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# Problem of hydroxyapatite dispersion in polymer matrices: a review

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**Abstract** This review summarizes recent work on manufacturing biocomposites suitable for bone tissue engineering. There is a great need to engineer multi-phase (i.e. composite) materials that combine the advantages exhibited by each component of the material, with a structure and composition similar to that of natural bone. The discussion concentrates on the preparation of nanocomposites containing hydroxyapatite particles (one of the most widely used bioceramics materials) with polymer matrices. Special attention is paid to the preparation of nanocomposites with individual (non-aggregated) nanoparticles because this is a key problem in nanotechnology industrialization. Controlling the mixing between so two dissimilar phases is a critical challenge in the design of these inorganic-organic systems. Several approaches that may be applied to overcome this problem will be described in this review.

## 1 Introduction

### 1.1 Hydroxyapatite

Hydroxyapatite (HA) is one of the most widely used bioceramic materials in the field of biomaterials and tissue engineering, because it is a major mineral constituent of the bone matrix. HA occurs in the form of nanocrystals with dimensions of about $4 \times 50 \times 50$ nm. The minerals are indirectly bound to collagen through non-collagenous proteins such as osteocalcin, osteopontin or osteonectin, which make up approximately 3–5% of the bone and provide active sites for biomineralization and also for cellular attachment [1–3]. Natural HA and synthetic HA can differ in their chemical composition and behaviour. It is known that most synthetic HAs are stoichiometric, with a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$. By contrast, human bones do not have pure or stoichiometric HA. Human bones contain other ions, mainly $CO_3^{2-}$ and traces of $Na^+$, $Mg^{2+}$, $Fe^{2+}$, $Cl^-$, $F^-$. The Ca/P molar ratio in bone is lower than 1.67, compared to a molar ratio of Ca/P in synthetic HA [4–7]. This ratio can be an important factor in cell adhesion, proliferation and in bone remodelling and formation [8]. Calcium-deficient HA is of greater biological interest because the mineral portion of the hard tissue is primarily carbonate substituted calcium-deficient HA [5, 6, 9–11], with a Ca/P ratio of about 1.5, which is chemically and compositionally similar to tricalciumphosphate but structurally similar to stoichiometric hydroxyapatite [10]. Mg-substituted HA prepared by Landi et al. [12] made the material more biological in terms of composition, morphology and crystallinity. HA is an ionic crystal that has a hexagonal structure with the space group $P6_3/m$. The lower crystallographic symmetry induces mechanical anisotropy along each axis [13]. It has also been shown that the *a*,*b*-plane is bio-active while the *c*-plane is bio-inert [14]. Therefore, a specific crystal orientation is required in order to use HA as a biomaterial. Several studies have been conducted for controlling the particle orientation of HA [13, 15, 16].

### 1.2 Preparation of hydroxyapatite particles

Recently, nanoscale HA (10–100 nm) has received much attention owing to its superior functional properties. It has

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been proven that the nano-HA is superior to conventional micro HA for promoting osteoblast adhesion, differentiation and proliferation, osteointegration, and deposition of calcium-containing minerals on its surface, which leads to enhanced formation of new bone tissue within a short period [17].

Nano-hydroxyapatite powders can be synthesized by a variety of methods including solid state methods [18], which usually give a stoichiometric and well-crystallized product, but they require relatively high temperatures and long heat-treatment times and wet chemical methods [19–24], where nanometer size powders can be prepared. However, their crystallinity and Ca/P ratio depend mainly upon the preparation conditions and are in many cases lower than for well-crystallized stoichiometric HA. A hydrothermal process [25–27] usually produces HA powders with a high degree of crystallinity and a Ca/P ratio close to the stoichiometric value. However, the obtained powders are usual in agglomeration and the size distribution is usual in a wide range. Other techniques are mechanochemical [28, 29], pH shock wave [30], and microwave processing [31]. A newly developed hydrothermal microemulsion technique [32, 33] was used to synthesize nanopowders, nanoneedles and nanowires, and this method is considered as an effective, convenient and mild synthetic methodology. Microemulsion not only can serve as a nano-reactor to control the particle size and size distribution in the processing reactions but also inhibits excessive agglomeration of particles. Zhou et al. [34] have used the nanoemulsion technique for synthesising of nano-sized carbonated HA, spherical in shape. The nanospheres were in an amorphous state and became highly crystalline after calcination at 900°C. HA can also be processed from animal bone [4, 35, 36] and coral exo-skeleton [37, 38]. Highly textured HA can be fabricated by slip casting using a well-dispersed suspension in a high magnetic field followed by sintering above 1373 K [16].

### 1.3 Physical principles of miscibility

The interfacial strength between filler and polymer is a very important factor in making HA/polymer composites. Lack of adhesion between the two phases will result in an early failure at the interface and thus in a decrease in the mechanical properties, especially the tensile strength. Wetting is important in the bonding or adherence of the two materials (e.g. the filler surface and the polymer); and depends on the hydrophilicity or polarity of the filler and the available polar groups of the polymer. Hydrophilicity refers to the ability of the molecule to bond transiently with water through hydrogen bonding, while hydrophobic substances interact within themselves and with other substances through van der Waals forces and have low or no capacity to form hydrogen bonds.

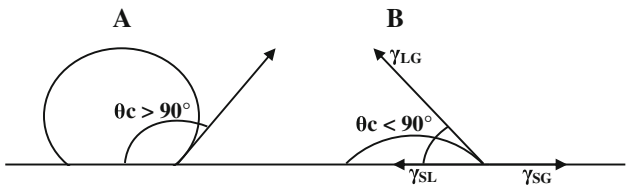


**Fig. 1** Wetting of different fluids: fluid with very little wetting (**a**), fluid with more wetting (**b**)

Wetting depends on the energies (or surface tensions) of the interfaces involved such that the total energy is minimized. The degree of wetting is described by the contact angle $\boldsymbol{\theta c}$, the angle at which the liquid–gas interface $\gamma_{LG}$ meets the solid–liquid interface $\gamma_{SL}$ (Fig. 1). If the contact angle is <90° (Fig. 1b, the fluid will spread to cover a larger area of the surface and so the wettable surface may also be termed hydrophilic. A contact angle of 90° or greater (Fig. 1a), generally characterizes a surface as non-wettable, referred to as hydrophobic.

A useful parameter for gauging wetting is the spreading parameter **S** according to Eq. 1 [39]:

$$\mathbf{S} = \gamma_{SG} - (\gamma_{SL} + \gamma_{LG}) \quad (1)$$

When $S > 0$, the liquid wets the surface completely, when $S < 0$, there is partial wetting.

Neuendorf et al. [40] have used wetting experiments and surface tension measurements to determine the work of adhesion between biodegradable polymers and HA, with specific reference to the role of humid environments. All the polymers are found to exhibit lower contact angles (60°) on the ceramic with work of adhesion values ranging between 48 J $m^{-2}$ for poly(ε-caprolactone) and 63 J $m^{-2}$ for polylactide. Surface wettability [41] and value of a Critical Surface Tension [42] that can influence the biological response to implant materials and affect protein adsorption (connection with thrombosis phenomena) can be also used for characterization of biomaterials.

## 2 Preparation of hydroxyapatite/polymer composites

A composite material consists at least two chemically identified phases which are separated by interface(s). The properties of composites are strongly influenced by a number of factors e.g. filler shape, size and size distribution, properties and volume percentage of filler, matrix properties (e.g. molecular weight), dispersion of filler particles in the polymer matrix and the state of the filler/matrix interface. In the case of biocomposites, other factors such as biocompatibility of the filler or matrix, the

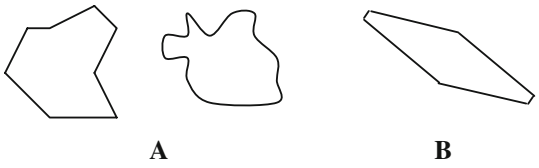


**Fig. 2** Shapes of particles: irregular (**a**), acicular (**b**) [44]

degradation rate of matrix and nontoxicity [43] must be considered.

Physical characteristics such as shape, size, and size distribution of filler particles are very important in determining the mechanical properties of a composite. For mathematical modelling of the mechanical behaviour, the filler particles are considered to have a spherical shape. In reality, however, reinforcing particles may have an irregular (Fig. 2a), acicular (rod, needle) (Fig. 2b) or platey shape [44]. Commercially available HA particles can be irregular in shape, and are composed of tightly bonded HA crystallites. It is known that natural bone is constituted of nanosize blade-like crystals of hydroxyapatite grown in intimate contact with collagen fibers [1, 45].

Irregular shape is preferred to the spherical shape, because the molten polymer can penetrate to the particle surface during high temperature composite processing and can form a mechanical interlock with the particle, while the smooth surface of spherical particles does not provide such a locking mechanism. Consequently, the absence of chemical bonding between the polymer and the particle will influence the mechanical properties of the composite. Bhowmik et al. [46, 47] have proved with the use of Steered Molecular Dynamics that mineral-polymer interactions have a significant role in the mechanical response of the polymer. They have evaluated the load-deformation behaviour at the interfaces in a polyacrylic acid/hydroxyapatite composite. It was found that the energy required to pull the polymer into close proximity with HA is significantly higher than in the absence of the mineral. Strong interfacial bonding can effectively transfer the load from the matrix to the reinforcement and weak interfacial bonding can deflect an advancing crack thus providing enhanced fracture toughness and avoiding catastrophic failure.

From a chemical point of view, we can distinguish between several ways to incorporate inorganic systems in organic polymers depending on the interactions between the components: materials with strong (covalent, coordination, ionic) interactions between the two components; weak interactions (van-der-Waals, hydrogen-bonds, hydrophilic–hydrophobic balance); or without chemical interactions between the two components. Four types of mutual arrangements of nanoparticles to polymer chain have been classified by Kickelbick [48]—see Fig. 3: (1) inorganic particles embedded in inorganic polymer,

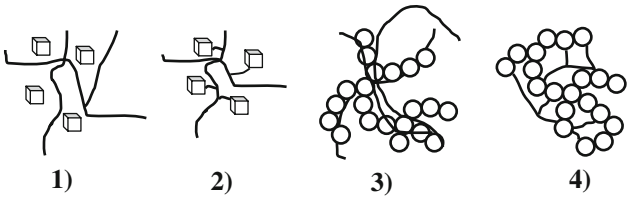


**Fig. 3** Four types of mutual arrangements of nanoparticles to a polymer chain: (1) inorganic particles embedded in an inorganic polymer, (2) incorporation of particles by bonding to the polymer backbone, (3) interpenetrating network with chemical bonds, (4) inorganic–organic hybrid polymer [48]

(2) incorporation of particles by bonding to the polymer backbone, (3) an interpenetrating network with chemical bonds, (4) an inorganic-organic hybrid polymer.

Another parameter that can affect the mechanical properties is the amount of ceramic filler. Espirages et al. [49] have found that the higher the amount of HA in bioactive acrylic bone cements the higher were the compressive and tensile moduli. As the percentage of HA increased to 20 wt%, the heterogeneity of the material was higher. Then the HA ceramic particles, start to act as stress concentration points giving rise to a more brittle material with a lower ultimate tensile strength [21, 49, 50]. Fine particles tend to combine together and form strongly bonded aggregates which may further produce larger structures, agglomerates. The principal adhesion forces: interlocking, electrostatic, van der Waals, liquid bridges, solid bridging, respectively, are applied with increasing agglomerate strength [44].

To form high quality and high performance ceramic polymer composites, the particle aggregates or larger structures termed agglomerates (Fig. 4) must be broken

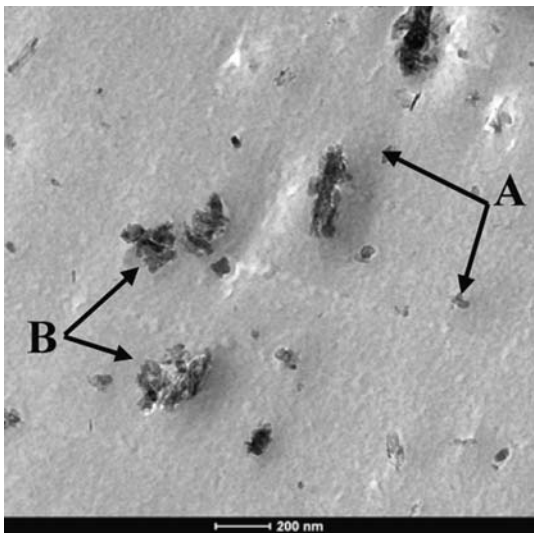


**Fig. 4** TEM image of an HA/polysiloxane composite with homogeneously dispersed particles (**a**) and HA agglomerates (**b**)

down during composite processing into primary particles which are sufficiently dispersed in the polymer matrix. Therefore, specially designed processing equipment is often required, which produces shear forces large enough to overcome various particle adhesion forces. The input energy must be carefully controlled because excessive energy input can cause the fragmentation of primary particles accompanied by heat generation, and this can result in thermal degradation of the polymer matrix. Analysis of the efficiency of particle dispersion in a polymer matrix can be assisted by employing scanning electron microscopy, transmission electron microscopy and image analysis techniques. An example of HA/polysiloxane composite (particles size 20–70 nm) with both, relatively homogeneously dispersed HA particles (a) and HA particle agglomerates (b) is shown in the TEM image (Fig. 4). The existence of HA particles was proven by EDS analysis. For every filled polymer system, there is a maximum volume fraction of particles that can be incorporated into the polymer matrix.

For theoretical analysis, it is normally assumed that the particles have a mono-modal size distribution. In reality, most ceramic powders have broad size distributions, or exhibit bi-modal size distributions. This bi-modal size distribution (Fig. 5) [44] is more efficient, because the small particles can occupy the space between large particles leading to high bioceramic content per unit volume in the composite.

Biocompatible and bio-stable polymers include a few polymers that are potential matrices of bone analogues: poly(methyl methacrylate) (PMMA) [51], polyethylene (PE) [52, 53], polypropylene (PP), polyurethane (PU), polytetrafluoroethylene (PTFE), poly(vinyl chloride) (PVC), polyamides (PA) [54, 55], polycarbonate (PC), poly(ethylene terephthalate) (PET), polyetheretherketone (PEEK) [21], polysiloxanes [56–58], epoxy resin [59] and polystyrene (PS) [60]. Recent tissue engineering has given the significant attention to biodegradable polymers, the main class of which comprises bioresorbable compounds known as alpha-polyesters or poly (alpha-hydroxy) acids, including polylactides (PLA) [5–7, 9, 61–68], polyglycolides (PGA) [6], and their copolymers (PLA/PGA) [6], poly(ε-caprolactone) (PCL) [69–71], poly(vinyl-alcohol) [72, 73], polyhydroxybutyrate (PHB) [74–77], polyanhydrides [78, 79]. Polymers very often display insufficient cell adhesion; their surfaces are hydrophobic or weakly hydrophilic, hindering cell growth in a three-dimensional architecture [21]. Suchý et al. [80] have studied the wettability of glass fibers/siloxane composites in a tissue culture medium and they have found that it is dependent on open pore size. The values of the contact angles for pore sizes 200–400, 400–600 and >600 μm were 33°, 64° and 67°, respectively. These values indicated that the surfaces are wettable i.e. suitable for bone cells in-growth. The wettability of PLGA and PLGA–collagen hybrid sponges were determined by Chen et al. [81]. The contact angles of water on PLGA and collagen-coated PLGA films were 76.3° and 31.3°, respectively. It follows that PLGA sponge was relatively hydrophobic and the wettability of the PLGA–collagen hybrid sponge increased with hybridization with collagen.

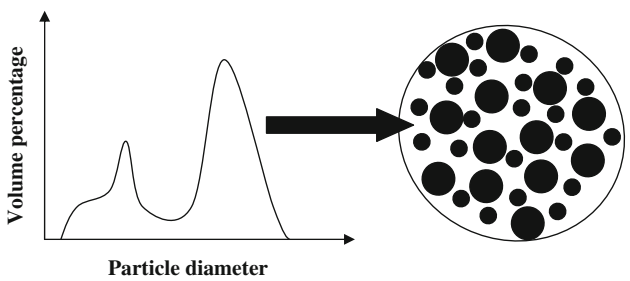


**Fig. 5** Bi-modal size distribution and dispersion of bi-modal size distributed ceramic particles in a polymer matrix [44]

Though many studies have been carried out to reproduce bone artificially mainly with the use of HA and collagen [82], bone is not in a reality a simple mixture of HA and collagen fibers. Bones contains other components such as glycoproteins and glycosaminoglycans, which play a pivotal role in bone metabolisms [83]. In this regard, attempts to mimic the real bone structure and composition have been made with the use of chondroitin sulphate [84, 85], chitosan [86–88] and hyaluronic acid [89–91]. The selection of a polymer matrix of a composite is based on the clinical requirements.

### 2.1 Techniques for HA/polymer nanocomposites preparation

Nanocomposites can be made, conventionally, by several techniques which can be summarized as follows:

1) Thermo-mechanical methods incorporate the impregnating of a porous bioceramic matrix with a polymer or incorporating of bioceramic particles into the polymer matrix using conventional plastics processing technologies [21, 68, 76]. The manufacturing process consists of compounding, milling and compression or injection moulding. The compounding process is mainly crucial in composite production for achieving a homogeneous distribution of bioceramic particles in the composite where the processing parameters such as temperature, screw/rotor speed and processing time should be strictly controlled. Prior to compression or injection moulding, the milled, compounded materials must be dried to avoid the formation of air bubbles at

the processing temperature. Moulding temperature and pressure are two key parameters, which depend on the melting behaviour and viscosity of the composite. For heat-sensitive polymers the moulding temperature must be carefully selected in order to avoid thermal degradation of matrix polymers.

2) Physico-chemical methods incorporate the precipitation of mineral crystals in situ in the polymer matrix (coprecipitation, biomimetic process) or dispersion of bioceramic particles in the polymer solution with subsequent consolidation (solvent casting). The selection of a suitable solvent and the polymer solution concentration are very important factors for satisfactory dispersion and even distribution of bioceramic particles in the final product. Other processing parameters such as mixing mode, stirring time and gelation rate also affect the quality of composites.

Mathieu et al. [68] have compared three methods for preparing a homogeneous blend of hydroxyapatite with a poly(L-lactic) acid. First, the dry process consists in mixing ceramic powder and polymer pellets before a compression moulding step. The second technique was based on the dispersion of ceramic fillers into a polymer–solvent solution. The third method was a melt extrusion of a ceramic/polymer powder mixture. Mixing dry powders led to a ceramic particle network around the polymer pellets, whereas the solvent and melt methods also produced a homogeneous dispersion of hydroxyapatite in the matrix also in the case of other polymers such as poly($\varepsilon$-caprolactone) [68], poly(hydroxybutyrate-co-hydroxyvalerate) [75, 76], poly(D,L-lactide) [5], poly(etheretherketone) [21] and polyamide 66 [55] as proved by the improved of mechanical properties. The main drawback of the solvent casting method is the risk of potentially toxic organic solvent residues. The melt extrusion method has been shown to be a good way to prepare homogeneous ceramic/polymer blends.

Instead of the traditional composite methods, Li et al. [78, 79] have used the in situ polymerization, where after dispersing HA nano-needles into the methacrylate anhydride monomer by long-time ultrasonic treatment, UV was used to initiate the photopolymerization to produce a crosslinking network. The following heat treatment increased the conversion of the anhydride monomers and decreased the toxicity. HA particles were homogeneously distributed at the nanosize scale and the improvements in the mechanical properties (compressive strength, compressive elastic modulus, flexural strength and flexural modulus) were observed.

With the modifications to the solvent casting method described above, a simple technique (Solvent Casting and Particulate Leaching) can be used to produce bioactive and biodegradable composite scaffolds as an excellent 3D substrate for cell attachment and migration in bone tissue engineering applications [92, 93]. Polymer, after dissolution in a suitable organic solvent, is cast into a mould filled with porogen particles. After solvent evaporation, the composite structure in the mould is immersed in a liquid and the porogen is dissolved. To overcome the problem of using organic solvents and solid porogens a technique using gas as a porogen has been developed (Gas Foaming) where polymer structures prepared by compression moulding are placed in a chamber with high $CO_2$ pressure for several days, and then the pressure is a gradually restored to atmospheric level. During this procedure the pores are formed by the carbon dioxide molecules resulting in a sponge like structure. The main drawback of this technique is the use of the excessive heat during compression molding and by the fact that the pores do not form an interconnected structure. A Freeze-Drying technique [94] is also a commonly employed for the fabrication of scaffolds. This technique does not require a solid porogen. The polymer is dissolved into a suitable solvent then water is added and the two liquids are mixed in order to obtain an emulsion. Then the emulsion is cast into a mould and quickly frozen by immersion into liquid nitrogen, followed by freeze-drying to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure but with relatively small pores and irregular porosity. Similar to the previous technique, the Thermally Induced Phase Separation procedure (TIPS) [63, 95] requires the use of a solvent with a low melting point that is easy to sublime. After cooling below the melting point of the solvent and some days of vacuum-drying to sublime the solvent a porous scaffold is obtained.

Among the existing methodologies, in situ formation of nanocomposites by forming nanoapatite crystals in the presence of polymers by the co-solution, co-precipitation method is one of the most attractive routes, since it avoids extensive particle agglomeration if a mechanical mixing between nanopowder and selected polymer is adopted. For hydroxyapatite in situ formation solutions of various compounds e.g. $Ca(NO_3)_2$, $Na_3PO_4$, $(CH_3COO)_2Ca$, $H_3PO_4$, $Na_2HPO_4$, $CaCl_2$ or $Ca(OH)_2$ have been used. This method was successfully used for nano-HA/polyamide composites [54, 96]. Polyamide (as a polar polymer) has good biocompatibility with human tissue, probably due to its similarity to collagen protein in chemical structure and active groups [97]. The formation of chemical binding between n-HA and polyamide, e. g. hydrogen bonding and/or carboxyl–calcium–carboxyl ($[-COO^-]-Ca^{2+}-[-COO^-]$) complex, allowed the uniform dispersion of n-HA in polyamide matrix and high HA contents (over 60%) [54]. A comparison was made of the mechanical properties of composites with n-HA and $\mu$-HA. The results showed that the bending strength and tensile strength of n-HA/PA66 composite increased with

increasing content of n-HA, but decreased with increasing $\mu$-HA content [96]. For chitosan/hydroxyapatite nanocomposites co-precipitation [86] or the in situ hybridization method [87] were used. These methods enable the precipitation of chitosan/hydroxyapatite layer-by-layer. Because chitosan dissolves well in aqueous solutions of various organic acids, the effect of organic acid solutions on the final composite microstructures was investigated by [86] using chitosan dissolved in acetic acid, lactic acid, malic acid, and citric acid. Calcium phosphate formed crystalline HA when acetic acid and lactic acid were used while it was found to be amorphous when organic acids having more than two carboxyl groups were used. According to the reactions in [98], it has been concluded that the growth of HA crystals is inhibited by organic acids having more than two carboxyl groups, which strongly bind to HA surfaces via a $COO^{-}-Ca^{2+}$ bond. Kato et al. [99] have studied a series of nanocrystalline hydroxyapatite/polymer composites via an in situ synthesis method and have found that the crystallization of the hydroxyapatite was retarded in the presence of the ionized polymers that were employed, and they showed that crystallization is concentration-dependent. A number of earlier studies indicated that polyacrylic acid (PAA) is an inhibitor for the crystallization of apatitic crystals [99–101]. The carboxylic group attaches strongly to calcium atoms and forms chelating structures, as shown by the experimental and modeling studies performed by [47, 102, 103]. Interaction between ionized PAA anions and Ca cations to form $PAA^{-}-Ca^{2+}$ intermediate compound delays the desired interaction of P and Ca ions in forming the apatite. However, PAA can act as a suitable site for nucleation for HA crystallization. This fact was used for preparation of needlelike calcium-deficient apatitic crystals with controlled aspect ratios and a core-shell configuration via in situ formation in the presence of PAA [10]. This approach can also be used in the preparation of nanocomposites.

In the in situ techniques, nanocomposites are generated inside a polymer matrix from precursors, which are transformed into the nanoparticles by appropriate reactions. The process of polymer shell formation on preformed inorganic cores, which is realized by polymerization of the monomers with dispersed organic nanoparticles is a so-called ex-situ approach. Particles coated by a polymer shell are considerably more stable against aggregation owing to their greatly decreased surface energy in comparison with bare particles [104].

Many studies have reported the processing of nano HA/collagen nanocomposites. Tampieri et al. [105] have produced and compared artificial bone like tissue HA/collagen composites prepared by using two different methodologies: (1) dispersion of the inorganic component (HA) in a collagen aqueous suspension and then freeze dried and (2) direct nucleation of an apatitic phase on assembling collagen fibrils. Composites obtained using first way were similar to uncalcified natural collagen. The crystallite sizes were not uniform, and were often aggregated and randomly distributed into the matrix, proving that there is no real interaction of HA with collagen fibers. However, the second method allowed the direct nucleation of HA nanocrystals on self assembler collagen fibers. In this case, the two components (HA and collagen) exhibited strong interactions, highlighted by several analysis techniques, which show a complete analogy of the composite with calcified natural tissue. It is well known that the natural bone consists of nano-sized blade-like crystals of HA grown in intimate contact with an organic matrix rich in collagen fibers. This is also the main reason why processing of bone graft nanocomposites using strategies found in nature, so-called biomimetic process, has recently received much attention compared to conventional methods.

### 2.2 Biomimetic process

The term biomimetic process can be defined as a microstructural process that mimics or inspires the biological mechanism, in part or as a whole [106]. This biological process generates highly ordered materials with hybrid composition, complex texture, and ultra-fine crystallites through hierarchical self-assembly, and begins by designing and synthesizing molecules that have the ability to self-assemble or self-organize spontaneously to a higher order structure. A key step in the biomimetic bone graft production is attributed to the crystal growth of apatite phase onto a collagen matrix. In real bone, three collagen molecules form a 3-fold spiral structure via hydrogen bonding [1]. The amino acid residue of each collagen molecule is almost 1000, about 20% of which have carboxyl (–COOH) or amino groups ($–NH_2$) on their outside of the 3-fold spiral. Calcium ($Ca^{2+}$) and phosphate ions ($PO_4^{3-}$) exist on the HA nanocrystal surface. The self-organization had occurred from electrostatic interactions between such ions and functional groups, e.g. $Ca^{2+}$ vs. $COO^{-}$ or $PO_4^{3-}$ vs. $NH_3^{+}$ [107–109]. It was noticed that the nanocrystals of HA elegantly aligned with their c-axis preferentially oriented along the collagen fibers, which indicates a close interaction between HA and collagen phases [84, 107]. Later, other groups [110, 111] that have demonstrated the nucleation of HA crystals onto collagen, have found that the mechanical reliability of the nanocomposite does not attain the properties of the host bone. In order to enhance the mechanical properties of the mineralized collagen, a glutaraldehyde-crosslinked porous HA/collagen nanocomposite was developed [112–115]. The interconnecting 3D porous structure of the composite provides a large surface area for cell attachment, supports cellular growth and offers sufficient space for nutrient transportation [116].

The biggest practical problems with collagen type-I are its cost, and the poor definition of commercial sources of this material, which makes it difficult to follow up on well controlled processing. Therefore, collagen type-I can be replaced by a gelatin precursor [117–119]. For gelatin crosslinking, a sugar such as glucose or fructose [120] was used. An interesting method for the self-assembly of HA coatings onto collagen membrane involved soaking the collagen membrane in a simulated body fluid (SBF) solution with and without citric acid [121]. Interestingly, the membrane soaked in SBF with citric acid has gradually stimulated the nucleation of HA crystals. Though citric acid has been known as an inhibitor for the formation of calcium phosphates, Rhee et al. [98] indicated that citric acid had a very good ability to induce the carbonate-containing HA formation on a bio-inert collagen membrane within a limited concentration range, 0.3–2 mM. The hydroxyl group of citric acid may adhere to a collagen membrane surface through the hydrogen bonding between the OH group of citric acid and the CO and NH groups of collagen. Negatively charged citric acid can act as a nucleus for a HA crystal. Studies were made of nucleation of HA in simulated body fluids onto different monolayers, such as arachidic acid with carboxyl groups [15, 122] or alkanethiols [123] having $CH_3$, $PO_4H_2$, COOH, $CONH_2$, OH, and $NH_2$ terminal groups. The growth rate decreased in the order $PO_4H_2 > COOH \gg CONH_2 \sim OH > NH_2 \gg CH_3 \sim 0$. These results confirmed that apatite formation initiated via calcium ion-adsorption through complexation with a negative surface charged.

Although many research studies for artificial bone production have been carried out using HA and collagen, real bone contains other bioorganics. An attempt was therefore made to mimic and combined both the bioorganic composition of bone and the peculiar configurational arrays of HA nanocrystals on bioorganics such as chondroitin sulphate [84, 85]. Other polymers such as poly(vinyl alcohol) [72] and poly(lactic acid) [124] were used for preparation of 3D porous nanocomposite suitable for tissue regeneration using a biomimetic strategy.

The ultimate goal of scaffold design is to produce an ideal structure that can replace the natural extracellular matrix until the host cells can repopulate and resynthesize a new natural matrix. Nanofibers can be manufactured by various methods e.g. the sol–gel method, the phase-separation technique or electro-spinning. Electro-spinning has recently been introduced as the most promising technique for manufacturing in vitro fibrous scaffolds for tissue engineering applications, with the fiber diameter ranging from a few microns to <100 nm [125]. It has been observed that cells proliferate intensively on nanofibers because of the high surface area-to-volume [126]. This technique is used to fabricate nanofibrous structures from natural and synthetic polymers, such as collagen [127–132], chitosan [133], silk fibrin [134] and poly(DL-lactide-co-glycolide) [126, 135, 136], poly(lactide) [137–139], polyurethane [140], polycaprolactone [141, 142] and collagen-glycosaminoglycan scaffold [143, 144]. The electrospinning method enables the production of hydroxyapatite nanoparticle loaded polymer (e.g. collagen, polycaprolactone, polylactide) nanofiber. These nanofibrous scaffolds modified by HA to hydrophilic (in the case of hydrophobic polymer) are highly porous and offer a biomimicking structure for adhesion, accommodation, proliferation and mineralization of osteoblast cells and synthesis of biominerals [145–151]. Zhang et al. [152] have used electrospinning for preparation of fibrous poly(butylene succinate)/wollastonite/apatite scaffold, followed by a biomimetic process.

To overcome the problem with agglomeration of HA particles during the mixing process a 12-hydroxystearic acid ($C_{18}H_{36}O_3$) [153], as a mediator has been introduced between the hydrophilic HA and the hydrophobic PLA fibers. These mediators or surfactant (**surf**ace **acti**ve **a**g**ent**) are compounds that are amphiphilic, having both, a hydrophilic end, and a hydrophobic end, usually a long chain hydrocarbon fragment. Surface modification of HA nanoparticles using a surfactant mediator is another promising technique for preparation of homogeneous nanocomposites.

### 2.3 Surface modification of HA nanoparticles

The purpose of the surface treatment is not only to guarantee the even distribution of HA particles at a high loading level in the polymer matrix but also to prevent or delay the debonding process of HA particles from the polymer matrix. However, surface modifiers must satisfy several requirements e.g. no toxicity, biocompatibility and no changes in the biological or physico-chemical properties of the fillers.

Various ways have been developed for HA surface modification. The HA surface was modified with hexanoic $CH_3(CH_2)_4COOH$ and decanoic $CH_3(CH_2)_8COOH$ acids by Tanaka et al. [154]. Hexanoic and decanoic acids were strongly hydrogen-bonded to the surface P–OH groups of HA. When the modified samples were outgassed at 500°C, acids were removed and the surface P–OH groups were revived. Treatment with carboxylic acid, which has a longer alkyl group than acetic acid, is better for making the HA surface hydrophobic. Moreover, the interaction between HA and carboxylic acids is of great interest in medical fields because animal organisms contain various kinds of carboxylic acids existing in proteins, lipids, vitamins, and metabolic inhibitors. Kim et al. [71] have developed a novel nanocomposite system consisting of HA

and poly(ε-caprolactone) with oleic acid as an amphiphilic surfactant. Oleic acid belongs to the fatty acids family, it is generally noncytotoxic and has no influence on human osteoblast cell proliferation [155]. Another modifier belonging to the same group is stearic acid, which has been used by Li et al. [156]. They found that the optimal addition was about 3 wt% without changing the particle size and with good bioactivity in vitro. Shimabayashi et al. [157] have used a sodium dodecyl sulphate (SDS) as a surfactant for the systems HA-SDS-polyvinylpyrolidon (PVP) and HA-SDS-bovine serum albumin systems. SDS is bound to HA through an electrostatic attractive force between a negative charge of $DS^-$ and a localized positive charge on the surface of HA. Hydrophobic interactions of SDS with PVP on the surface of HA are important factors in the understanding of the mechanisms of the formation of mammalian hard tissues. These tissues are composed of HA, proteins and amphiphilic compounds, which interact with each other in hard tissue [158].

An alternative means of surface modification for HA involves esterification reactions. Surface modification of HA with dodecyl alcohol [159] results in stable HA suspensions in ethanol. The dodecyl groups react with acidic phosphate sites on the HA surface to form esterified HA particles. The number of surface P–OH groups on non-stoichiometric HA is 2.6 groups $nm^{-2}$ [160]. Hence regulation of surface P–OH groups by surface modification may be expected to change various properties of HA, e.g. acidity and basicity, affinity, reactivity to molecules, catalytic activity and increasing electrophoretic mobility, which may improve the adsorption properties of proteins. Surface modification of HA with alkyl phosphates has been performed by [161, 162]. In 1964, Nelson and Toy synthesized monoalkyl phosphates by the reaction of alcohols (R–OH) with pyrophosphoric acid ($H_4P_2O_7$) according to reaction 1 [163]:

$$\mathrm{R\text{--}OH + H_4P_2O_7 \rightarrow ROPO(OH)_2 + H_3PO_4} \quad (1)$$

Tanaka et al. [164, 165] have modified the HA surface using pyrophosphoric acid (PP). Since the HA possesses three kinds of P–OH groups, the pyrophosphoric acids react with surface P–OH groups and form additional surface P–OH groups and phosphoric acids. With increasing PP concentration, the pyrophosphoric acid reacts with the remaining surface P–OH groups according to the reaction shown in Fig. 6 [164].

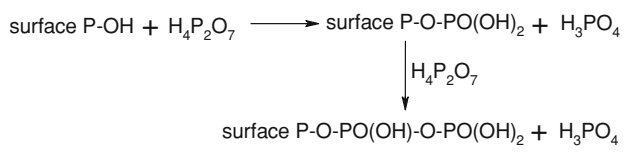


**Fig. 6** Modification of HA surface by pyrophosphoric acid [164]

Some of the additional surface P–OH groups can be hydrogen bonded among the particles. As a result, the modified particles were more aggregated than the unmodified ones, leading to a reduction in the effective surface area. Moreover, the additional amount of $PO_4$ was increased with an increase in PP concentration, leading to a decrease in the Ca/P molar ratio from 1.62 to 1.35.

Silanes are popular and widely used such as bonding agents, adhesion promoters, and for bridging organic and inorganic components. The silane coupling agent will not increase the cytotoxicity of the end products [59], but according to Dupraz et al. [166] free silane is biotoxic to tissue cell. Its applications have been reported in fabricating both, a HA-filled dental composite [167] and a bone-repairing material [168]. A biological basis for the role of silica in bone formation was established by Carlilse [169] in a study deals with a role of silicon in bone calcification. Nanophase hydroxyapatite particles were coated with varying amounts of silica (5–75 wt%) via the hydrolysis of tetraethyl orthosilicate by Borum et al. [170]. Hench et al. [171] have shown the integral role that silica plays in the bioactivity and osteogenic potential of Bioglass. The surface chemistry and structural basis for enhanced HA and bone formation in Bioglass and related glass ceramic system has been revealed in studies due to the high density of surface silanol groups (Si–OH) that exist on amorphous silica. Silanol groups tend to be more effective at inducing HA formation than highly crystallized or heat-treated silica surfaces [172]. The surface of HA particles was treated by repeated modification with hexamethyldisilazane $[(CH_3)_3Si]_2NH$ (HMDS) in hexane and using thermal treatment by Tanaka et al. [173]. HMDS reacted with surface P–OH groups of HA to yield surface $Si–(CH_3)_3$ groups and the surface of the HA became hydrophobic. When the modified materials were heated to 500°C in air, the surface $Si–(CH_3)_3$ groups formed three kinds of surface Si–OH groups. Surface Si–OH groups formed in this way and the remaining surface P–OH groups can react with HMDS by repeating the modification, resulting in an increase in the surface Si atoms according to a mechanism proposed by Tanaka et al. [173] (Fig. 7). The modified material has the surface $Si–(CH_3)_3$ or Si–OH groups.

Wen et al. [58] have used a vinyl triethoxy silane that was hydrolyzed into silanol in acid aqueous solution of ethanol according to reaction 2 [58]:

$$\mathrm{CH{=}CH_2Si(OCH_2CH_3)_3 + 3H_2O \xrightarrow{H^+} CH{=}CH_2Si(OH)_3 + 3C_2H_5OH} \quad (2)$$

The hydrolysate reacted with active surface groups of HA particles such as $OH^-$, $Ca^{2+}$ and $PO_4^{3-}$. Then hydrophobic organic groups were formed on the surfaces of the HA particles [174]. Vinyl groups of the hydrolysate, which

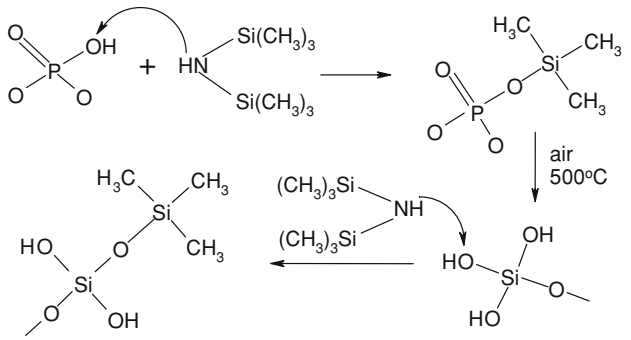


**Fig. 7** Mechanism of repeated modification of HA with hexamethyldisilazane using thermal treatment according to Tanaka et al. [173]

covered the surfaces of the n-HA particles, reacted with vinyl groups of silicon rubber under the action of vulcanizer (peroxide) and chemical bonds were achieved. The HA micro particles were subsequently modified with amino groups with the use of $\gamma$-aminopropyltriethoxysilane [175] to prepare HA/silicon composites suitable for percutaneous implant materials. Deb et al. [176] have used $\gamma$-methacroyloxytrimethyl silane as a coupling agent for micro HA as filler in a two-paste bioactive bone cement. Damia et al. [57] have prepared a silane solution by mixing equimolar amounts of [3-(2-aminoethylamino)propyl]trimethoxysilane and glycidoxypropyltrimethoxysilane to prepare a bioactive coating. In the works presented by Wang et al. [52, 53], two approaches were used to improve the interfacial condition: the use of silane (3-trimethoxysilylpropylmethacrylate) treated HA as the filler and the application of polymer grafting (acrylic acid) for polyethylene. Results indicated that the silane coupling agent also facilitates polymer penetration into the cavities of the HA surface during composite processing, which enhances mechanical interlocking between the HA and the matrix for the resultant composites. Liu et al. [26] have prepared polyacrylic acid (PAA) coating HA and they found that PAA had almost no additional effect on the mechanical properties of composites HA/polyethylene glycol/poly(butylene terephthalate) composites, either in the dry state or in an aqueous environment. The surface grafting reactions of a series of isocyanates with hydroxyapatite particles at different temperatures were studied by [177]. The reactivity of isocyanates towards the surface hydroxyl groups of HA was strongly affected by the structure of the isocyanates. The results showed that both hexamethylene diisocyanate (HMDI) and isocyanatoethyl methacrylate (ICEM) react readily with HA while ethyl isocyanate acetate (EIA) and butyl isocyanate (BIC) have lower reactivity towards HA particles. Therefore ICEM and HMDI, as suitable coupling agents, were used to introduce polyethylene glycol to the surface of HA particles [178] or to couple poly(methylmethacrylate) to HA particles through isocyanateoethylmethacrylate [51]. A novel method of grafting ring-opening polymerization of L-lactide (LLA) in the presence of stannous octanoate onto the surface of hydroxyapatite nanoparticles was developed by Hong et al. [62]. PLLA was directly connected onto the HA surface through a chemical linkage, i.e. carbonyl group of PLLA on the surface of PLLA-g-HA. In this way, the g-HA particles can easily be dispersed in the PLLA matrix and strongly tethered to the molecular chains of the PLLA matrix. Grafted-PLLA molecules played the role of tie molecules between the fillers and the PLLA matrix, and the g-HA particles played the role of heterogeneous nucleating agents in the crystallization of the PLLA matrix [61]. The effect of surface-modified HA with ethylene glycol of a new type nanocomposite consisting of HA/PCL on biocompatibility was described by Lee et al. [70]. In vitro biological evaluation showed that the existence of modified HA in nanocomposites offered favourable environments for protein adsorption and cell adhesion and proliferation. A novel approach for HA surface modification was described by Lee et al. [179]. First, in situ synthesis of surface thiol-functionalized HA (HA-SH) was realized by adding 3-mercaptopropionic acid during hydrothermal synthesis of HA, (Fig. 8a) [179]. This was followed by grafting polymerization of ethylene glycol methacrylate phosphate (EGMP) by radical chain transfer generating the sulphur-centred radicals on the HA surfaces (Fig. 8b) [179], which initiated the surface grafting polymerization of EGMP (Fig. 8c) [179]. The colloidal stability of PolyEGMP-grafted HA over synthesized HA nano-crystals in water increased dramatically without inter-crystal aggregation.

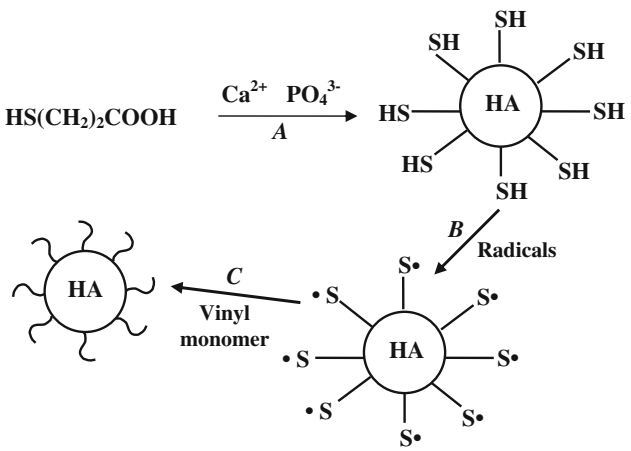


**Fig. 8** HA surface modification by grafting polymerization according to Lee et al. [179], *A*-surface thiol functionalized HA, *B*-sulphur centred radical on HA surface, *C*-surface grafting polymerization of EGMP

## 3 Conclusions

Nowadays, multiple methods are available for treating bone defects e.g. autografting and allografting. These are considered good therapies, but they have limitations, such as the supply of an autograft and the possibility of pathogen transfer from an allograft. Because bone has the exceptional ability of self-repair, the use of synthetic bone grafts is a great challenge for man to produce what nature has made. Although there has been good progress in bone grafting using synthetic bone grafts, the way in which they execute their functions in vivo can be quite different, and most of them differ from natural bone either compositionally or structurally. A single-phase material does not always provide all the essential features and therefore, there is a great need to engineer multi-phase materials (composites) with a structure and composition similar to that of natural bone.

This review has highlighted the problem of dispersion of hydroxyapatite particles in polymer matrices. It has discussed approaches to overcome this problem, which is significant for the attempt to produce biocomposites with a qualities equivalent to those of natural bone. Homogeneously dispersed nanoparticles in the polymer matrix play a key role, mainly in mechanical properties. The interfacial strength between filler and polymer is a very important factor, because lack of adhesion between two phases will result in early failure. Moreover, nanoscale-organized HA/polymer nanocomposite provides a better substrate condition for cellular interaction, particularly in the cell adhesion and proliferation state, when compared with the conventional composite.

Several approaches can be used to overcome this problem, e.g. hydroxyapatite particle modification or biomimetic process. A biomimetic strategy (using strategies found in nature) that either mimics or inspires biological mechanisms and may replicate the natural process is a convenient tool for preparing of biocomposites with features of natural bone, both in the main composition and in the hierarchy microstructure.

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