentrations along with the method of gel growth.

Starch gel contains OH^- groups that provide binding sites for Ca^{2+} and PO_4^{3-}, thus favoring hydroxyapatite crystallization. A detailed study on the state of art of Biopolymer/Calcium phosphate scaffolds for bone tissue engineering has been recently reported[97]. In this article, authors have reviewed the recent progress made on the usage of the naturally derived biopolymers as well as methods applied to generate biomimetic biopolymer/calcium phosphate composites along with their applications in bone tissue engineering

In all the investigation cases, starch has been used due to its polar nature that enhances the adhesion with hydroxyapatite and leads to a composite material. The ions generating solutions usually contain $CaCl_2$ [92,98–100]or $Ca(NO_3)_2$ [93,101], and Na_2HPO_4 [92,98–100] or $(NH_4)_2HPO_4$ respectively. A precipitate is obtained after processing of these solutions. This substance is further dried for isolating hydroxyapatite crystals and sometimes calcined[101]. Suprabha et al. have reported an extensive study on the synthesis and sintering of biomimetic hydroxyapatite nanoparticles for biomedical applications[101]. In this work, hybrid organicinorganic approach was used to synthesize particle of Hap using the protein: bovine serum albumin. The influence of different concentration of bovine serum albumin on particle size, degree of crystallinity and morphology of biomimetically synthesized hydroxyapatite particles was studied in deatil[101]. **Figure 5 & 6** shows the different morphologcal images of the synthesized particles and clearly depics the effect of the protein concentration.

The type of calcium phosphate that may be synthesized following these methods depends on the pH maintained during preparation. Hydroxyapatite was obtained at pH maintained at 9 [93], 10-10.5[99,100] and 11 [102]. Maintaining the pH at approximately 4.5 led to obtaining of brushite and octacalcium phosphate, with Ca/P ratios of 1.02 - 1.64[92]. The synthesis of calcium phosphates in starch gels begins at the negatively charged surface of starch particles from within the gel, where interactions with Ca^{2+} ions take place. An important role in crystal formation is played by the pores from the gel, which store Ca^{2+} ions and PO_4^{3-} groups and become nucleation centers for crystals. Further, the calcium phosphate nuclei join the starch molecular chains through hydrogen bonding and lead to

heterogeneous precipitations of calcium phosphate crystals[103,104]. The hydroxyapatite prepared by these methods was studied along with the starch matrix and after isolation. The material was found to exhibit a low degree of crystallinity[99,100,102]. The gel matrix has been reported to control the hydroxyapatite's growth and agglomeration[93]; higher starch concentrations contribute to a decrease of crystals dimensions[99]while increasing their specific surface and reducing agglomeration predispositions[93]. Lei et al in their studies have reported on the scalable synthesis of non-agglomerated and low-aspect ratio hydroxyapatite nanocrystals using gelatinized starch matrix[93]. Synthesis of non-agglomerated, low-aspect ratio hydroxyapatite and nanocrystals in large quantities was carried out in a facile manner with large yield. **Figure 7-9** shows the different SEM/ TEM images of the synthesized particles. It was concluded from the study that gelatinized starch matrix can be used without the surfactant and this technology can be easily extended to the production of another nanoscale particles.

It was confirmed from different studies that from starch solutions, it is possible to synthesise various crystal morphologies such as irregular rods and needles of hexagonal plates[99,100,102].

In vitro testing of hydroxyapatite synthesized from starch solutions revealed that it does not induce cytotoxic effects[100]. Also, bioactivity evaluation showed that a new layer of carbonated hydroxyapatite is formed on the material's surface after 14 days from immersion in simulated body fluid[99]. Another biomimetic process for preparing calcium phosphates was used for improving the surface properties of a composite based on starch and ethylene vinyl alcohol, known as SEVA-C. This process implies treating composite disks with sodium silicate followed by immersion in simulated body fluid[105,106]. This method allowed the preparation of composite materials with bioactive surfaces without any toxic effects. The biomimetic coating developed through this method have osteoconductive potential, and the final materials are considered adequate candidates for medical applications[106].

An "alternate soaking method" was used for formation of hydroxyapatite coating on hydrogen disks prepared from starch and N-vinylpyrrolidone (NVP)[107]. This method involved

preparation of starch/NVP hydrogel through irradiation-induced copolymerization, followed by alternate immersion of hydrogel disks in calcium chloride ($CaCl_2$) and sodium hydrogen phosphate (Na_2HPO_4). The hydroxyapatite crystals grown through the alternate soaking method were strongly attached to their supports due to the bonding (chemical) among hydroxyl and amino groups within the hydrogel and P-O and O-H groups within the crystals. The newly formed hydroxyapatite quantity was found to be dependent on the number of soaking cycles which was also correlated with a better thermal stability of the composite material. However, increasing the hydroxyapatite concentration in the composite was associated with a lower compression strength, possibly due to ceramic particles agglomeration (which induced a heterogeneous structure, with poor adhesion between phases). In vitro evaluation confirmed the non-toxic effect of the composite material and the increased hydroxyapatite concentration contributed to improving material's hemocompatibility. Also, on composites surfaces, there was formation of a carbonated hydroxyapatite layer due to the immersion in simulated body fluid predicting their bioactivity[107].

Mixing and heating

The preparation methods based on mixing and heating, were used for developing bone cements starting from the precursors namely starch and calcium phosphates and drug-delivery systems starting from starch and calcium sulfate[108]. Other studies aimed to obtain starch-gelatin-hydroxyapatite composites used for adhesives[109] and scaffolds[48,49]. For the preparation of bone cements, amorphous calcium phosphate, dicalcium phosphate and 0-0.2% starch were mixed with distilled water at ½ liquid/solid ratio. In general, the interaction between the aforementioned calcium phosphates and aqueous media leads to the formation of hydroxyapatite as final product but the transformation is not always completed. However, in this case, the starch addition led to the removal (or at least decrease below the detection limits) of dicalcium phosphate residues from cement's structure, hydroxyapatite being the only crystalline phase identified in XRD analyses. Starch addition also contributed to a more compact morphology, due to the dispersion of starch

particles between ceramic ones, and improved material's mechanical particles by reducing its porosity[110].

The procedure for developing gelatin-starch-hydroxyapatite scaffolds and adhesives included the solubilization of gelatin and starch in water, at approximately 60°C[48,49,110]. During adhesives' preparation, the gelatin-starch solution was mixed with 0-0.3%wt. hydroxyapatite until homogenization and the prepared suspension was subsequently heated at 60°C for 48h. Porous materials were obtained by this method, with interconnected pores and pore dimensions smaller than 1µm[109]. For scaffolds development, after the preparation of polymeric gel, 20-50%wt. hydroxyapatite was added following homogenization and cooling of the mixture. The composite was soaked in trisodic citrate for polymer cross-linking, then the materials were microwave vacuum dried[48,49]. Microwave drying allow the preparation of porous materials, with pores being formed due to pressure differences between inside and outside of the material, mainly due to steam generation. If microwave drying is performed in vacuum, beside pore formation, a rapid drying of the materials occurs. Prolonged drying improved composite's mechanical properties while the released thermal energy contributes to further polymer cross-linking[28,111].

After drying, the diameter of composite samples was found to be increased with approximately 40 % (from 3.5 to 5 cm), without any influence from material's composition. The morphological analysis revealed a homogenous distribution of ceramic particles in the polymeric matrix for hydroxyapatite concentrations above 20%wt. [48,49] (composites with 20%wt. hydroxyapatite contained micrometric ceramic clusters, formed by agglomeration of nanometric particles). Samples porosity was evaluated at 70-80% and pore diameters varied between 10 – 300µm[48,49] . XRD analysis showed that hydroxyapatite crystals formation is controlled by the gelatin and starch concentration in composites. Besides the hydroxyapatite phase, the analysis identified trisodic citrate residues from cross-linking. FT-IR analyses showed that starch matrix interacted with hydroxyapatite during preparation and led to the development of a carbonated hydroxyapatite[48,49]. The materials could not be fractured during compression testing. Rather the samples suffered a

densification, regardless of hydroxyapatite concentration. These results suggest that hydroxyapatite's fragility can be overcome by combining it with starch and gelatin[48,49,11148,49].

Electrospinning

Electrospinning has also been used in the preparation of the sustainable biomaterials for biomedical bone substitution applications[112,113]. Adequate levels of porosity for bone regeneration were also achieved by combining electrospun silk fibroin[112]. In this study, the nanobiocomposite scaffolds were developed from silk fibroin nanofiber-porous starch via wet electrospinning employing the methanol coagulation bath. The chopped electrospun nanofibers were subsequently incorporated into the starch matrix and were subjected to particulate leaching and freeze-drying. It was concluded from the study that the accumulation of silk fibroin nanofibers within starch matrix results in the reduction in the porosity, mean pore size, and water uptake of the fabricated scaffolds. Bioactive composite scaffolds from polycaprolactone nanofibers-chitosan-oxidized starch for bone regeneration were also prepared and characterized[113]. In this study, chitosan was combined with different quantities of oxidized starch using reductive alkylation process. It was concluded from the study that with the incorporation of higher amounts of starch, the porosity, mean pore size, and water uptake of the composite scaffolds increased. However, an opposite trend was obtained for compressive strength and modulus.

Blends of starch with poly (ε-caprolactone) – SPCL were also wet-spun for preparing starch-based scaffolds which were further cell cultured[114]. In this work, the impact of the porosity of starch-based fiber mesh scaffolds on the proliferation and osteogenic differentiation of bone marrow stromal cells cultured in a flow perfusion bioreactor was studied in detail. From the detailed investigation, it was demonstrated that the sequential development of osteoblastic cells is influenced by the scaffold structure and the porosity. A study on the combination of bioactive starch-based scaffolds and human adipose stem cells for bone tissue engineering was also carried out[115]. It was concluded from the study that the combination of a calcium silicate solution and wet-spinning technology facilitated the synthesis of starch-based scaffold having Si–OH functional groups. The beauty of this work lies in the elimination of the usage of chemical modification of the fiber mesh or calcium phosphate coating or to procure the bioactive scaffold.

Precipitation

Precipitation was used for the preparation of hydroxyapatite-starch materials in combination with other polymers like silk fibroin[61] and chitosan[57]. The preparation consists in precursor's homogenization at constant pH (9-11) and mixing until a precipitate was obtained. Composites preparation ends with ageing, washing, filtering and drying at 60-85°C[49,61]. The morpho-structural studies performed on composites obtained through precipitation were aimed to identify the role of starch in mixing hydroxyapatite with other polymers. For hydroxyapatite/chitosan mixtures, starch addition contributed to a better dispersion of ceramic particles within the material[57]. In this work, ternary nanocomposite system containing chitosan/ hydroxyapatite and starch (n-HA/CS–ST) was synthesized at room temperature. **Fig. 10-11** shows the SEM and EDX micrographs of (a) n-HA/CS, (b) n-HA/CS–ST and their respective SBF study after 2, 4 and 8 weeks (c–h). Rod shape n-HA. This work addressed different namely mechanical strength, biocompatibility and cytotoxicity. **Fig. 12** demonstrate the possible interaction between different components in n-HA/CS–ST nanocomposite.

Starch addition was directly correlated with a decrease in particles dimensions in starch/hydroxyapatite/silk fibroin composites (20-80nm), compared to binary composites. Also, higher starch concentrations influenced the hydroxyapatite crystals shape, enhancing their rice-grain appearance. High polymeric concentrations also increased composites porosity while decreasing pores' dimension and contributing to the development of a nanoporous structure[61]. Structural analysis performed on composites obtained through precipitation showed that increasing the starch concentration in hydroxyapatite/silk fibroin materials is accompanied by modifications of crystalline lattice parameters, crystallinity and crystallites dimensions. Those modifications are further associated with an increase in compressive strength. Typical values for compressive strength vary from 1.5 MPa in hydroxyapatite/starch to 3-7 MPa in hydroxyapatite/starch/silk fibroin and 9 MPa in hydroxyapatite/starch/chitosan[57,61]. Similarly with hydroxyapatite-starch materials prepared through other methods, materials obtained through precipitation proved their non-toxic behavior and bioactivity through in vitro testing.

Processing methods

Traditional processing methods: twin-screw extrusion and injection molding

Twin-screw extrusion is frequently used for processing starch based materials because shear forces ease the starch manipulation and enhances its gelatinization[47]. Twin-screw extrusion has been frequently used for incorporating hydroxyapatite in starch blends, especially SEVA-C, SCA (based on starch and cellulose acetate) and SPLC (based on starch and polycaprolactone)[67,106,116–120]. The studies performed on extruded mixtures of hydroxyapatite and starch blends evaluated the mechanical[119,121] and biological properties[116–118,120] of the obtained products. In general, the addition of hydroxyapatite in starch blends increased their stiffness and decreased the mechanical strength[122]. Mechanical testing performed in static regime showed that tensile strength of SEVA-C and hydroxyapatite based materials was correlated directly with hydroxyapatite concentration and indirectly with its particles size[121]. Dynamic mechanical analysis between -20 to +120°C did not reveal any significant influence of hydroxyapatite addition up to 30°C. The main material modification was an increase of moisture content on materials surface, observed at 60°C; authors attributed to a possible hydrolysis of polymeric molecules at higher temperatures. The events revealed by dynamic mechanical analysis of SEVA-C/hydroxyapatite composites are a possible indicator for in vivo degradation of these materials[121].

Injection molding was also used for processing SEVA-C/hydroxyapatite composites in order to form compression testing specimens. Testing results indicated that composites were characterized by an anisotropic behavior, with directionally improved mechanical properties. However, the variation range of processing parameters was limited due to molecular degradation of the polymeric components[122]. In general, the hydroxyapatite-starch based materials that were obtained by extrusion of injection molding were considered as one of the most promising biomaterials. After extrusion, their biocompatibility and imunocompatibility was studied in terms of osteoblast adhesion and cellular proliferation[122], cytokine secretion from in vitro mononucleate cells[120], leucocyte adhesion and activation[118] and host-tissue response after implantation in rats[116]. Despite

their nontoxic behavior, the composites based on starch blends which were subjected to thermal degradation induced by processing may release a higher content of molecular chains responsible for morphological modifications and even cellular death in the adjacent tissue. So, the use of such materials in medical applications was proposed to be possible only if the preparation and processing methods are optimized for avoiding the fabrication of thermos-degradable products[119].

Additive manufacturing: Perspectives of using selective laser sintering, tridimensional printing and material extrusion (robocasting) in fabrication of hydroxyapatite-starch materials:

Among the additive manufacturing methods, selective laser sintering, tridimensional printing and robocasting are suitable technologies for producing porous or dense bodies starting from ceramic powders and various additives[123,124]. The successful application of additive fabrication methods depends equally on the fine adjustments of processing parameters and on the material characteristics of raw materials: powders, binder or/and suspensions. When referring to powders, one of the main challenges in additive manufacturing is arranging the particles in thin layers. This is mainly influenced by (a) particle's dimensions, which is recommended to be between 15-35 μm. This recommendation is based on the average thickness of a printed layer (30-150 μm) and on the observation that particles with dimensions smaller than 5 μm are prone to agglomeration[125]; (b) powder flowability, required for constructing thin layers, improving resolution and simplifying the removal of trapped powders and (c) powder stability, required for an optimum binder spreading. Butscher et al have reviewed the structural and material approaches to bone tissue engineering in powder-based three-dimensional printing[126]. In this study, different aspects on the synthesis of bone tissue engineering scaffolds based upon three-dimensional printing (3DP) were discussed in detail. **Fig.13** shows the mechanical properties of natural materials in comparison with bulk materials for medical purpose. Binders characteristics of interest were: (a) wettability, which influences the dimensional accuracy of printed structures (too high wettability may lead to excess spreading of the binder while insufficient one will affect the mechanical integrity of the structure)[126] and (b) reactivity against the powder, which shall not modify the phase composition of the material; also,

the reaction among the powder and binder shall be rapid enough to avoid unwanted binder spreading and slow enough to allow the fusion of printed layers[126].

Ceramic suspensions shall possess a pseudoplastic nature adequate for passing through a nozzle, maintaining a stable shape after injection and supporting multiple layers, without generating materials defects. Notable examples of printed materials which may further inspire the additive manufacturing of starch and hydroxyapatite materials are: starch/dextran/cellulose[124], starch/cellulose[123] and starch/hydroxyapatite[108].

Tridimensional printing of hydroxyapatite/starch composites was found to be based on calcium sulfate and starch as raw materials. In the first stage, 85% wt. calcium sulfate and 15% wt. gelatinized starch were mixed and further printed in cylindrical shapes using a water-based binder. Hydroxyapatite was obtained after printing through immersing the printed bodies in disodium hydrogen phosphate solution for 48 h at 80°C. This method led to fabrication of structures with 64 % porosity and 0.15 µm pore dimensions. XRD analyses showed that the material embodies hydroxyapatite as a single crystalline phase and TEM analysis revealed its organization in rod-like crystals. The samples were further tested as antibiotic-delivery systems[108]. Important steps in ceramic printing were taken by selective laser sintering, an additive manufacturing method whose performances depend on proper adjusting of laser power and scanning rate, as well as on the powder particles dimension[127,128]. Selective laser sintering was tested for fabricating hydroxyapatite bodies[129,130] and starch blends[124].

Applications

A frequent objective for preparing hydroxyapatite/starch composites is fabrication of scaffold-type products[48,49,57,61,116–120,131]. A description of these bone reconstruction products and their associated requirements is briefly presented in the next part of this study, dedicated to hydroxyapatite porous products, and in dedicated reviews[132–135]. Scaffold for tissue fabrication were reviewed in an excellent article[133]. In this article, the concept of tissue engineering was introduced and it explained

in detail the relationship between materials science and tissue engineering. Different scaffold design principles were explained in detail in this article. **Figure 14** shows the tissue engineering concept[133]. Other potential uses for hydroxyapatite/starch composites are:

- *Adhesives* – The use of starch for preparing adhesives is attributed to the hydroxyl groups (OH^-) available in the material, which enable the hydrogen bonding between polymeric chains, thus enhancing adhesives fluidity[136]. Testing of adhesive properties of a hydroxyapatite/starch composite confirmed material's stability and strong adhesion to a glass surface in aqueous medium[109].
- *Bone cements* – starch addition in bone cements prepared from calcium phosphates improved composites resistance to degradation. For bone cements, this property is important for preventing cement washout before solidification. The addition of a small starch quantity also seem to improve materials injectability: a composite paste with approximately 1% wt. starch was completely extruded with lower shear forces than cements without starch. However, this improvement was not observed for higher starch quantities. The solidification time of bone cements increased with approximately 10 minutes after addition of 2%wt. starch[110].
- *Bone waxes* – bone wax is used for avoiding cancellous bone bleeding during surgical interventions. Due to its hemostatic properties, chitosan was used with starch and hydroxyapatite for adapting composite's properties for using it as bone wax. Preparation of a mixture consisting of oxidized starch, deacetylated chitosan and hydroxyapatite powder led to the formation of a predominant elastic paste with a malleable shape and adjustable viscosity (by water addition)[137].
- *Drug-delivery systems* – hydroxyapatite coating of SEVA-C (obtained through immersion in Ca^{2+} and PO_4^{3-} - rich solutions) can be further functionalized by the incorporation of specific drugs for treating bone pathologies. An example is sodium clodronate, which also contributed in the improvement of the mechanical properties of the composites evaluated through micro-hardness testing. Effective dosing of incorporated drug was able to maintain adequate levels of cellular viability and osteoclastic profiles without affecting the coating properties[105].

Another evaluation was performed by incorporating various types of antibiotics (gentamicin, vancomycin and fosfomicin) in hydroxyapatite/starch composites derived from calcium sulfate. Samples microstructure did not suffer major alterations except for an antibiotic layer on hydroxyapatite surface in samples impregnated with vancomycin. The preparation method did not affect antibiotic efficiency, which was maintained for 12 month after processing. However, only vancomycin addition led to satisfactory in vitro evaluation, because the samples containing gentamicin and fosfomicin exhibited a cytotoxic potential which disappeared after 2-4 days from the extraction. It was concluded that while hydroxyapatite/starch may be considered a suitable support system for drug delivery, the materials morphology and toxicity may be influenced by the type and concentration of incorporated drug[108,138].

Starch as porogen agent in sintered hydroxyapatite

Porous calcium phosphates are used in reconstruction of bone defects which are generally caused by trauma, inflammatory processes or osteoporosis[139–141]. Material's porosity ensures an extended surface contact between the ceramic implant and host tissue which contribute to develop a stronger interface thus reducing implant's mobility[139]. Besides this, the set of calcium phosphates characteristic also supports bone regeneration. A tridimensional porous structure used in bone reconstruction is defined as a "scaffold"[132,134,135,142,143]. In order to ensure its functionality, an ideal structure of this type must be bioresorbable (with a degradation/resorption rate adapted to bone regeneration rate) and biocompatible to provide a surface chemistry adequate for differentiation, cellular adhesion, and proliferation to exhibit mechanical properties adapted to the local biomechanics. Based on their use, scaffolds may be classified into the following categories:

- *Osteoconductive scaffolds:* produced from materials that support cellular migration and growth as well as ensure the mechanical support needed during bone regeneration[142,144–146].
- *Carrier scaffolds:* autogenic osteogenic cells are inserted in the scaffold for promoting osteoinduction. Also in this category, the porous drug-delivery devices[145,147–151].

A proper identification of the porosity requirements depends on the final use of the materials. Until now, some requirements for bone regeneration refer to pore dimensions/diameter[152,153], which shall be correlated with the dimensions of bone cells and other functional units within bone tissue*: (a)* ***100-200 µm*** are needed for cells accommodation; *(b)* ***75-100 µm*** ensure the development of non-mineralized osteoid tissue *(c)* ***10-75 µm*** allow the penetration of fibrous tissue within the scaffolds, thus providing the mechanical support during healing.

Another requirement for scaffolds porosity is pore interconnection (the existence of access routes between pores which encourage the penetration of bone cells and blood vessels[154], for a proper cellular migration and nutrients transport to the newly-formed tissues[150,155,156]. For ceramic implants, the recommended dimensions for pore interconnections are 20-50 µm[154]. These dimensions are smaller because interconnections only act as an access path while the pores have the main role in regeneration. For drug-delivery devices, pore interconnections contribute to a homogenous drug distribution within the entire volume of the scaffold[148]. Pore interconnections are also named "mesoporosity" [154]. Considering the specific requirements for ceramic scaffold porosity, proper morphological characterization programs need to be defined in the important stages of scaffold production. At least an equal importance needs to be assigned to compositional, structural and biological characterization, which shall confirm that the materials and products are adequate for the use in medical applications. A particular attention shall be directed towards mechanical characterization due to its inverse correlation with porosity.

Starch's role in fabrication of porous products

Selection of appropriate methods for ceramic scaffolds fabrication is currently targeted to allow the construction of complex tridimensional geometries while fulfilling the porosity requirements specific for bone regeneration. Multiple preparation, processing and consolidation methods for obtaining porous ceramics have been detailed in dedicated studies[154,157,158]. Some of these methods use special substances for pore generation, which are often named porogen agents. The general procedure consists in incorporating the porogen agent in the main material and then eliminating it

by a method which will form pores in the initial locations of the porogen. The traditional methods for eliminating the porogens are heating and dissolving[154]. Polymers such as naphthalene, starch or poly (methyl methacrylate) are eliminated through heating, while sugars and salts are eliminated by dissolving them in an appropriate solvent[154,159–161]. The processes involved in constructing the porous structures either use exclusively solid phases (as in pressing) or combine them with liquid ones ("wet processing methods" or "casting methods").

Even if the use of starch as porogen agent is possible both through dry or wet methods, second ones better explore the key-properties of starch: swelling and gelatinization in presence of water. Starch may be used alone or in combination with other porogens and is able to define a porous structure without affecting its biocompatibility, since starch is biodegradable. Starch incorporation as porogen agent was described for alumina[162–165], hydroxyapatite[166–168], β-tricalcium phosphate[169,170], α-tricalcium phosphate[170] and combination of the latter two ones[150,171].

In presence of gelatinized starch, the ceramic particles cohere and consolidate a solid body[164,166,168,172]. The inspiration for this consolidation method is attributed to the processing of starch-based polymers, for which the addition of water lowers starch melting temperature and plasticizes the materials. Water evaporation leads to pore formation through "foaming". Besides its pore forming ability, starch also contributes to consolidation of green ceramics and ensures their mechanical stability until sintering. The heating of starch in presence of water (at approximately 70°C) leads to swelling. During mixing, the available water quantity is decreased by starch swelling so the mobility of ceramic particles is drastically reduced until it ends with their consolidation in a solid body. The crystalline components of starch are absorbed at the ceramic particles' surface and act as a binder thus further improving the mechanical resistance of the consolidated green part[168].

In hydroxyapatite fabrication, starch consolidation was used for obtaining porous ceramic structures, as alternative to polymeric sponge method [167]and foaming methods[150,172] for avoiding risks like development of density gradients or particle segregation during elimination of liquid phase[164,168]. One of the major challenges in ceramic processing through casting methods is

preparing a well-dispersed suspension. The rheological properties of ceramic suspensions are influenced by the type and quantity of dispersing medium, the particle size of ceramic powders, and by the type and quantity of additives used for slurry preparation. In an obvious manner, the quantity of water used as dispersing medium is a key-step in starch consolidation of hydroxyapatite slurries. Moreover, other substances may be added in starch-ceramic mixtures, with different purposes: deflocculant/dispersing agents (acetone, ammonium polycarbonate[172], and sodium polyacrylate), foaming agents (sodium lauryl sulfate, concentrated foam bath or lubricants[148](magnesium stearate). To ensure a proper green part density, the solid content incorporated in the ceramic slurry needs to be maximized as possible. The explanation for this requirement is that once the solids concentration is increased, the liquid between the ceramic particles (which destabilizes the green part) is decreased and the material may maintain a shape more easily. Ceramic slurries obtained with unprocessed commercial hydroxyapatite particles, water and sodium acrylate as a dispersing agent are able to embody approx. 15% vol of solids (but this concentration can be increased up to 60%vol. if the hydroxyapatite is previously calcined, due to an increase in its crystallinity and/or particle dimensions). Starch addition was associated with a decrease in slurry viscosity, because the starch lessens the inter particle bonding. However, this may be partially influenced by the dispersing agents, especially at low shear forces. Besides starch consolidation, which is the key transformation in the forming stage, the porous body development begins after heating of the green part. The pore forming is possible due to thermal degradation of starch and the pores correspond to the shape, quantity and particle size of gelatinized starch[168]. Besides the complete degradation without release of toxic effects, the starch is used as a porogen agent also for enhancing pores interconnectivity[150].

Similar to other organic materials, the thermal degradation of starch begins with evaporation of the free water from material's structure, followed by the release and evaporation of structural water and loss of plasticity. Water evaporation ends at approximately 120℃, and thermal degradation of starch continues with forming (350 - 800℃) and oxidation of a carbonaceous residue. Based on this

information, it is expected that starch addition will influence the material properties of hydroxyapatite only in the preliminary sintering stages.

Materials characterization

Physico-chemical properties

The initial composition of ceramic slurries is important for controlling the dimensional accuracy of scaffold-type bodies. The effect of starch addition upon the density of the green parts was studied for alumina by comparing theoretical density values with experimental ones[173]. The study revealed a good correlation between calculated and measured parameters only for suspensions with low starch concentrations. At higher starch contents, the differences between theoretical and measured values suggested that significant contractions take places during sintering. For hydroxyapatite, starch addition did not significantly alter the relative density of the green part, so it is assumed that starch does not improve the packing ability of such mixtures[173]. This is explained by the differences in the surface areas of hydroxyapatite (2 m^2/g) and starch (0.333 m^2/g). The theory behind improving the packing behavior of a ceramic slurry is represented by the Furnas model and suggests the replacement of a fraction of fine particles with the same quantity of coarser ones[174,175] . This is how shape and dimension of precursors particles are involved in optimization of porous bodies through casting methods[150,173]. Various shapes and dimension of hydroxyapatite particles were described in numerous preparation and characterization studies[176–180]. Similarly, the variety of extraction sources for starch induces a significant variability in starch particles shapes and dimensions. Starch particles may have oval, elongated, polygonal, spherical, lenticular or irregular shapes[165] and are groped, based on their dimensions, in: large (above 25µm)/ medium (10-25µm)/ small (5-10µm) and very small articles (below 5µm)[42].

Morphology (porosity)

The morphological studies performed on green parts formed after starch consolidation of hydroxyapatite revealed that it is possible to ensure a homogenous distribution of starch between

hydroxyapatite particles if slurry preparation methods are carefully controlled. Few material defects were observed in the green parts prepared with approx. 40%wt. starch as cracks and large pores. These defects were intensified after heating due to severe contraction of starch particles[173]. Besides green parts morphology, many studies aimed to evaluate the porous structure of sintered bodies. The main indicators used in evaluation were medium, pore dimension[171], the porosity[167,171,181] and pore interconnection[162]. The different methods used for incorporating starch as porogen agent led to porosities between 0.47% and 80%. Most of the methods lead to formation of interconnected pores[150,167,168] with a round shape[150]. The pore size distribution is influenced by the size of starch particle and their contraction during sintering. A bimodal particle size distribution, easy to use due to the variability of starch morphologies, may simultaneously contribute to micro- and macroporosity in sintered bodies. Besides microporosity, small-sized porogens may contribute to pore interconnection (because smaller spheres exhibit more contact points). The morphological aspects related to sintered bodies porosity are influenced by the type of starch and concentration, sintering temperature along with the use of other porogens or sintering additives. The morphological difference were explained by the different mechanisms of pore formation employed for each starch type. While soluble starch was subjected to gelatinization during forming (which allowed the consolidation of the green parts and the risk of pore-closing during sintering), the insoluble starch particles agglomerated in presence of water thus contributing to development of interconnections[182].

Using insoluble starch, even in combination with soluble starch, appear as an efficient approach for enhancing pore interconnectivity, this phenomenon is mostly due to starch particle agglomerations. Hence, it is a localized process which is difficult to control. In more recent studies, the constant improvement of preparation methods based on soluble starch and addition of complementary porogens or sintering additives that can be easily dispersed within the entire body volume led to pore interconnection. However, since the pore interconnectivity is assessed mostly qualitatively, a proper evaluation of this indicator requires further research. Besides starch type, starch

concentration in the ceramic mixture clearly influences the porosity of the sintered structure[167,168,171], but the results are strongly influenced by the preparation methods. The forming methods appear to have a significant influence, with porosity for pressed mixtures[171] significantly lower versus cast ones[167].

Another influencing factor upon the porosity of sintered hydroxyapatite is the sintering temperature[148,150]. Usually, hydroxyapatite-based ceramics are sintered at 800 - 1300°C and this range is adequate for the effective removal of starch. For samples with 30%wt. starch (without other additives), sintering at higher temperatures (1350°C) led to a porosity similar with heating performed 1300°C. Based on this observation and taking into account the economic aspects of preparing these ceramic materials, it is possible to lower the sintering temperature to 1250-1300°C. The sintering duration (range 1-5 hours) did not influence the porosity of porous hydroxyapatite tablets prepared with starch[148]. The presence of other porogen agents and/or additives enhance pore formation[148,150,167,168,171] and ensures their proper size distribution[167]. A suggestive example of the influence of a sintering additive may be found when comparing the porosity of two materials prepared through similar methods, with 20%wt. starch, formed by uniaxial pressing and sintering at 1250°C [148]. Addition of 2% wt. magnesium stearate as a lubricant in the mixture increased the porosity up to 64.5%[148]. Incorporation of various additives[150,167] (foaming agents or lubricants[148] which improve the wetting of ceramic particles) is also recommended for a better pore interconnectivity.

Morphological evaluation of sintered hydroxyapatite-starch mixtures leads to optimistic perspectives of using starch as porogen agent. However, careful control of every preparation step is necessary to avoid risks such as:

- *Dimensional change of sintered body*, due to material contraction during heating. Contraction depends on the packing abilities of ceramic mixtures and is avoided by using slurries with high loads of ceramic particles[150]. Since starch concentration does not significantly influences the

hydroxyapatite green parts[173], the main influence upon structures contraction is the sintering temperature.

- *Macroscopic defects in sintered bodies.* Thermal treatments performed during preparation of hydroxyapatite and 20-50 %wt. starch (sintered at 800-1250°C) led to ceramic materials with "potholed surface texture". The main cause is considered the poor blending uniformity which allowed the porogen agglomeration. The solutions proposed for avoiding these defects are shape and particle dimension optimization, adequate selection of sintering additives and use of a proper homogenization method[148].
- *Microcracks development.* Shell-shaped microcraks were observed near large pores after the sintering of hydroxyapatite-starch mixtures at 1100°C. The results were explained by the swelling of starch particles during gelatinization, which press the ceramic particles in the adjacent area and create more compacted areas. In the drying process, the starch granules were contracted more in comparison to the ceramic matrix, leading to detachment of compacted areas and development of microcracks. These defects may be avoided through proper selection of shape and size of starch particles used as porogen[167].

Mechanical properties

Bone regeneration, intensively influenced by the porosity, is a long-term objective of a modern bone substitution material. Besides porosity, the similarity between a scaffold and the original bone shall manifest for the mechanical properties, which shall be adapted to local biomechanics. The mechanical characteristics of a scaffold have a significant role in the short-term success of an implanted medical device, by ensuring the stability and mechanical strength required until bone regeneration. In order to have the proper dimensions, stability is also required during preparation and processing of the bone scaffolds. This is often a difficult aim when processing ceramic powders and suspensions. Starch consolidation ensures the mechanical stability of green parts. An improvement on green parts compression strength was also observed up until 30 % vol. starch in

hydroxyapatite suspensions, followed by a decrease at 40 %vol. due to starch particles contraction during drying[173].

For sintered parts, the relationship among porosity and mechanical properties is intuitive: the more porous is a material, the less mechanically resistant it will be. In this case, starch addition has only an indirect influence, considering its direct influences upon scaffold's porosity. The mechanical properties evaluated after sintering hydroxyapatite-starch mixtures were: compression strength[148,150], fracture toughness[167,182], and flexural strength[167,168]. Thus, after sintering at 1250°C, at 67% porosity, hydroxyapatite's compression strength was approximately 30 MPa[150]. Compression behavior, evaluated in terms of friability of sintered hydroxyapatite tablets was influenced by concentration of the starch used as porogen: friability increased from 0.2% up to 0.7% when starch concentration was increased from 20%wt. to 37.5%wt. Further increase in starch concentration, up to 50%wt., led to 0.5% friability. Tablets friability increased when higher sintering temperatures were applied[148].

Fracture toughness was evaluated at 0.21MPa for hydroxyapatite with approximately 70% porosity (after sintering at 1100°C)[167,168]. Even if an exponential dependency of fracture toughness to material's porosity was observed (with increased incidence of intergranular fracture due to a weakening of grain boundaries), a proper correlation between mechanical properties and porosity is difficult to define due to multiple uncertainties induced by other factors (mainly the various interactions between sintered body's cracks, hydroxyapatite grains and pores) [167]. In another study, the fracture strength of hydroxyapatite sintered at 1200°C was modified based on the solubility of the starch used as porogen: for 30 %wt. starch used in the mixture, fracture strength was equal to 86.7 N for water-soluble starch versus 51.2 N for insoluble starch. The difference is attributed to porosity differences developed in the presence of the two different types of starch [182]. *The flexural strength* varies between 10-21 MPa for materials with approximately 45% porosity and decrease at 2-5 MPa for 70% porosity. Similarly to other mechanical properties, proper evaluation of flexural strength is difficult due to the differences between porosity, pore size and/or interconnection

degree[167,168]. Finally, fabrication of sintered hydroxyapatite after starch addition is not possible with high starch concentrations due to poor mechanical properties.

Structure

Only few studies have analyzed the structure of hydroxyapatite starch-mixtures[34,183], although some information about the structure of the green parts may be extrapolated from structural studies of hydroxyapatite-starch composites. For the sintered parts, XRD analyses performed after sintering at 1100°C[34,183], 1200°C and 1250°C revealed only peaks characteristic to hydroxyapatite which suggests that the ceramic does not react with starch during processing[182]. After heat treatments performed at high temperatures, no porogen residues were observed[148].

Biological properties

Similar to structural studies, the biological properties of the ceramics obtained through sintering of hydroxyapatite-starch mixtures were not thoroughly assessed[167]. However, the results suggests that starch was completely eliminated after sintering and it is expected that the biological properties of these materials will resemble closely the ones of hydroxyapatite. If the lack of toxic degradation products is confirmed experimentally, the fabrication of porous products using starch as porogen may gain advantage over other methods for preparing porous ceramics, which use potentially toxic porogens, like PMMA[158,164,166,174,182]. In vitro evaluation of sintered scaffolds showed that hydroxyapatite remained after starch removal promotes the cellular growth and has a non-toxic nature[183]. Another biological property, biodegradation, is manifested through physico-chemical dissolving of ceramic grains, which leads to a decrease of the intergranular cohesion in near-pores areas. The process continues with grains detachment in these areas, so migration of material from the implants, which coincides with a decrease in the material's mechanical properties. SBF immersion testing of hydroxyapatite prepared through starch consolidation revealed that after 21 days of immersion, the material does not show degradation signs[167]. The in vivo response of hydroxyapatite implants prepared with starch is still in its early stages. A preliminary study evaluated some hollow hydroxyapatite space-maintaining devices in 3 patients. The devices were

implanted for 8 weeks before prosthetic treatments. At 6 weeks after the process of implantation, the radiological evaluation confirmed new bone formation, without any loss or bone tissues of infection signs. Histological evaluation of hydroxyapatite explants revealed that they maintained integrity and did not generate any adverse effects. Also, newly-formed bone tissue was observed at the surface and within the pores devices[184].

Conclusions and future perspectives

This review covered the perspectives of using hydroxyapatite and starch mixtures as biomaterials with different applications in bone substitution. Two main approaches may be distinguished based on the role of starch within the material: hydroxyapatite-starch composites and porous hydroxyapatite ceramics obtained with starch as porogen agent. Hydroxyapatite-starch composites have been tested for various medical applications such as adhesives, bone cements, bone waxes or scaffolds. However, due to the different research aims, the variability of the raw materials, as well as the numerous preparation methods interferes in the proper characterization of hydroxyapatite-starch interactions and properties. Until further improvements of hydroxyapatite-starch composites, the use of starch particles as porogen agent in sintering hydroxyapatite-based ceramics is considered a safe approach in terms of toxicity because the processing at high temperatures allows the complete degradation of starch without release of toxic products.

The information gained by the research in the hydroxyapatite-starch composites is useful in controlling the properties of green parts having a major contribution in assuring the dimensional accuracy of the final products. The method for preparing hydroxyapatite-starch green parts, often named “starch consolidation”, allows molding in complex tridimensional shapes and ensures the mechanical stability until sintering without any modification of the ceramic material. However, the use of the starch gels needed by starch consolidation is possible only in a limited temperature interval, around 70°C. The starch composition, the hydroxyapatite particles dimension and the mixing method also influence the outcomes of starch consolidation. Also, other challenges related to hydroxyapatite such as the poor mechanical performance of porous structure are not addressed by

the use of starch, since the polymer is entirely removed by heat treatment. Moreover, at high starch concentrations, post-sintering machining is difficult and in some cases the sintered parts lose their integrity without any external influence. To date, the use of hydroxyapatite and starch mixtures for preparing materials with different applications in bone substitution is only partially researched and documented, so further information is required for establishing fabrication protocols. After a moderate expansion of this research field in 2000-2010, the starch-hydroxyapatite mixtures have recently regained attention as potential biomaterials with recent studies providing useful insights on precursor selection and slurries optimization. Novel research hypotheses regarding hydroxyapatite-starch materials are expected due to recent technological advancements in the field of ceramic fabrication, which allow the fine tuning of *in situ* different processing constraints including temperature and concentration and overcome the limitations induced by traditional fabrication methods.

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Authors contributions

All authors contributed equally in preparing this work.

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Figures

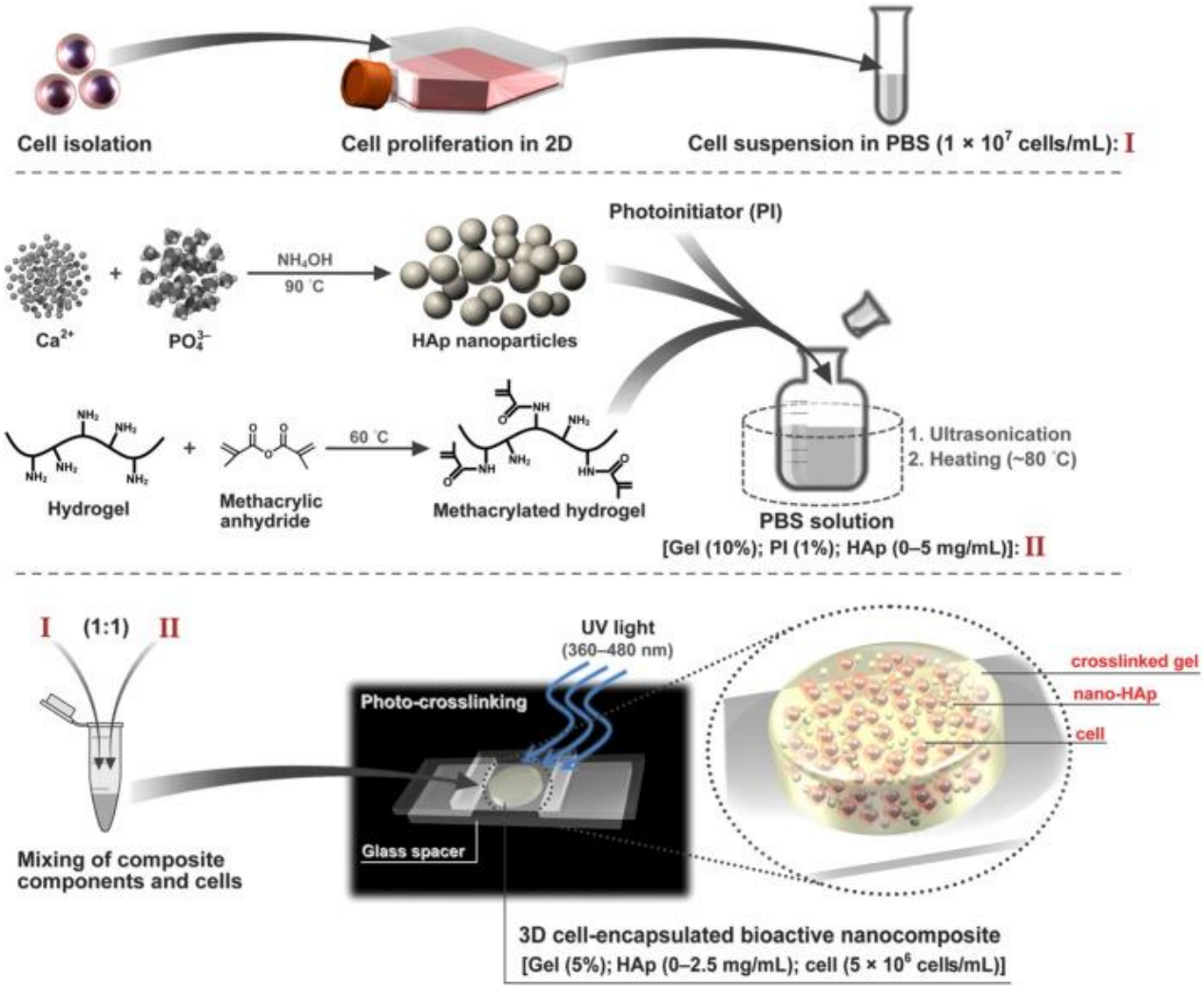

Fig. 1. Schematic representation of the steps required for fabrication of 3D cell-laden HAp/hydrogel nanocomposites[31]. Reprinted with permission. Copyright 2015 Elsevier.

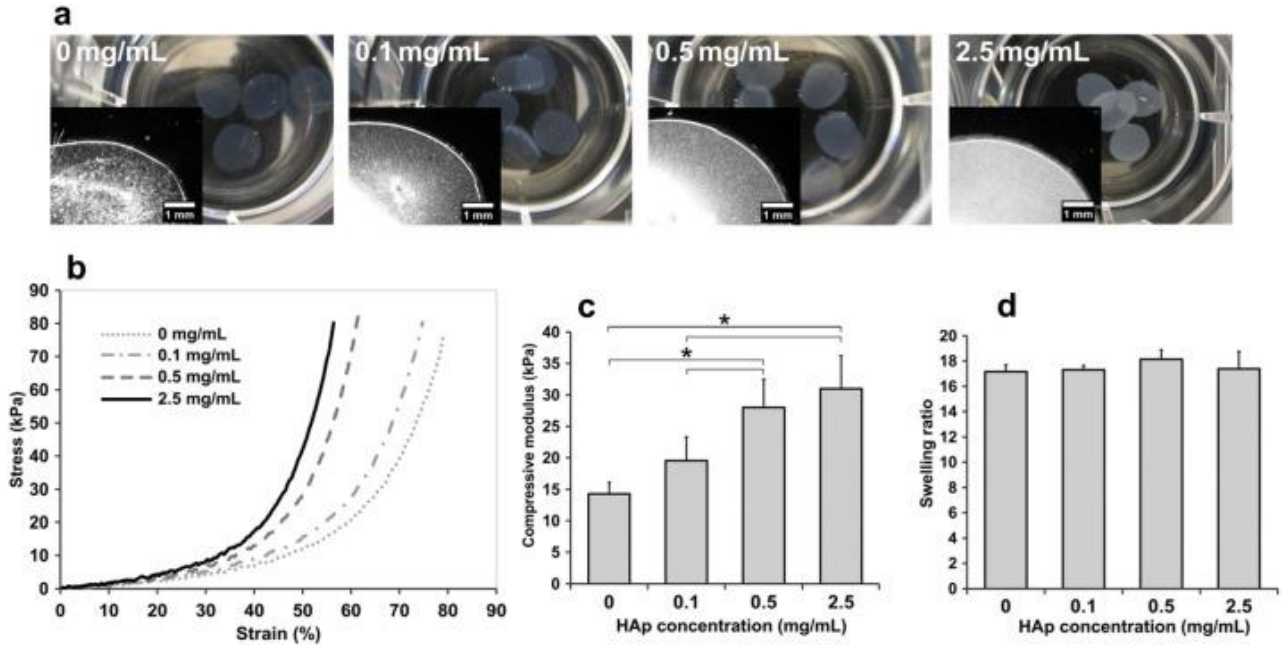

Fig. 2. Structural characterizations of the as-prepared hydrogel/HAp nanocomposites containing different concentrations of HAp nanoparticles: (a) Appearance of nanocomposites immersed in PBS along with their bright-field microscopy images (inserts); very bright parts of the first three microscopic images were due to the roughness of the surface, bubbles on the surface and/or curvature of the circular samples; (b) representative stress–strain curves recorded for the fully swollen hydrogel nanocomposites; (c) compressive modulus of nanocomposites calculated from initial slope of the stress–strain curves; results are mean ± SD of five measurements, and asterisks (*) indicate the significant difference ($p < 0.05$) between the experimental groups; (d) swelling ratio (mean ± SD of five measurements) after 24 h equilibration in PBS at 37 °C, showing no significant difference ($p < 0.05$) between the experimental groups[31]. Reprinted with permission. Copyright 2015 Elsevier.

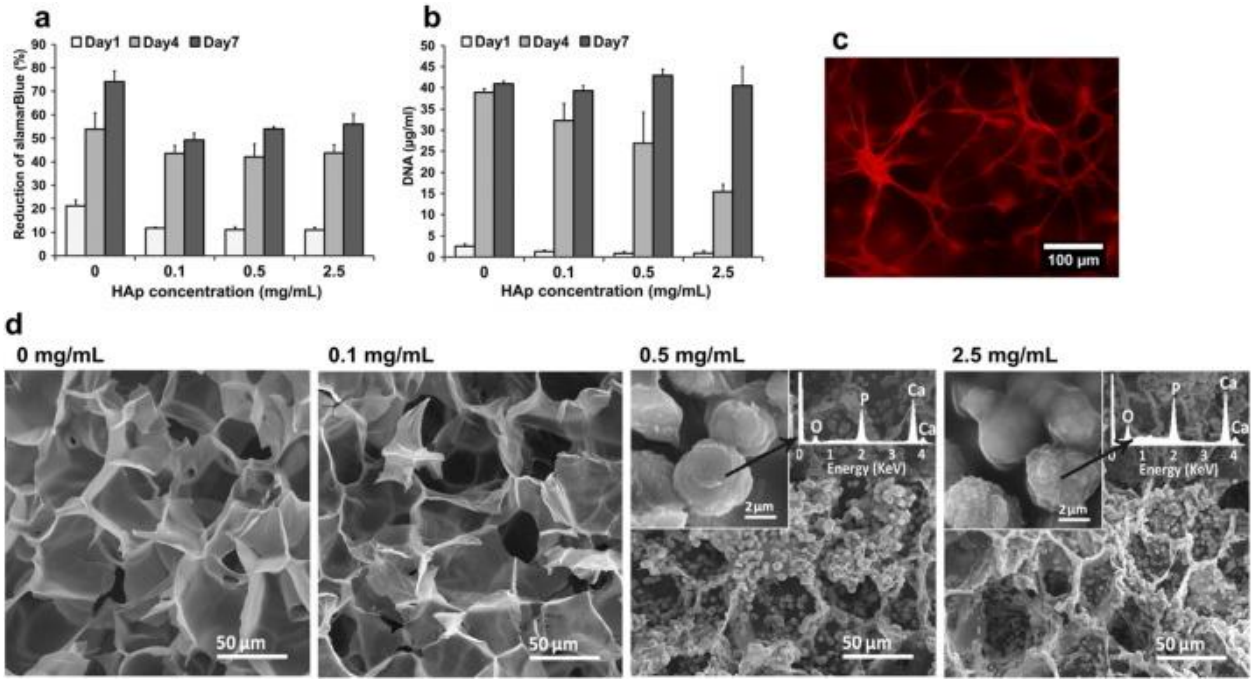

Fig. 3. Cell metabolic activity (a) and cell proliferation (b) of 3T3 fibroblasts encapsulated in hydrogel/HAp nanocomposites containing different concentrations of HAp nanoparticles after 1, 4, and 7 days of culture. Results are mean ± SD of six measurements. All changes between days of culture were significant ($p < 0.05$). (c) Representative fluorescence image of the cytoskeletal F-actin fibers of MC3T3-E1 preosteoblasts encapsulated in hydrogel/HAp nanocomposite with 0.5 mg/mL HAp content after 14 days of encapsulation. (d) SEM cross-sectional micrographs of hydrogel/HAp nanocomposites containing different concentrations of HAp nanoparticles after 30 days of incubation in SBF at 37 °C; the inserted images show the SEM micrographs at a higher magnification along with the corresponding EDX elemental analysis[31]. Reprinted with permission. Copyright 2015 Elsevier.

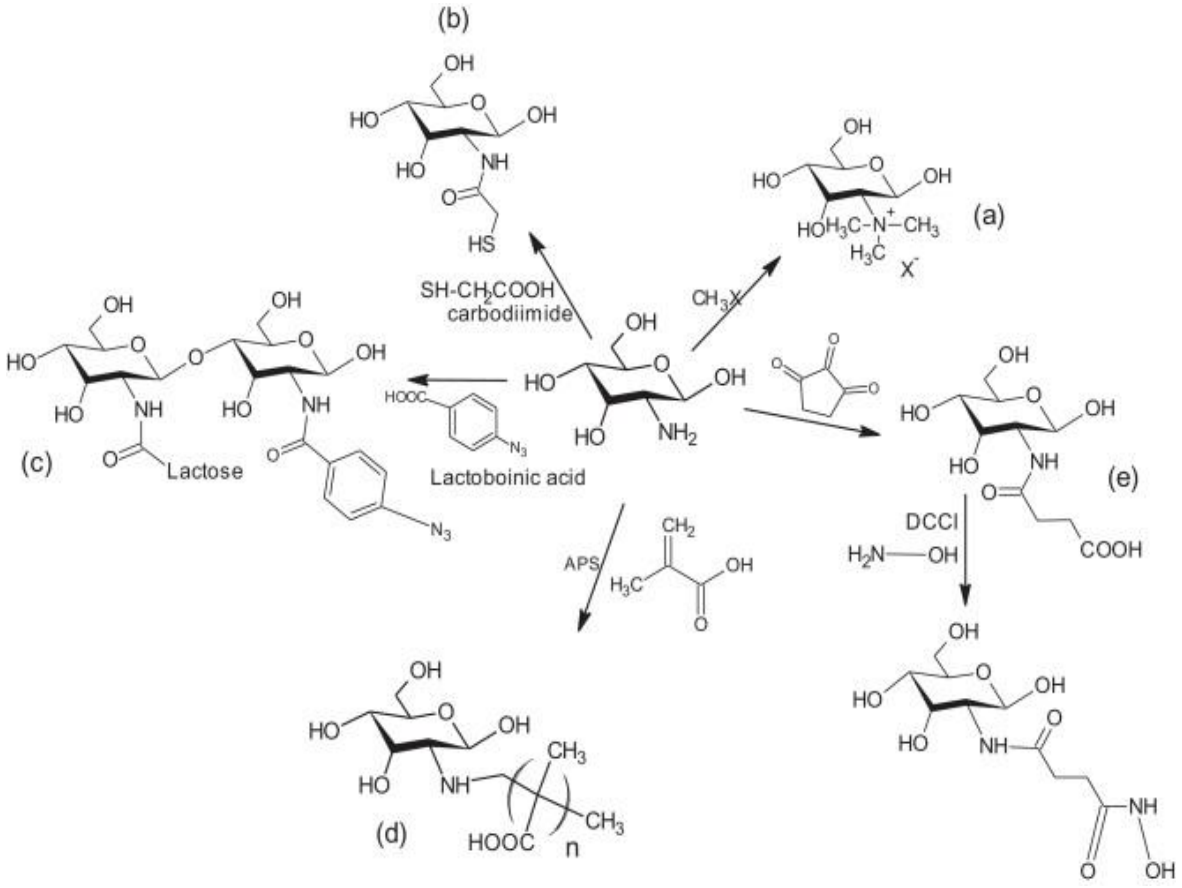

Fig. 4 (a). Chemical modification of chitosan for different applications: (a) methylation; (b) thiolation; (c) azylation; (d) co-polymerization; (e) N-succinylation[58]. Reprinted with permission. Copyright 2013 Elsevier.

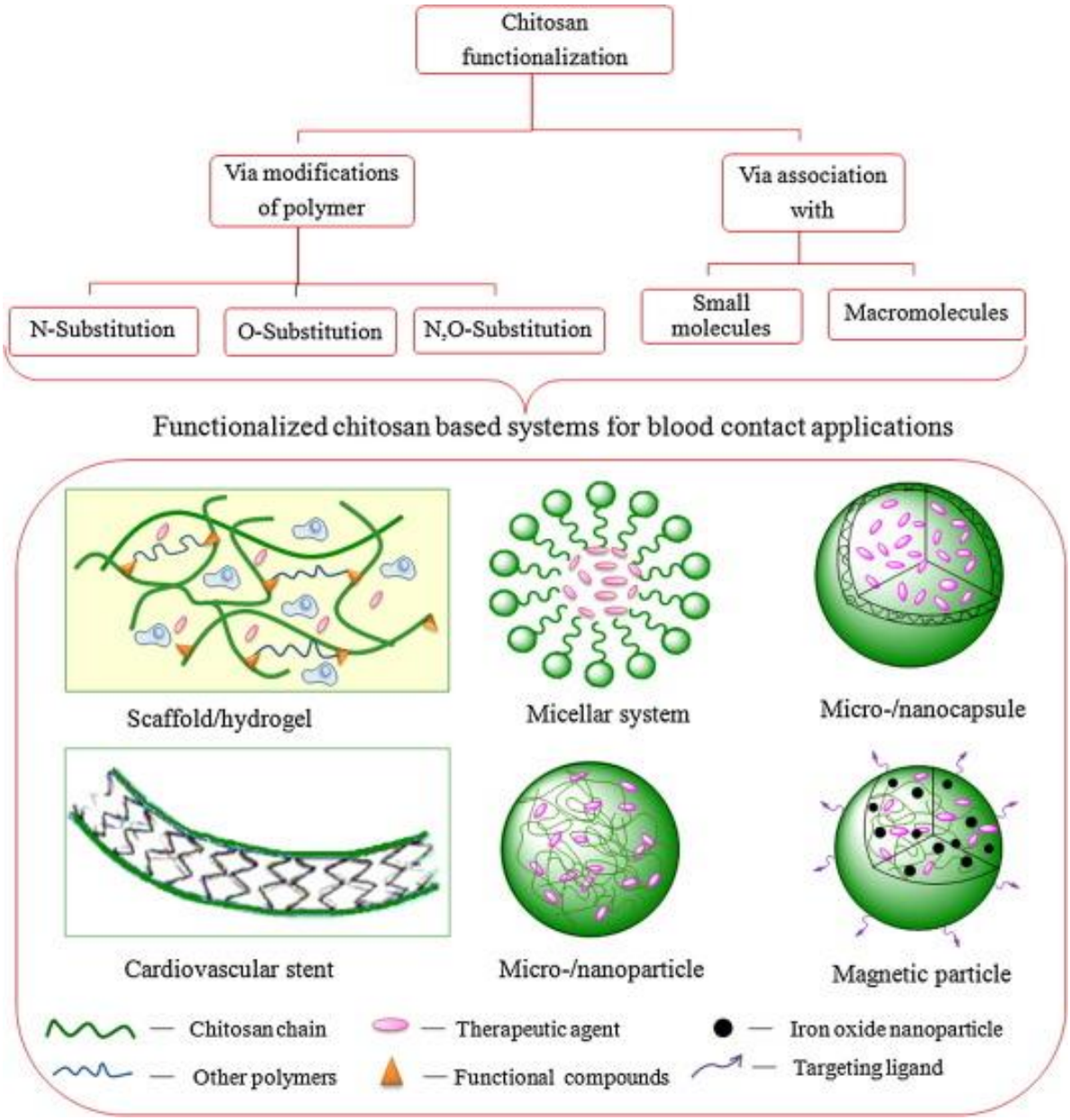

Fig. 4 (b). Strategies used to improve the blood compatibility of the chitosan[59]. Reprinted with permission. Copyright 2014 Elsevier.

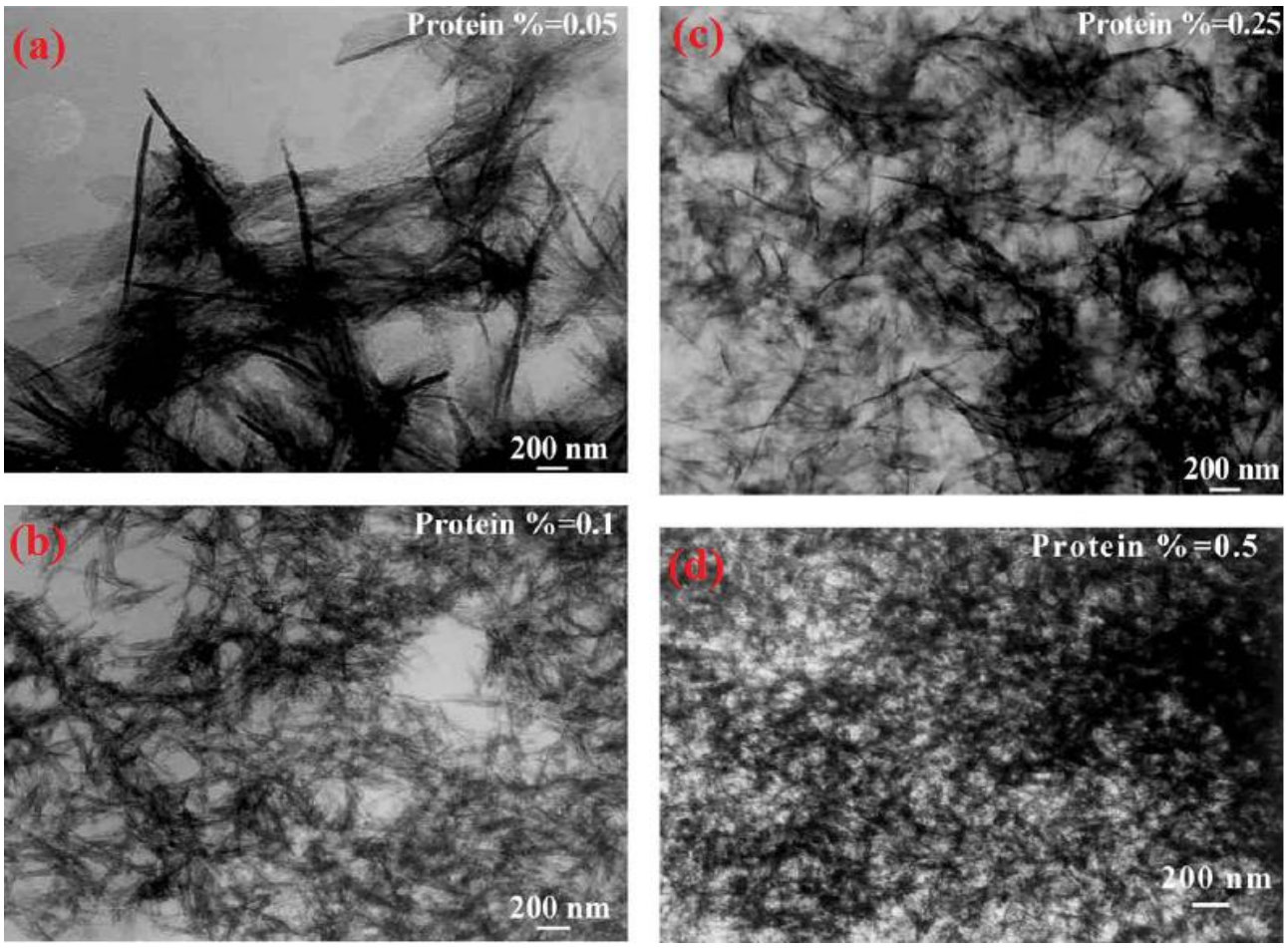

Fig. 5 TEM Bright field images of nanosized HAp particles synthesized in different protein concentrations; HAp particles in (a) 0.05% BSA, (b) 0.1% BSA, (c) 0.25% BSA and (d) 0.5% BSA[101]. Reprinted with permission. Copyright 20006 Springer Science + Business Media, LLC

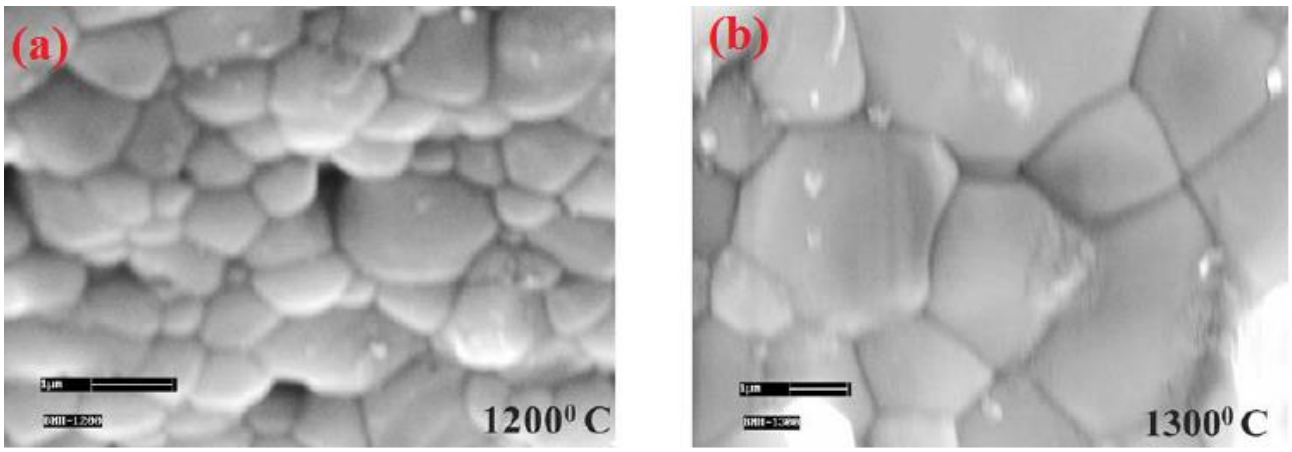

Fig. 6 SEM studies on sintered HAp (a) HAp nanopowder sintered at 1200◦ C and (b) sintered at 1300◦ C[101]. Reprinted with permission. Copyright 20006 Springer Science + Business Media, LLC

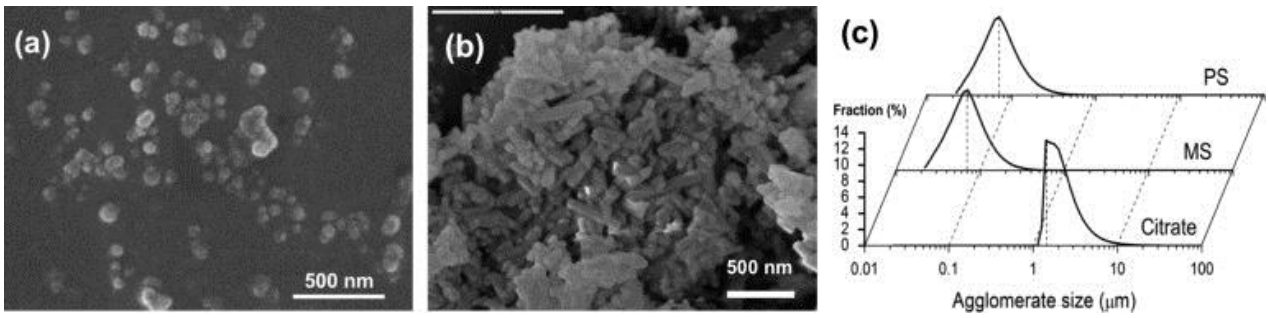

Fig. 7. SEM images of HA nanocrystals synthesized with (a) gelatinized PS matrix and (b) citrate, and (c) DLS size distributions of HA agglomerates synthesized with gelatinized starch matrices and citrate[93]. Reprinted with permission. Copyright 2013 Elsevier.

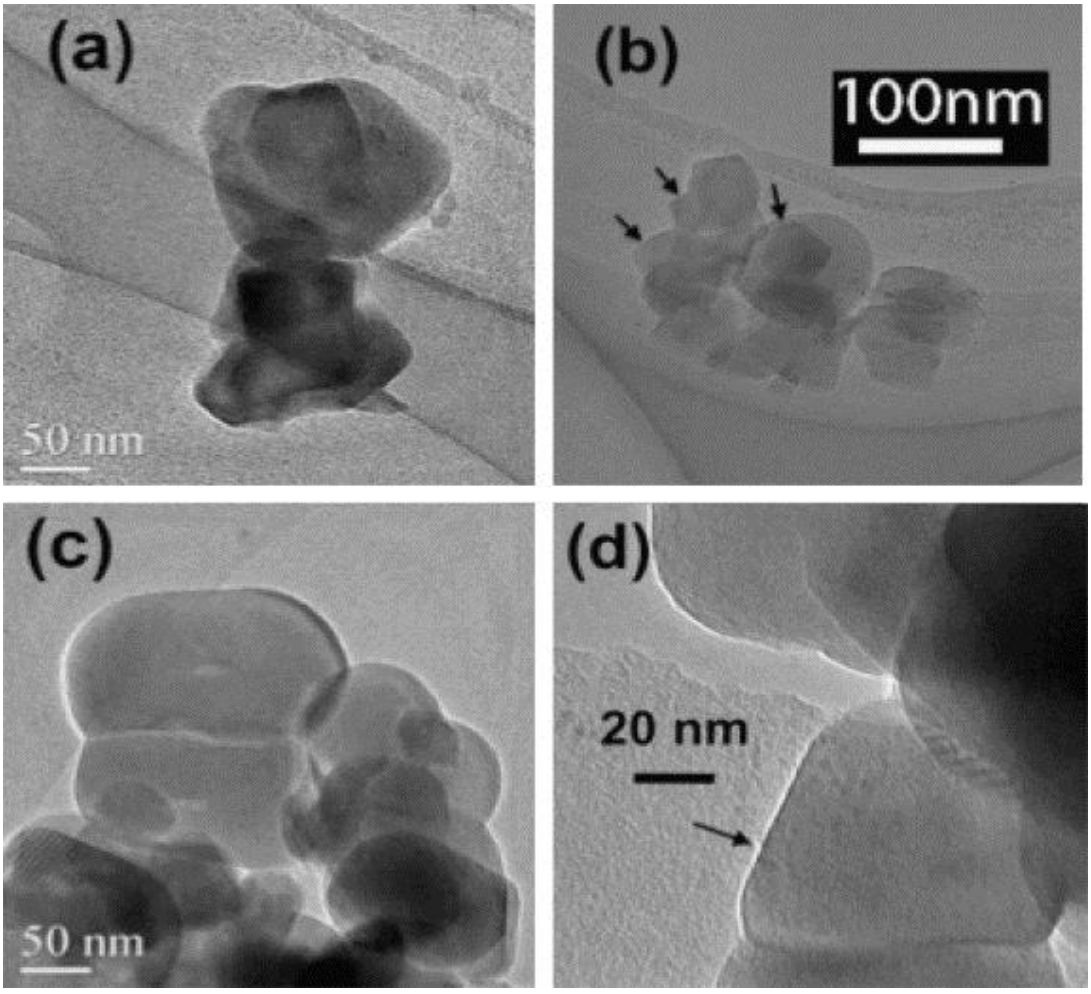

Fig. 8. TEM images showing a variety of low-aspect ratio morphologies of HA nanocrystals synthesized with gelatinized starch matrices: (a) irregular, (b) cubic (arrows) and spherical, (c) spherical and twined-capsule, and (d) triangular (arrow)[93]. Reprinted with permission. Copyright 2013 Elsevier.

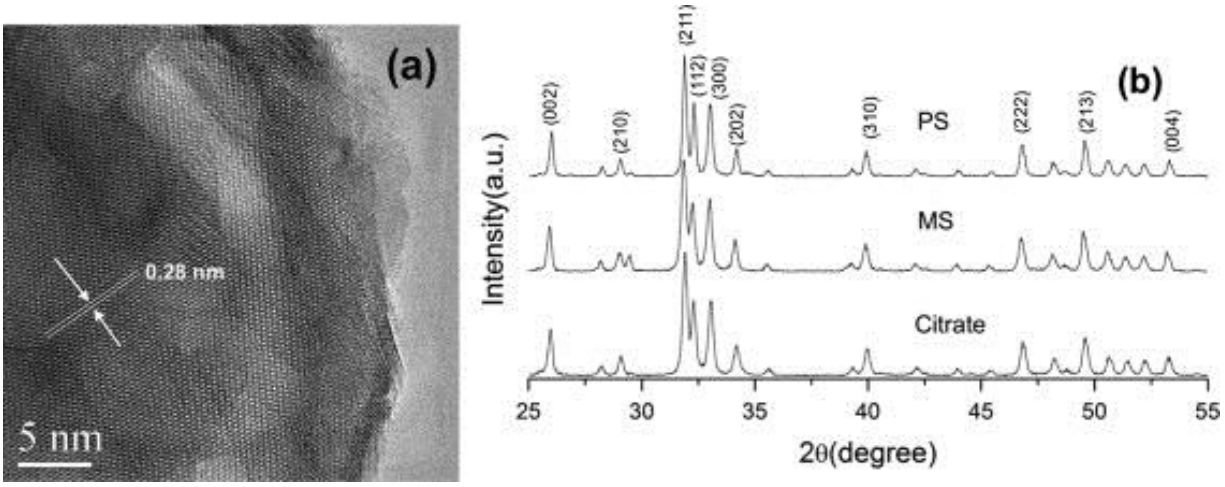

Fig. 9. (a) HRTEM image of HA nanocrystals produced with the gelatinized MS matrix, d=0.28 nm corresponding to the interspace between (2 1 1) planes (d_{211}=0.2814 nm by JCPDS no. 09-0432) and (b) XRD patterns of HA nanocrystals produced with gelatinized starch matrices and citrate[93]. Reprinted with permission. Copyright 2013 Elsevier.

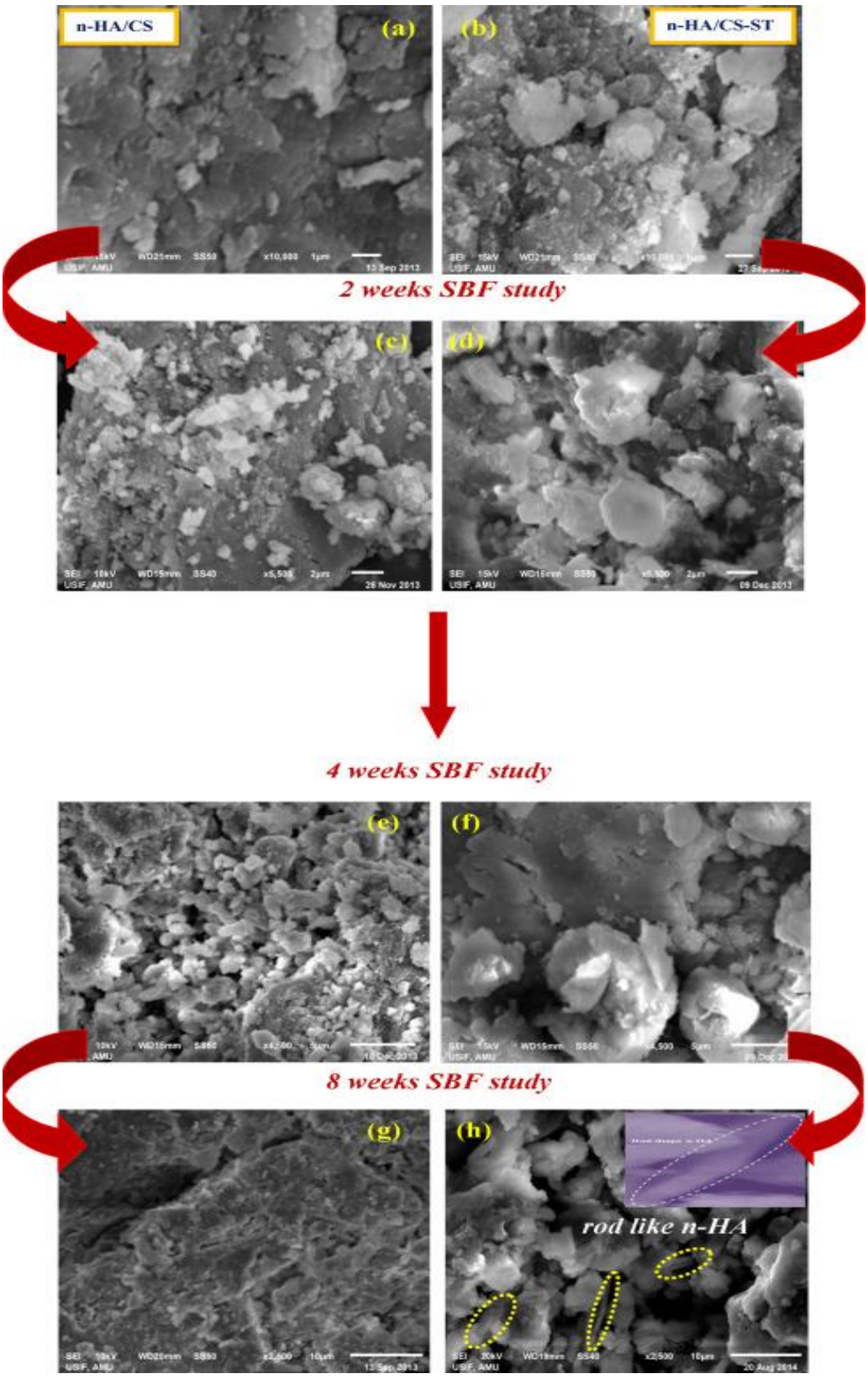

Fig. 10. SEM micrographs of (a) n-HA/CS, (b) n-HA/CS–ST and their respective SBF study after 2, 4 and 8 weeks (c–h). Rod shape n-HA[57]. Reprinted with permission. Copyright 2013 Elsevier.

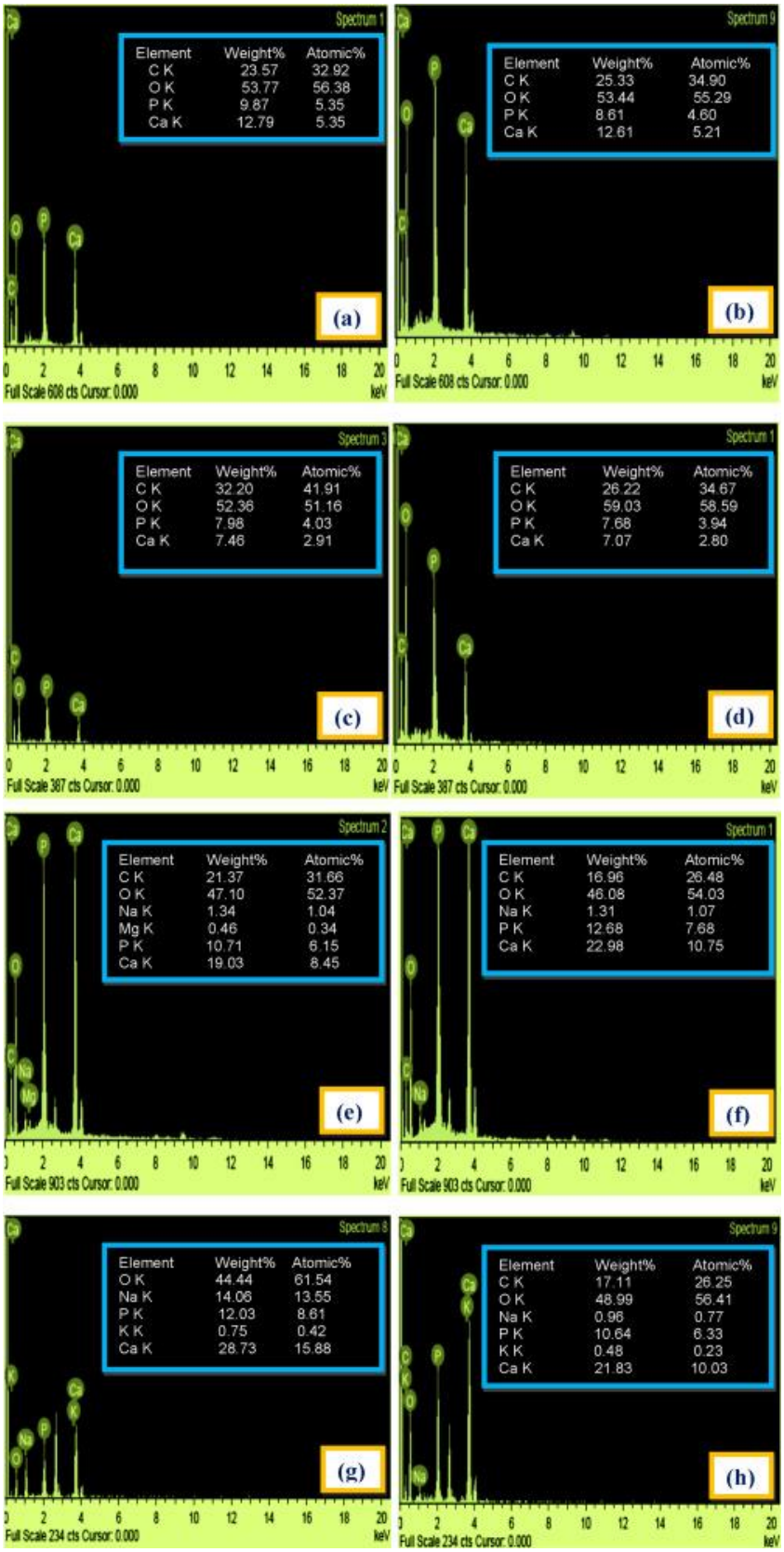

Fig. 11. EDX micrographs of micrographs of (a) n-HA/CS, (b) n-HA/CS–ST and their respective SBF study after 2, 4 and 8 weeks (c–h)[57]. Reprinted with permission. Copyright 2013 Elsevier.

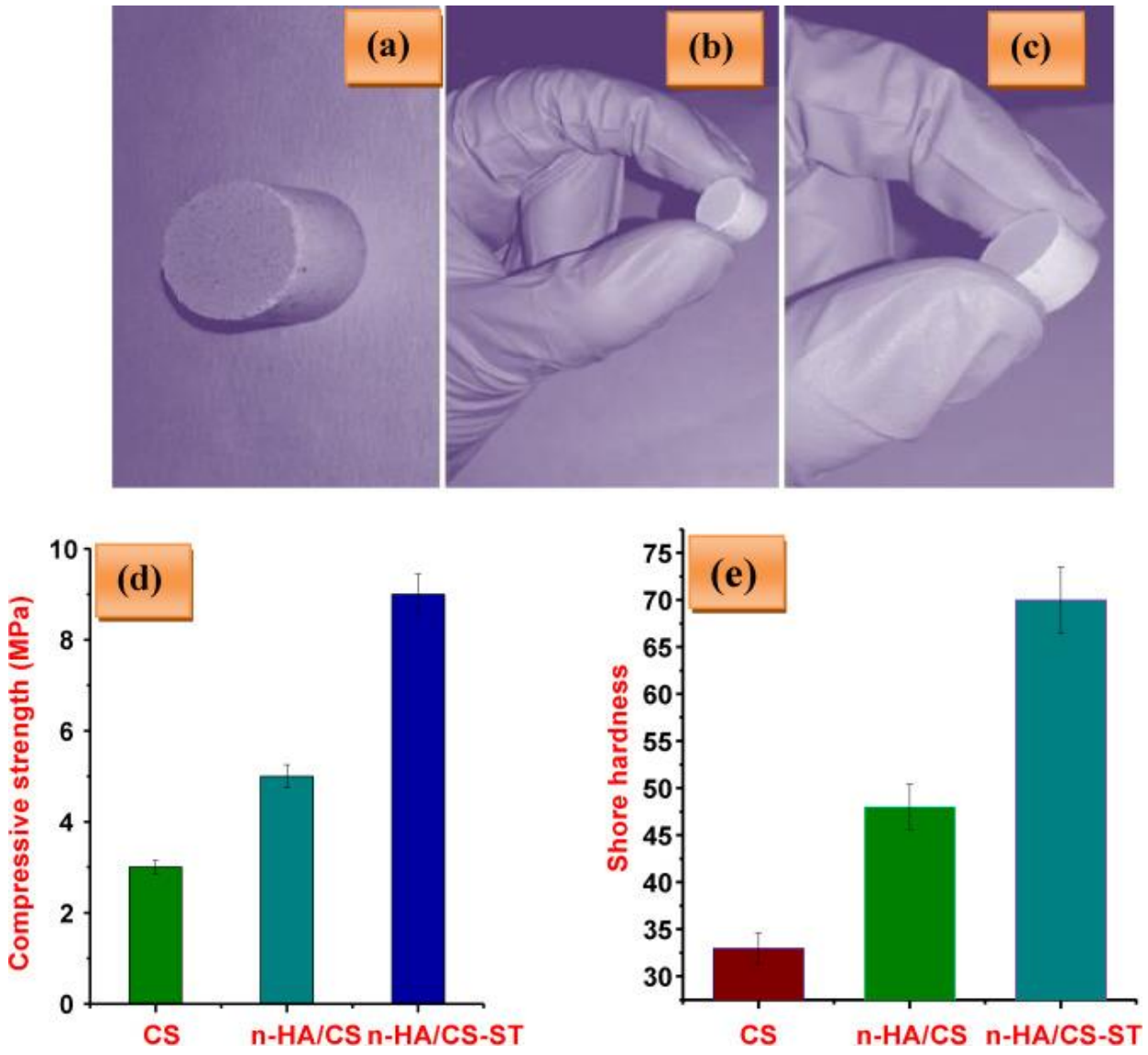

Fig. 12. Pellets display: (a) CS, (b) n-HA/CS and (c) n-HA/CS–ST scaffolds; mechanical properties: (d) compressive strength and (e) shore hardness[57]. Reprinted with permission. Copyright 2013 Elsevier.

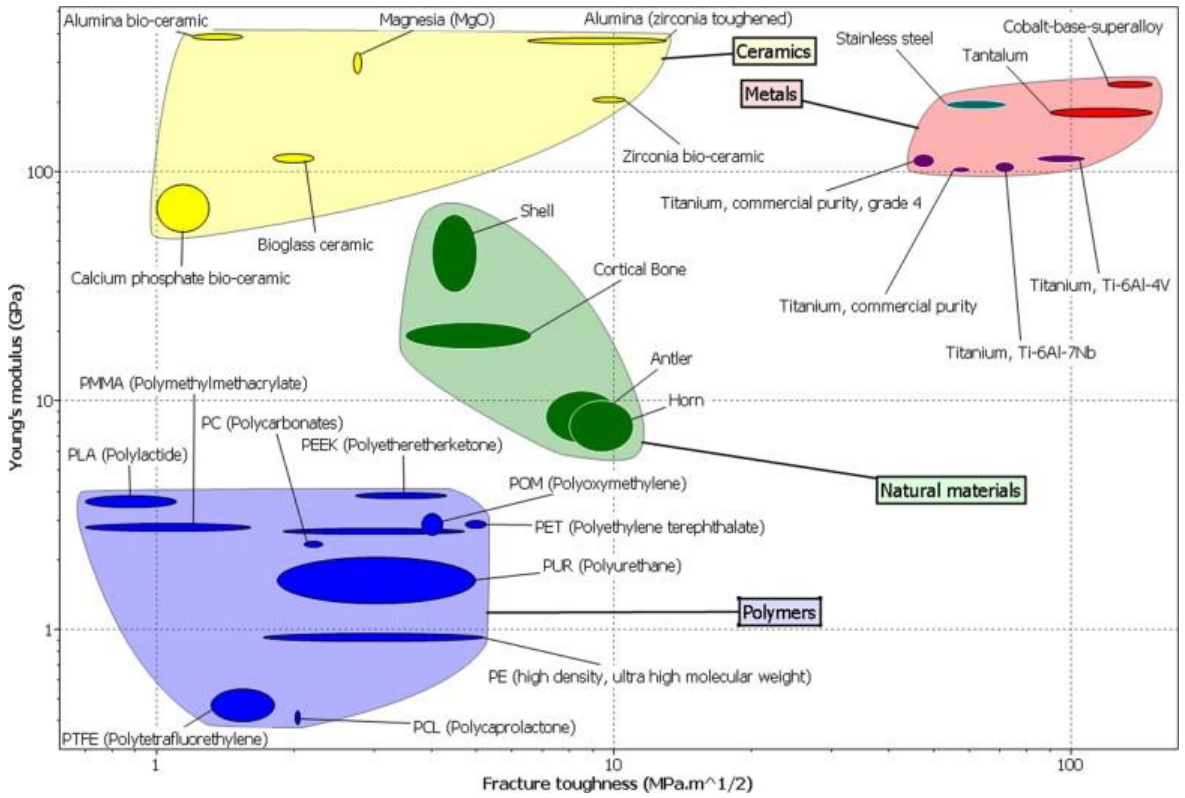

Fig. 13. Mechanical properties of natural materials in comparison with bulk materials for medical purpose (graph constructed with CES Selector 5.1.0) [126]. Reprinted with permission. Copyright 2011 Elsevier.

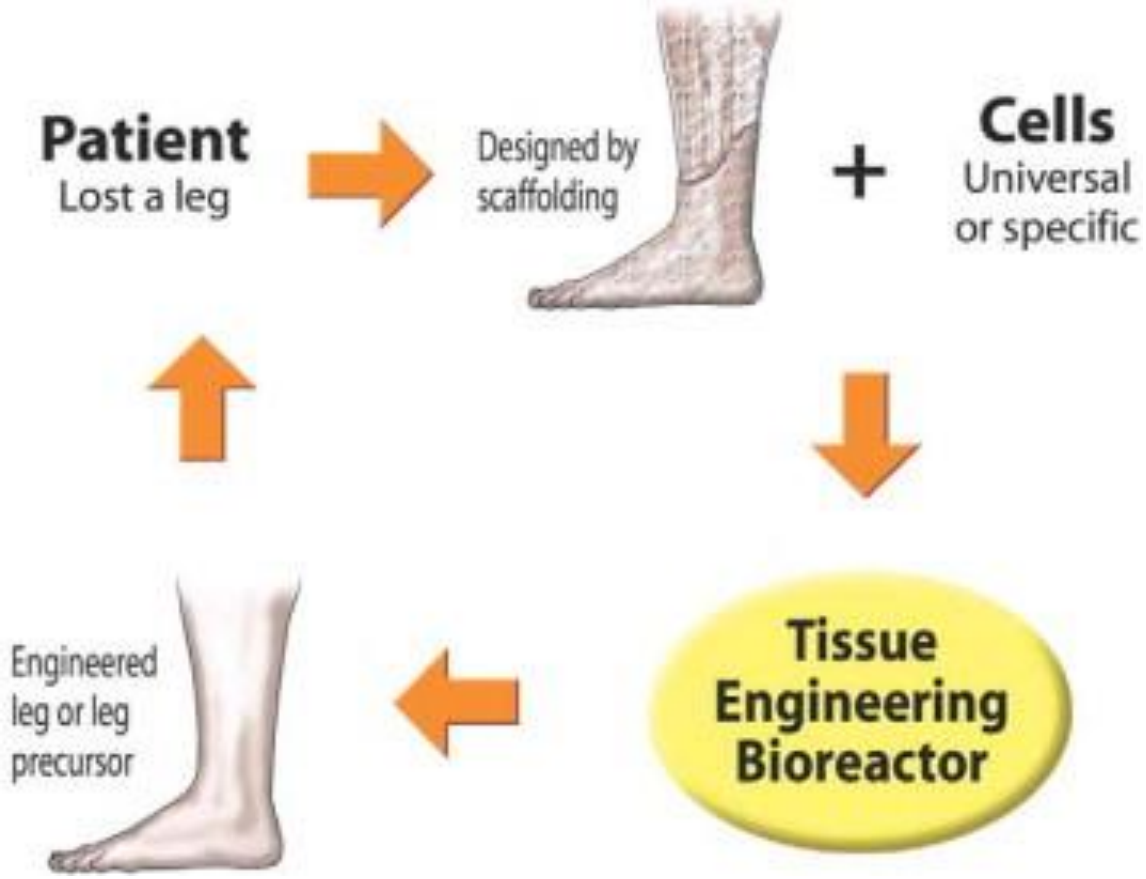

Fig. 14. Schematic diagram showing the tissue engineering concept using a hypothetical example of leg regeneration. Scaffolding materials (temporary synthetic extracellular matrices) are designed as a three-dimensional mirror image, on which cells grow and regenerate the needed tissues. Because the scaffolding materials are biodegradable, they will resorb after fulfilling the template function and leave nothing foreign in the patient[133]. Reprinted with permission. Copyright 2004 Elsevier.

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Dr. Habil. Florin Miculescu is Full Professor in the Metallic Materials Science and Physical Metallurgy Department at the Politehnica University from Bucharest, Head of three laboratories within his department and President of the Romanian Society for Biomaterials (2014-2017). He has participated in five postdoctoral stages in Europe and USA and applied his expertise in various research projects related to materials science, engineering, and technology (manager of 8 national and over 50 projects for private companies in the last 15 years). His research activities in the fields of biomaterials, nanomaterials and materials synthesis, processing and characterization are also presented in over 85 ISI-Web of Science articles (impact factor 35 as main author), 5 books and 8 book chapters. He constantly supervises a heterogeneous team of PhD, MSc and BSc students and he is teaching materials science & engineering, biomaterials characterization methods and electron microscopy & microanalysis courses.

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Vijay Kumar Thakur

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Lucian Toma Ciocan

In 2002 Lecturer Dr. Lucian Toma Ciocan graduated the University of Medicine and Pharmacy "Carol Davila" from Bucharest, Faculty of Dental Medicine. In 2005 he completed the specialization in Oral Surgery and a Master in biomaterials field at the Department of Bioengineering and Biotechnology, Faculty of Materials Science, Politehnica University from Bucharest. Currently he is Lecturer at Dental Technology and Dental Materials Department. His special interests are related to dental prosthetics, causes of technological errors in metal-ceramic adhesion, osteointegration of metal implants depending on the degree of surface preparation, mixed metal-ceramic crown. In the last years he published articles and books related to mentioned subjects and he obtained few national and international awards.

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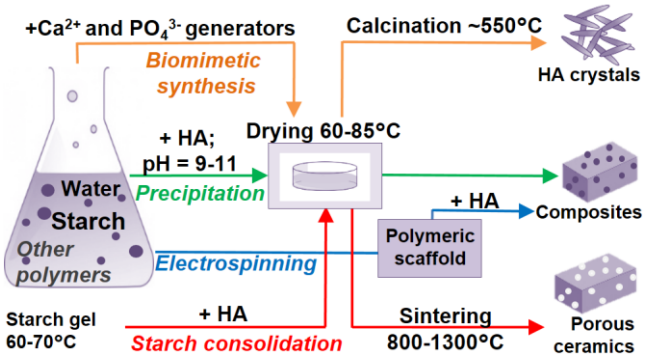

Synopsis: Hydroxyapatite and starch are natural materials which can be used to develop innovative biomedical devices in a sustainable manner.

2017-09-05

Progress in hydroxyapatite-starch based sustainable biomaterials for biomedical bone substitution applications

Miculescu, F.

American Chemical Society