JOURNAL OF NANO- AND ELECTRONIC PHYSICS
Vol. **10** No 6, 06003(11pp) (2018)

ЖУРНАЛ НАНО- ТА ЕЛЕКТРОННОЇ ФІЗИКИ
Том **10** № 6, 06003(11cc) (2018)

Design and Fabrication of Polymer-Ceramic Nanocomposites Materials for Bone Tissue Engineering

L.F. Sukhodub, K. Dyadyura*

Sumy State University, 2, Rymskyi-Korsakov Str., 40007 Sumy, Ukraine

(Received 22 July 2018; revised manuscript received 28 November 2018; published online 18 December 2018)

This study intends to present a review of the different types of ceramic-polymeric nanocomposites. The present article has as its main goal to analyze the most recent reported studies based on polymer-ceramic nanocomposites produced for bone replacement and regeneration. Scaffold fabrication methodology, mechanical performance, biocompatibility, bioactivity, and potential clinical translations are discussed. Some of the most popular processing methods to produce bioceramic structures are discussed, with an emphasis on the production of HA scaffolds. The numerous scaffold processing methods currently adopted to fabricate porous bioceramic structures and their current limitations, are discussed and comparisons are made to understand the rationale and motivation for the research. Structural, morphology, micrograph and chemical composition of this nanocrystalline composites were characterized by XRD, AFM, SEM and FTIR, respectively.

Keywords: Nanohydroxyapatite, Scaffolds, Composites, Biodegradable polymers, Regenerative medicine, Tissue engineering.

DOI: 10.21272/jnep.10(6).06003 PACS numbers: 87.85.J –, 87.64.Bx, 87.64.Ee

1. INTRODUCTION

Restoration of bone tissue is a complex process due to the coordinating influence of central and local regulatory systems on structural repair of bone tissue in the area of injury [1-3]. Further development of the theoretical foundations of the mechanisms of reactivity and regeneration of bone tissue with a directed effect on the healing processes is one of the most important directions of the current stage in the development of ideas about reparative regeneration [4, 5]. Nowadays, the goal of bone tissue engineering is not to permanently replace the damaged bone tissue by synthetic scaffold, but provide a support for its regeneration as long as necessary. The scaffold should help to stimulate bone growth and attract newly formed bone tissue, before being remodeled [6, 7]. The principal of bone tissue engineering is based on using natural or synthetic scaffolds that are biocompatible and similar (mechanically, chemically and biologically) to the native extracellular matrix (ECM) of human bone [8]. Constructing a viable graft depends, in part, on choice of scaffold material [9]. Because a human body is a complex and sensitive system, the requirements for scaffold materials are manifold and extremely challenging.

The success of orthopedic implants strongly depends on its interaction between its surface and the surrounding tissues after implantation [10]. At present, perfect orthopedic implants are inaccessible due to inadequate osteointegration, which increases the risk of implant failure [11]. Implants used, as a rule, lack three important characteristics of living tissues: the ability to self-repair; the ability to maintain a blood supply; and the ability to modify their structure and properties in response to environmental factors such as mechanical load [12]. Due to the basis of growth and regeneration of bone tissue are nanoscale processes, the focus of researchers today focuses on the creation of nanocrystalline structure of new materials (nanocomposites) [13] with biomimetic morphology, which corresponds to the physical, chemical, mechanical, biological characteristics of living tissue [14, 15].

The authors' research is aimed at the development of nanostructured hybrid materials based on synthetic calcium of deficient hydroxyapatite (CdHAp) in combination with natural biodegradable polysaccharides for the purpose of their further use as scaffolds of bone-forming cells, as well as for the controlled delivery of a dosage amount of anti-inflammatory drugs. The aim of the present article is to review and update various aspects involved in incorporation of synthetic nanohydroxyapatite into synthetic polymers, in terms of their potentials to promote bone growth and regeneration in vitro, in vivo and consequently in clinical applications.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Composition of Bone

Bone is a composite natural living tissue which comprises of an organic phase in which calcium containing inorganic phase crystals are embedded. Bone by weight contains about 30 % matrix, 60 % mineral (for example, $Ca_{10}(PO_4)_6(OH)_2$) and 10 % water (for example, $CaHPO_4 \cdot 2H_2O$) (Fig. 1) [2, 16]. The bone matrix is primarily collagen which responsible for the tensile strength. The mineral component of bone is calcium phosphate, which imparts compressive strength to the bone tissue [3]. The Figure 1 shows the model for bone matrix organisation. The model has lamellae with an apatitic core (see Fig. 1), surrounded by a structure similar to octacalcium phosphate – citrate as the disordered hydrated region cover-ing the lamellae surface. These phases have multiple compo-

* dyadyura@pmtkm.sumdu.edu.ua

nents which consist of, in decreasing proportions: minerals, collagen, water, non-collagenous proteins, lipids, vascular elements, and cells (Table 1). There are two types of bone tissue, cortical (compact), and cancellous (trabecular). The cortical bone has higher mineral contents than the cancellous bone [17]. In addition, given the presence of spaces within the structure of cancellous bone, the latter is more osteogenic than cortical bone [18]. Compact bone has Young's modulus of elasticity ranging from 7-30 GPa and compressive strength in the range of 131-224 MPa [2, 4], while Young's modulus and compressive strength for trabecular bones are 50-100 MPa and 5-12 MPa respectively [17, 18].

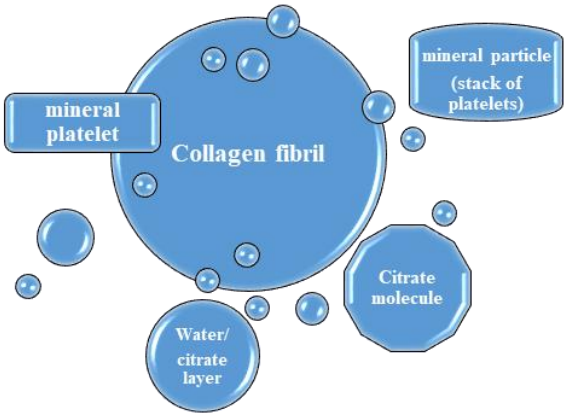


Fig. 1 – The schematic view of the bone matrix organisation (consists of the molecular components: water, biological apatite, collagen and other proteins): the schematic view of the detailed structural model of bone mineral showing how citrate anions and water bind the mineral platelets together

While the type I collagen is mainly responsible for the bone tensile strength, the embedded mineral in the matrix provides the compressional and torsional strength in bone. The water and cellular phases do not do much for the bone mechanical strength and stiffness, but contribute by decreasing the brittleness and enhancing the resilience and toughness.

Besides, many kinds of essential trace elements including silicon (Si), fluorine (F), zinc (Zn), strontium (Sr) [29, 30, 31], magnesium (Mg), boron (B), and copper (Cu), sodium (Na), manganese (Mn), carbonate (CO_3), potassium (K), chlorine (Cl) etc. present in biology bone, which play an important role in bone growth or can have an effect on bone metabolism [19, 20, 21].

Table 1 – Composition of natural bone

Structure of the bulk			
Inorganic Phase	Wt %	Organic Phase	Wt %
Hydroxyapatite	≈ 60-70	Collagen	≈ 20 30
Carbonate	≈ 4	Water	≈ 9
Citrate	≈ 0,9	Non-Collagenous proteins (osteocalcin, osteonectin, oeteopontin, thrombospondin, morphogenic proteins, sialoprotein, serum proteins)	≈ 3
Sodium	≈ 0,7		
Magnesium	≈ 0,5		
Other traces (Cl^-, F^-, K^+, Sr^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+})		Other traces (Polysaccharides, lipids, cytokines)	
		Primary bone cells (Osteoblasts, osteocytes, osteoclasts)	
Mechanical properties of the collagen matrix		*E* = 1-2 GPa, *UTS* = 50-1000 MPa	
Mechanical properties of the calcium phosphate mineral		*E* = 130 GPa, UTS = 100 MPa	
Compressive strength of cortical bone		131-224 MPa	
Compressive strength of cancellous bone		5-12 MPa	
Young's modulus of cortical bone		7-30 GPa	
Young's modulus of cancellous bone		50-100 MPa	

While the type I collagen is mainly responsible for the bone tensile strength, the embedded mineral in the matrix provides the compressional and torsional strength in bone. The water and cellular phases do not do much for the bone mechanical strength and stiffness, but contribute by decreasing the brittleness and enhancing the resilience and toughness.

Besides, many kinds of essential trace elements including silicon (Si), fluorine (F), zinc (Zn), strontium (Sr) [22], magnesium (Mg), boron (B), and copper (Cu), sodium (Na), manganese (Mn), carbonate (CO_3), potassium (K), chlorine (Cl) etc. present in biology bone, which play an important role in bone growth or can have an effect on bone metabolism [23, 24].

Bone is a dynamic living tissue which constantly undergoes remodeling. The old bone is removed by osteoclasts whereas new tissue is formed by osteoblasts. Osteoclasts dissolve the mineral phase via locally decreasing pH, resulted a small cavity created on the surface. Subsequently, bone forming cells called osteoblasts begin to fill in the cavity with the osteoid which mineralized in new bone tissue [25]. Osteoblasts activity decreases with age leading to imbalance of resorption and formation of new bone.

The processes of new bone formation and extracellular matrix deposition are regulated by a range of growth factors and biomolecules. Bone morphogenic proteins (BMP) play a critical role in bone and cartilage development, and have the ability to trigger proliferation and differentiation of osteoprogenitor cells [26].

Bone substitutes are formed by a biomaterial scaffold that acts as mimetic ECM to induce new functional bone regeneration. Natural bone has an architectural design consisting of nanoscale to macroscopic dimensions, which provide it with stable mechanical properties. In order to mimic the nanostructure in natural ECM and mechanical characteristics of the natural bone, vast research has focused on manipulating polymeric scaffolds at the nanostructure dimension, for instance by incorporation of nanoparticles, nanotubes and nanofibers into the polymer matrix [27].

Scaffolds by design are not intended to be permanent implants and will ideally facilitate host cells to deposit ECM and replace the scaffold structure over time [28, 29]. Different biomaterials have been employed to mimic ECM.

The main disadvantages of metals and ceramics are

that they lack degradability under biological conditions and that their processability is extensively limited as opposed to polymers, which offer wide design flexibility [16, 17].

During scaffold manufacture it would therefore seem logical to include a combination of materials to create a composite scaffold, potentially allowing greater scaffold bioactivity and structural biomimicry to be achieved [30, 31]. Scaffold bioactivity is also increased by incorporating materials that possess the ability to interact with or bind to living tissues. Increased scaffold bioactivity can in turn lead to improved bone cell ingrowth (osteoconduction), stable anchoring of scaffolds to host bone tissue (osseointegration), stimulation of immature host cells to develop into osteogenic cells (osteoinduction) and increased vascularisation [32, 33]. Enhanced osteoconductive properties and osteoinductive behavior can be achieved using composite scaffolds with ceramics [34] and incorporating growth factors; all this provides osteogenic response [35, 36]. At the beginning of the sequence, lining cells on the surface of bone become activated and retract.

Growth factors also have a significant role to play in successful bone tissue engineering scaffolds.

In light of the above, there is considerable ongoing effort to address the design of composite materials, which include ceramics and polymers, to mimic the microstructural features of bone. Hydroxyapatite (HA) and tricalcium phosphates (TCP) have predominated these studies, because they resemble the natural inorganic component of bone and possess osteoconductive properties [21, 24].

2.1.2 Hydroxyapatite

Apatite is a common term for crystalline minerals and can be represented by the formula $M_{10}(ZO_4)_6X_2$. Each component (M, ZO4, and X) in the formula can be replaced by a large number of different ions listed below [37]:

$M = Ca^{2+}$, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, H^{+}, Na^{+}, K^{+}, Al^{3+} and other

$ZO_4 = PO_4^{3-}$, AsO_4^{3-}, VO_4^{3-}, SO_4^{2-}, CO_3^{2-}, SiO_4^{4-} and other

$X = OH^{-}$, F^{-}, Cl^{-}, Br^{-}, O^{2-}, CO_3^{2-}, vacancies and others

Calcium phosphates (CPs) are the most important inorganic constituents of biological hard tissues [42]. CPs are a group of minerals containing calcium ions (Ca^{2+}) together with orthophosphates (PO_4^{3-}), metaphosphates ($P_2O_7^{4-}$) and occasionally hydrogen (H^{+}) or hydroxide (OH^{-}) ions.

CaPs can be obtained in different crystalline or amorphous phases, depending on the synthesis conditions (see Fig. 2). These materials differ in Ca/P molar ratio and both physicochemical and biological properties, such as solubility, biodegradability, and bioactivity [1].

Two different crystallographic structures have been proposed for biological apatites [8]: (1) hexagonal (not close packed), space group $P63/m$, with lattice parameters $a = b = 9.432$ Å, $c = 6.881$ Å, and (2) monoclinic with lattice parameters $a = 9.421$ Å, $b = 2a$, $c = 6.881$ Å,

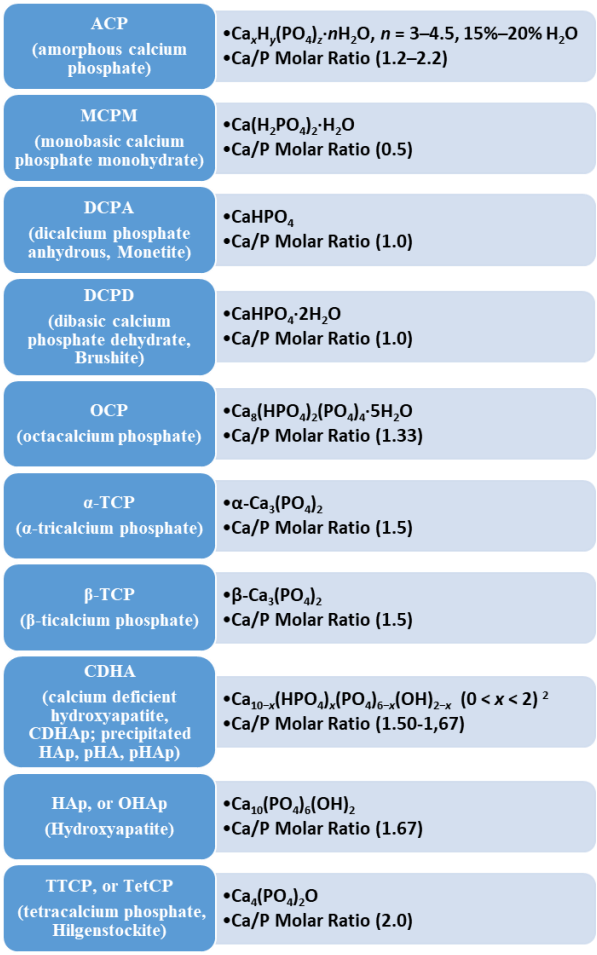


Fig. 2 – Calcium phosphates of biomedical interest

$\gamma = 120°$. These two structures share the same elements, with a stoichiometric Ca/P atom ratio of 1.67. The major difference between them is the orientation of the hydroxyl groups. In the hexagonal HAp, two adjacent hydroxyl groups point at the reverse direction, while in the monoclinic form – hydroxyl groups have the same direction in the same column, and an opposite direction among columns.

They are present in bone, teeth, dentine and cartilage. CPs based biomaterials made of the combination and processing of these materials are brittle. For this reason, these materials are used primarily as fillers and/or as coating materials. The proposed mechanism of CPs integration into bone tissue is through an initial process of dissolution and resorption, followed by a subsequent precipitation of a carbonate-substituted calcium deficient biological apatite. Biological apatite is a non-stoichiometric form of calcium deficient hydroxyapatite [36].

The hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] is the main inorganic mineral phase. The Ca/P ratio of HA is less than 1.67 [4]. Physiochemical characteristics of nHA such as its high melting point (does not melt in human body), its hard and wear resistance as well as its ability to resists surface reaction with certain fluids in the body, render nHA an interesting bioceramic material for bone tissue engineering (see Fig. 3). Hexagonal HAp is the common form in biology and medicine [3, 37].

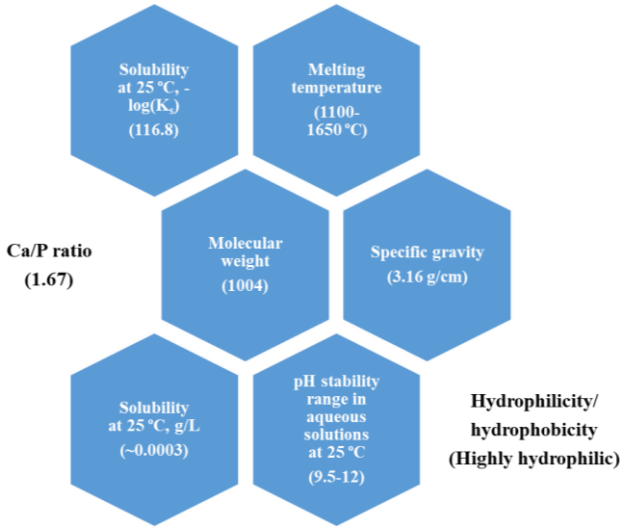


Fig. 3 – Physiochemical characteristics of synthetic nanohydroxyapatite

ISO 13779-6:2015 specifies requirements for hydroxyapatite powders used as a raw material for the manufacturing of surgical implants or coating of surgical implants.

Recently, studies in this area have shifted towards calcium phosphates with a Ca/P ratio lower than hydroxyapatite, since they have a higher degree of resorption, in particular, biomaterials that contain various calcium phosphates, which allows controlling the level of resorption of the implant in the body. Depending on the type of solid tissue, biological apatite exhibits various morphologies of the crystals [6, 7]. Synthetic HA when prepared via a high-temperature reaction is a highly crystalline ceramic. Although synthetic and natural HA differ in terms of physical microstructure, crystal size and porosity, chemical similarities to bone accounts for the osteoconductive potential [10]. One of the most important features of hydroxyapatite is its ability to replace ions (ion exchange). These replacements alter the lattice parameter and the solubility. The Ca^{2+} sites in bioceramics can be replaced by various monovalent (K^+, Na^+, Ag^+), divalent (Cd^{2+}, Zn^{2+}, Eu^{2+}, Sr^{2+}, Mg^{2+}, Ga^{2+} etc.), trivalent (Bi^{3+}, La^{3+}, Y^{3+}, Al^{3+} etc.), tetravalent (Zr^{4+}), and pentavalent (Ta^{+5}, V^{+5}, Nb^{+5}) cations, which controls the implant-environment interactions [1, 8, 17, 38]. Carbonate substitution in hydroxyapatite causes changes in several physical properties; a drop of the thermal stability and reduced crystallite volume was observed; the a-axial length decreases whereas the c-axial length increases compared to non-substituted ceramic apatites.

There is much interest on developing novel processing methods and improvement of existing fabrication methods to produce porous HA structures of controlled porosity and improved mechanical properties.

2.1.3 Methods of Ion Substitution in HA

Ions, and in particular Ag, can be incorporated in the HA structure with either of the following methods:

(i) Wet precipitation method, where all precursors are solubilized in water and precipitate at the same time [4, 8].

(ii) Ion exchange between the previously prepared pure HA and a metal salt solution by submerging HA in the salt solution [4, 9].

(iii) Sol-gel method [5], by which a colloidal solution is formed from monomers and will act as the precursor for an integrated network of ions and ceramic crystals.

(iv) Ultrasonic spray pyrolysis [13], which is an aerosol synthetic method with deposition of metals on nanoparticles.

(v) The microwave method [2, 4] regardless of the technique used, the resulting material may be then subjected to heat treatment by sintering or drying which can affect the final structure or the crystal size [2, 3].

As the main mineral component of bone ECM, hydroxyapatite (HA) has been used to increase biocompatibility, osteoconductivity, and osteoinductivity in different scaffolds [1, 2, 5]. Despite not being osteoinductive, hydroxyapatite is osteoconductive and able to directly bond to bone. HA enhance the attachment, differentiation, and proliferation of relevant cells (such as osteoblasts and mesenchymal cells). Its application in bone tissue engineering is, however, limited due to low mechanical strength and very slow biodegradation. The dissolution rate of synthetic HA is depended on the crystallinity, porosity and composition (impurities) of the HA phase; type, concentration and pH of the solution, degree of the solution saturation, solid/solution ratio, etc. However, properties such as its lack of flexibility and its brittleness make it difficult to form nHA into specific shapes for bone tissue engineering on its own [20]. These limitations could be overcome by incorporation of nHA into a suitable polymeric scaffold [38].

2.1.4 Polymer Ceramic Composite Materials

Biopolymer composites and nanocomposites have been investigated as the best approach to mimic natural bone properties and create an ideal artificial scaffolding for bone regeneration and repair. The combination of polymers and ceramic phases leads to composite materials with improved mechanical properties due to the inherent higher stiffness and strength of the inorganic material [4, 39]. Natural polymers (i.e., collagen, alginate, agarose, chitosan, fibrin and hyaluronic acid or hyaluronan) and/or synthetic polymers are generally considered as interesting materials to support cell ingrowth in most tissues (see Table 4).

Natural polymers offer the advantage of good biocompatibility and are bioactive as they can interact with the host tissues [39, 40]. Collagen based nanocomposites have been greatly investigated for bone tissue engineering applications. Collagen nanofibrous structure (50-500 nm) can have improving effect on cell attachment, proliferation and differentiation for bone regeneration. However, the natural and rapid degradability of this family of materials limits their applications [55]. Other naturally occurring materials used for bone tissue engineering include gelatine- and fibroin-based nanocomposites. Like the collagen family, these natural polymers also have limited applications due to the nature and rate of their degradation.

Table 4 – Mechanical properties of natural bone tissue compared with other degradable and non-degradable materials and their applications

Material Type	Compressive strength (MPa)	Tensile Strength (MPa)	Young's Modulus (GPA)
Human cortical	131-224	35-283	17-20
Human cancellous	5-10	1.3-38	0.05-0.1
Collagen	0.5-1	50-150	0.002-5
Chitosan	1.7-1.4	35-75	2-18
PGA	340-920	55-80	5-7
PLLA	80-500	45-70	2.7
D,L(PLA)	15-25	90-103	1.9
L(PLA)	20-30	100-150	2.7
PLGA	40-55	55-80	1.4-2.8
PCL	20-40	10-35	0.4-0.6
Hydroxyapatite	500-1000	40-200	80-110

Therefore, a thorough search of the literature reveals that synthetic polymers have been extensively studied as the basic materials for the purpose of fabricating tissue engineered scaffolds with potentials to promote in vivo bone ingrowth and subsequently repair or regenerate bone to replace missing tissue, as they come in various types including degradable and non-degradable, can be easily modified and also mass-produced [56-59].

These materials can be amorphous and crystalline with chains being linear, branched or cross-linked with other chains. They enjoy several advantages including versatility and processability, which enables imparting the desired morphology – i.e., porosity accommodating a wide range of pore sizes and shapes and desired mechanical response [41]. Physical-chemical properties of polymer matrices can be easily modified and the mechanical behavior and degradation rate can be suitably tailored by varying the chemical composition. However, these polymers show a bioinert surface that generally lacks bioactive functions for bone formation and, consequently, elicit minimal tissue response. The incorporation of additive chemical functionalities is therefore required in order to improve their chemical bioactivation [20].

2.1.5 Scaffolds for Tissue Engineering

Requirements for bone scaffold.

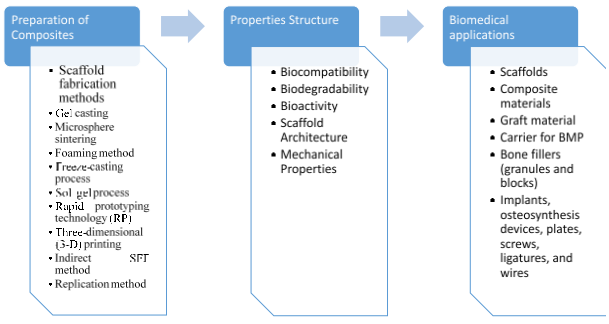
The ideal scaffold for bone tissue engineering should meet the following criteria [42].

The biocompatibility is an essential property; it means that the material must not elicit an inflammatory response nor demonstrate immunogenicity or cytotoxicity. The scaffolds are artificial, lattice-like structures capable of supporting a tissue formation (see Fig. 2). This three dimensional structures are typically engineered with pores allowing cells to migrate throughout the material and support vascularization of the ingrown tissue.

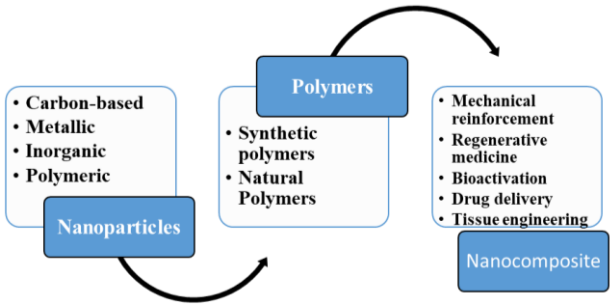
A blood supply in and around the implant would be created within a few weeks after implantation. Pores should be interconnected with pore size minimally 100 μm in diameter (ideally about 150-500 μm) [3, 4, 6]. Besides macropores, microporosity of the walls (< 100 μm) is desirable because it provides a larger surface area which is significant for protein adsorption, cellular adhesion and proliferation [3, 5]. Besides that, scaffolds should be further able to create a stable interface with the host bone without fibrous connective tissue. Its surface texture should promote cell adhesion and adsorption of biological metabolites [5, 6]. In addition, it should support cell differentiation and proliferation [26, 37, 41].

Over a few months' time, the scaffold ought to resorb in the body, leaving behind the natural tissue. Resorption kinetics should be equal to the bone repair rate in order to facilitate load transfer to developing bone. The by-products must not be toxic and should be easily eliminated via the respiratory or urinary systems [2, 3, 6, 16]. In addition to pore size and overall porosity, mechanoregulatory effects are thought to be key in influencing bone tissue growth and cellular differentiation *in vivo*. Mechanical properties should be ideally similar to those of the host bone to avoid several problems such as stress shielding. Stress shielding occurs if the Young's modulus of an implant is higher than that of bone. If it happens, the implant carries nearly all the load, the result bone becomes weaker, and the interface between the bone and the implant deteriorates [17, 42]. If a scaffold is unable to replicate the mechanical forces transferred to cells in physiological conditions, cells may be stimulated to differentiate away from an osteogenic lineage towards an undesirable morphology [32, 38]. The ideal scaffold would have a compressive strength comparable to cortical bone, which along the long axis is approximately 100-230 MPa, with a Young's modulus close to 7-30 GPa and a tensile strength of 50-151 MPa [9]. Ideally this compressive strength would be complemented by a porosity between 60 % and 90 % and an average pore size of > 150 μm [3, 4, 7]. Scaffold mechanical stiffness and porosity are directly conflicting physical properties, with mechanical strength inversely related to increasing scaffold porosity.

Tailoring scaffold mechanical performance to individual defects remains difficult, as anatomical loading conditions for individual defects are difficult to quantify [2, 3, 5, 7]. Achieving satisfactory mechanical performance requires a range of properties to be addressed during scaffold fabrication, including compressive, tensile, elastic and fatigue resistance, and successful replication of these properties can help stimulate osteogenesis and perhaps facilitate degrees of load bearing to occur [8, 43].



a)



b)

Fig. 4 – Synthesis and application of nanocomposites with improved properties for be applied in a range of biomedical applications:
the relationship between the composition structure, properties and processing of materials, design, reserch and develop new materials for a specific purpose;
b) nanocomposites are made by combining nanomaterials (carbon-based na-nomaterials, poly-meric nanoparticles, inorganic nano-particles, and met-al/ metal-oxide nanoparticles) with the synthetic or natural polymers.

The scaffold surface should also be optimised to facilitate cell attachment, proliferation and differentiation. To function properly, a variety of properties may be required [4, 7, 18, 42], some of which are listed in Table 2.

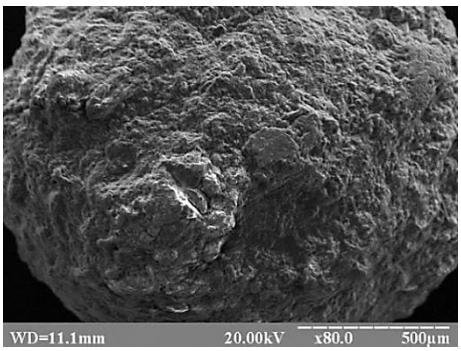
Table 2 – Properties required from calcium phosphates for medical applications

Property	Definition/Function
Bioactivity	The inherent ability of a material to participate in specific biological reactions or have an effect on living tissues
Biocompatibility	The ability of a material to perform with an appropriate host response in a specific application
Bioactive fixation	Reactive surfaces form chemical bonding with bone, thus minimizing the fibrous capsule formation
Biostability	The ability of a material to maintain its properties *in vivo*
Crystallinity	Higher level of crystallinity prevents fast resorption (dissolution) of the bioceramic in body fluids
Interfacial stability and good adhesion	Prevent mechanical failures under load-bearing conditions
Osseointegration	Direct anchorage of an implant by the formation of bony tissue around it without growth of fibrous tissue at the bone/implant interface
Osteoconduction	Ability to provide a scaffold for the formation of new bone
Osteoinduction	The process by which osteogenesis is induced. This term means that primitive, undifferentiated and pluripotent cells are somehow stimulated to develop into the bone-forming cell lineage
Resorption	Gradual degradation over time to replace the biomaterial with the natural host tissue
Therapeutic capabilities	Templates for the in situ delivery of drugs and growth factors at required times
Wettability	The property that indicates a material's ability to attract/repel water molecules

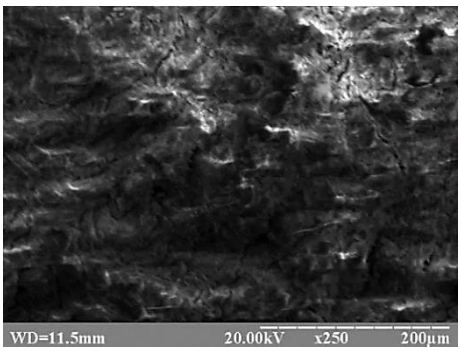
Hence, the intrinsic structure as well as the composition plays critical roles in the success of a scaffold.

Porosity also supports cell migration into the scaffold and improves available surface area for cell-scaffold binding and interaction with surrounding tissues (Fig. 5 a and b). Individual pore size within the scaffold is also an important consideration. It has previously been shown that scaffold pore density and size significantly impact upon cellular growth and attachment [41, 42]. As pore size decreases, the surface area of the scaffold increases. This increases the availability of scaffold ligands for cells to bind to and interact with.

A large variety of techniques have been used in the fabrication of 3D scaffolds, sometimes in combination. In general, it is difficult to create complex scaffold microarchitectures with precise control using conventional techniques. However, the integration into bone tissue engineering of 3D printing using computer-aided design



a



b

Fig. 5 – SEM image showing interconnected porous structure of hydroxyapatite scaffold

Table 3 – Comparison of scaffold fabrication methods

Manufacturing Method	Benefits	Potential Limitations
Solvent casting/ particulate leaching	Relatively simple technique that allows creation of scaffolds with regular porosity, controlled composition and pore size.	Use of organic solvents precludes cells and biomolecules being included directly in scaffolds. Can be difficult to control pore shape and interconnectivity. Limited thickness of structures and mechanical properties achievable.
Gas Foaming	Eliminates use of chemical solvents	High pressures involved prohibits inclusion of cells and bioactive molecules directly into scaffolds. Temperature labile materials may be denatured during compression moulding step. Difficult to control pore sizes and ensure interconnectivity
Emulsification Freeze-Drying	Does not require use of solid porogen	Requires use of organic solvents. Small pore size. Porosity often irregular. Long processing time.
Phase Separation	Eliminates leaching step of porogen Can be combined with other techniques easily	Small pore sizes limit use. Use of organic solvents inhibits use of bioactive molecules or cells during scaffold fabrication
Electrospinning	Creates scaffold with large surface area for cell attachment. Simple and inexpensive technique.	Organic solvents may be required, which can be harmful to cells Limited mechanical properties Difficult to incorporate precise microarchitecture into constructs
3D Printing SLA SLS FDM Inkjet Laser-assisted Microvalve Microextrusion	Complex 3D shapes with high resolution, controlled pore size & morphology and controlled internal structures can be fabricated. Improved capacity to incorporate vascular structures into constructs. Depending on technique used, cells may be included in high concentration directly in scaffold materials	Some techniques are limited by printable materials Set up costs can be expensive for machinery

(CAD) modelling has greatly increased scaffold manufacture precision and repeatability, with control over scaffold macro- and microporosity possible. The advantages and disadvantages of conventional scaffold manufacturing methods and more recent 3D printing techniques will therefore be discussed and summarized in this section (see Table 3).

Up to now, three methods have been used to prepare collagen/nHA scaffolds including: direct blending, SBF immersion and co-precipitation. Compared with nonuniform distribution of HA in direct blending and the slow and uncontrollable process of HA formation in SBF, spontaneous co-precipitation of collagen fibrils and nano-HA is considered as a promising way to achieving the same hierarchical structure of bone [3, 8]. The process of nucleation of HA nanocrystals onto collagen fibers is achieved in an aqueous suspension which containing high concentration of Ca^{2+} and PO43- in a certain ratio and triggered by raising pH of collagen solution to 9-10 at room temperature. The chemical interaction between HA and collagen results in the c-axes of blade-shaped HA nanocrystals aligning along collagen fibers which is similar to bone [5].

3. Hydroxyapatite Based Nanocomposite Ceramics

3.1 Characterization and Properties of the Nanocomposites

Nanocomposites are defined as a heterogeneous combination of two or more materials in which at least one is at the nanometer-scale [3, 5]. Because of the contrast in composition, interaction, and properties between dissimilar components in nanocomposites, several key factors affect the role that nanoparticles play as reinforcing fillers in a polymer-matrix.

A critical issue to pay attention to in developing nHA/polymer nanocomposites is the interfacial strength between the filler (nHA nanoparticles) and the polymer. A suitable polymer should have a chemical bonding composition that supports adhesion be-

tween its particles and those of nHA [8]. Otherwise, the lack of adhesion can lead to early failure of the two incorporated phases and hence affect the mechanical properties (in particular tensile strength) of the construct. Furthermore, the wettability of a polymer can play a crucial role in successful incorporation of nHA by affecting the type and strength of bonding and adherence of the nHA surface and the polymer [8, 36]. This phenomenon is determined by the polarity and available polar groups of the polymer, which can render the polymer hydrophobic or hydrophilic.

In addition, the method of incorporation of nHA into the polymer could have great impact on the properties of the final construct. The methods of delivering nHA into a polymer matrix can be divided into two main categories; thermo-mechanical methods and physicochemical methods [7, 8]. The former methods use conventional plastics processing techniques to impregnate a porous polymeric matrix with nHA. Examples of such methods are compounding, compression or injection moulding. Physico-chemical methods, on the other hand, use either co-precipitation of nHA crystals in situ into the polymeric matrix or use a solvent as a dispersion solution for nHA before being added to the polymer. Both thermo-mechanical methods and physicochemical methods have been shown effective for nHA incorporation into polymers, however, various limitations such as thermal degradability of heat-sensitive polymers caused by moulding temperature and pressure, solvent toxicity, and gelation rate exist [4, 9].

Nanocomposite scaffolds, in order to be considered as part of a valid bone regeneration strategy, have to be cytocompatible. Another essential property of cytocompatible scaffolds is its surface properties and surface to bulk ratio, which increases with increasing porosity or decreasing size in particulate systems. It can come as a result of the nanocomposite construction or it can be stimulated by specific procedures. Size and number of pores has to be taken in consideration as well. Porosity of the final regeneration scaffold is essential for providing physical structure for bone ingrowth. When considering applications where a considerable extension of bone needs to be regenerated porous scaffolds are preferred to particulate systems due to its superior mechanical stability. A porous scaffold can provide an ideal physical structure for bone cells to infiltrate the scaffold and to produce new bone. Additionally, it contributes for implant stability by biological fixation.

Other very important property of a nanocomposite surface is hydrophilicity. In fact, a certain affinity to water can help to immobilize growth factors and diffuse nutrients in bone native tissues and scaffolds, enhancing the adhesion of host cells. The nanocomposite's bioactivity can be defined as the ability of the ceramic component to establish a chemical bond with the host bone tissue. This includes enhancing the ability of apatite formation, osteoblast differentiation and bone matrix formation [3, 6, 16, 39].

Since bone regeneration strategies are commonly intended to serve as temporary replacement for the extracellular matrix, they should present, besides excellent biocompatibility, suitable biodegradability and sufficient mechanical strength to ensure tissue functionality [37, 41].

In other words, the mechanical load should be supported initially by the scaffold and gradually transferred to the newly forming bone, according to the biodegradation profile of the composite. Within a nanocomposite, it can be ideally considered that the bioceramic filler will induce a bioactive behavior towards bone regeneration by self-degradation, so the space formed by that degradation can be replaced by new bone. On the other hand, the polymer matrix would have to degrade slowly, to compensate the quick degradation of the nanofiller, so both materials end up giving space newly formed bone.

Nanocomposites implanted in vivo are often affected by bacterial colonization. If the aimed regenerating site is the alveolar bone, bacteria like Porphyromonas gingivalis, Streptococcus mutansand Fusobacterium nucleatum, which are the major pathogens of periodontitis and periimplantitis are likely to infect the nanocomposite and result in an inflammatory reaction and subsequent failure of the bone regeneration procedure. Therefore, the incorporation of an antibacterial component can be an effective way to improve the nanocomposite functionality.

The addition of other components to nanocomposites, can enhance their features. Adding silver nanoparticles confers antibacterial properties to the scaffold and titanium particles apparently increase bone formation in vivo.

The seeding stem cells from the host to the nanocomposite, increases its bioactivity. When implemented in the nanocomposite, stem cells receive specific stimuli and differentiate into bone-cells that have the ability to produce new bone. In the future, much more research is needed to understand the mechanism of nanocomposite-tissue interactions and to optimize the composition, structure and properties of different polymer-ceramic nanocomposites, in order to finally extract the full potential of nanocomposites for bone tissue regeneration.

Structural, morphology, micrograph and chemical composition of this nanocrystalline composites were characterized by XRD, AFM, SEM and FTIR, respectively.

Eligibility criteria included in vitro studies that evaluated the biocompatible and stimulating capacity of materials composed of nano-ceramic particles dispersed in a polymeric matrix with bone-related cells, in vivo studies that comprehended the investigation of the mechanical, structural and bioactive behavior of polymer-ceramic nanocomposites, clinical trials performed to evaluate the overall performance of nanocomposites in humans, specifically in the craniomaxillofacial region and pshysicochemical studies of novel regeneration systems including polymers and ceramic nanoparticles.

3.2 Synthesis Methods for HAp Nanoparticles

An important direction in the development of nanotechnologies and the creation of new nanostructured materials is associated with the study of the laws of synthesis, the study of phase-structure materials and physical and mechanical properties of nanocomposite multi-component protective coatings with different

chemical composition and internal architecture. Modern nanocomposite materials represent a complex non-equilibrium system in which nonlinear processes take place, including bifurcation and the formation of dissipative structures with phase transitions. Non-equilibrium conditions contribute to the formation of nanocrystalline and / or nanocluster structures with unique functional properties. Similar processes are described on the basis of physical mesomechanics and nonequilibrium thermodynamics. Currently, nanocluster structures are formed by different methods [2, 43]. The results of fundamental research are presented in many papers. In the overwhelming majority, certain questions of the applied nature of the simulation of physical laws are considered. Practical interest is the improvement of technologies for obtaining coatings from nanocluster structures with high physico-mechanical and operational properties through the targeted selection of technological parameters based on the fundamental physical principles of constructing a nanostructure in multi-element and multilayer systems. Formulating the conditions for nanocomposite materials and coatings with special properties will increase the efficiency of existing equipment, reduce energy costs and cost of the process [4, 9]. The project considers the physical aspects of the design of multi-element and multilayer nanostructures. Hypotheses and ideas are used from various fields of natural sciences, namely: solid state physics, plasma physics, physical material science, physical chemistry, condensed matter physics and consolidated materials. The conditions for the formation of multi-elemental and multilayer systems with hierarchical and / or adaptive behavior with a certain phase composition, structure, substructure, stress state and high functional properties are considered and formulated.Two approaches are desirable for reviewing the synthesis and properties of nanoobjects: microscopic and thermodynamic. Depending on the specific application it is possible to find nanocomposites in different forms. The synthetic form of HA is osteoconductive and can have a crystalline structure similar to the HA in bone. HA synthesized at high temperatures is highly stable and slower to resorb than its endogenous form and may stay at the site of implantation for many years. Tricalcium phosphate (TCP) is a bioceramic that exists in alpha (α-TCP) and beta cristal (β-TCP) forms [3, 8, 35]. α-TCP is the high-temperature and β-TCP is the low-temperature polymorph of TCP. The morphology of HAp nanoparticles depends on the precipitation conditions such as the concentration of reactants, ionic strength, pH, and temperature [39].

In ceramic-polymer nanocomposite processing, wet and dry methods are used very often, being the sol-gel technique one of the most used. An important class of nanostructured biomaterials on which intensive research has been carried out is nano-fibrous materials, especially biodegradable polymer nanofibers. In this specific material, nanofibers are dispersed in a biodegradable polymer matrix. The large surface-area-to-volume ratio of nanofibers combined with their porous structures favours cell adhesion, proliferation, migration, and differentiation, as it was already discussed. There are three main techniques to produce nanofibers: phase separation, self-assembly and electrospinning [15].

Self-assembly is the most complex technique and it allows the creation of nanofibers with very small diameters (a few to 100 nm). It basically consists on an autonomous organization of components that are able to assemble at the molecular level [15, 40]. Being a natural process for several essential biological components including nucleic acid or protein synthesis, self-assembly technology usually incorporates some specific biological components of the extracellular matrix (ECM), closely mimicking the ECM assembly process [40]. In this process, molecules require some specific configurations to be assembled into nanofibers. Molecules that meet this requirements are peptide-amphiphiles (PAs) [40], oligopeptides, synthetic diblock/triblock copolymers and dendrimers [41]. Self-assembly is the most complex technique and it allows the creation of nanofibers with very small diameters (a few to 100 nm). It basically consists on an autonomous organization of components that are able to assemble at the molecular level [15, 40].

It is proposed to use a cluster approach when considering the bundle process in multi-element and multilayer systems of nanoscale scale. Properties of the solid surface and its transformation are considered through the application of thermodynamic potentials and their associated quantities. Clusterization in this case is represented as a stage of phase segregation, that is, the decomposition of a supersaturated solid solution with positive free energy. This allows us to characterize the surface and nanoclusters with values of energy and entropy without resorting to atomic-molecular detail. The dynamics of transition in clusters and the region of existence of solid and liquid states are studied using Monte Carlo (MK) and molecular dynamics (MD) methods. In MK methods, the phase space is modeled by stochastic processes at a given temperature and potential. In the methods of MD, the equations of motion for each atom in a cluster in the field of given potential are uncoupled. In calculations of cluster dynamics, the pair potential of Lennard-Jones is used, and the primary coordinates correspond to a structure with a minimum potential energy. At the heart of the bundle process is the change in the nature of the interaction of various elemental atoms in the solid solution, which is determined by the parameters of near-ordering in the metallic subsystem. Concentration bundle with the formation of the modulated structure of the metal subsystem leads to the enrichment of one of the elements of the central region and, accordingly, the depletion of this element of the periphery of the formed crystallite. Optimization of modes of obtaining the structural state and physical properties of composites, formation of structural states and mechanical properties of composites under various thermomechanical influences will allow us to construct a model of contact loading of condensate systems in a state of decay and of layered structures

3.3 Nanostructural Components

Biopolymers and mineral compounds are the two main classes of material that typically conjoin to form composite biostructures. Polymers can be divided into two groups regarding their source: natural and synthetic. Regarding their response when applied to living tissues, polymers can be biodegradable or non-

biodegradable.

Cellulose, collagen, agarose, chitin or hyaluronan form the members of natural polymeric materials or so-called biological polymers. Recently [3, 8, 40], an antibacterial HA/Alg/CHX scaffolds for biomedicine, in particular for oral hygiene and potential dental treatment as a paste have been synthesized (Fig. 6).

Natural polymers such as collagen have been used for bone tissue engineering purposes. In contrast to natural polymers, synthetic polymers are also used in the bone engineering field.

Some examples of these polymers are poly-lactic acid (PLA), poly-glycolic acid (PGA), polyurethane (PU) and polycaprolactone (PCL), from which PLA and PLGA have receives the highest interest because of their biological properties and easy processability. In addition, poly(ε-caprolactone) (PCL), polyanhydrides, poly (vinyl alcohol) (PVA), polyurethanes and recently polyhydroxykanoates (PHA) which are linear polyesters of microbiological origin, have also been investigated for bone regeneration [5, 10].

Typically, the polymer-ceramic composites used for bone replacement and regeneration are systems in which a ceramic filler is dispersed within a polymer matrix. In nanocomposites, the dispersed filler material is at the nano dimension [3, 9, 29]. Another important consideration is the dispersion of the filler, since a well dispersed system yields more desirable composite properties.

The synthetic polymers are proven to be better for integrating nanocomposites, since the can be modeled to have suitable properties, conferring in the end better mechanical and biological behavior.

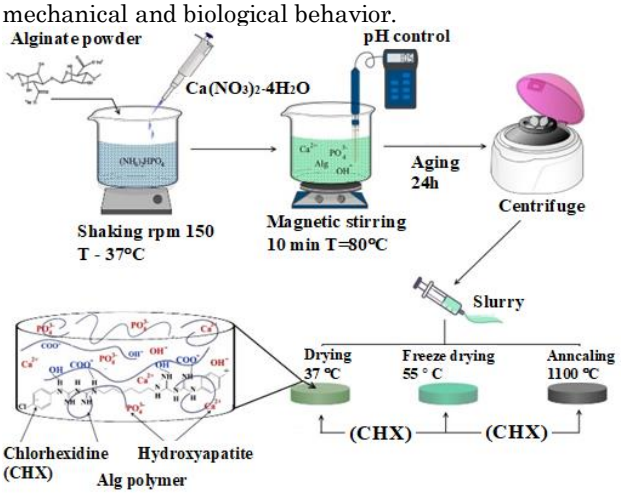


Fig. 6 – Scheme for the preparation of a HA/Alg/CHX composites [8]

Natural-based polymers have been gaining more and more interest in the latest years mostly since they can better mimic the extracellular matrix of bone, can provide an adequate environment for establishing a chemical bond with the inorganic nanofiller and also because they are able to generate non toxic products upon their degradation process.

The development of these new imp roved HAP based composites would require that their structure and mechanical properties be optimized to effectively mimic bone. The challenge will be to find suitable bioco mpatible additives wit h desirable properties that can be incorporated with the HAP to form a composite with superior mechanical properties without soliciting any unfavorable inflammatory responses. Recent studies of HAP based composites composed with blended natural polymers such as chitin; chitosan and collagen have shown both improved biocompatibility and mechanical properties.

CONCLUSION

There are further constraints faced by researchers undertaking collaborative, translational research. Biomaterials, regardless of whether they are permanent or biodegradable, naturally occurring or synthetic, need to be biocompatible, ideally osteoinductive, osteoconductive, integrative, porous and mechanically compatible with native bone to fulfill their desired role in bone tissue engineering. Achieving a successful balance in vivo between the properties of a scaffold favorable to cellular function, cellular viability and mechanical integrity under load bearing therefore remains challenging. Nanotechnology can provide an alternative way of processing porous bioceramics with high mechanical strength and enhanced bioactivity and resorbability. It has been proved that HAp nanoparticles (nano-HAp) are better positioned to serve as an apatite substitute of bone in biomedical applications than micrometer-sized hydroxyapatite (micro-HAp). Incorporation of nanohydroxyapatite into synthetic polymers has shown promising bioactivity, osteoconductivity, mechanical properties and degradation profile compared to other techniques previously considered. With the development of three-dimensional (3D) printing technology, the desired outer contour can be made to match the bone defect. But it is still impossible to simulate the complex internal patterns of nanocrystalline composition and collagen fibrous structures of the bone matrix frame. As the technology develops, the boundaries of what can be achieved may advance such that new types of tissues and organs can be produced with superior functionality to the human body.

Проектування та виготовлення полімер-керамічних нанокомпозитних матеріалів для інженерії кісткової тканини

Л.Ф. Суходуб, К.О. Дядюра

Сумський державний університет, вул. Римського-Корсакова, 2, 40007 Суми, Україна

У роботі представлений аналіз різних типів керамічно-полімерних нанокомпозитів. Основна мета даної статті полягає у аналізі останніх результатів досліджень полімер-керамічних нанокомпозитів, що розробляються для заміни та регенерації кісток. Обговорюється методологія виготовлення скафолдів, механічні характеристики, біосумісність, біоактивність та потенційні клінічні переходи. Обговорюються деякі з найпопулярніших методів обробки для виробництва біокерамічних конструкцій з акцентом на виробництві скафолдів на основі HA. Зроблено порівняння, щоб зрозуміти обгрунтування та мотивацію дослідження. Структурна будова, морфологія та хімічний склад цих нанокристалічних композитів досліджувалися відповідно методами XRD, AFM, SEM та FTIR.

Ключові слова: Наногідроксиапатит, Каркаси, Композити, Біологічно розкладені полімери, Регенеративна медицина, Тканинна інженерія.

REFERENCES

1. Fa-Ming Chen, Xiaohua Liu, *Prog Polym Sci.* **53**, 86 (2016).
2. S.A. Christopher, N. Satoshi, *Neurosurgery* 80(3S), S9 (2017).
3. J. Radhakrishnan, A. Manigandan, P. Chinnaswamy, A. Sethuraman, S. Subramanian, *Biomaterials* **162**, 82 (2018).
4. V.M. Kuznetsov, L.B. Sukhodub, L.F. Sukhodub, *J. Nano-Electron. Phys.* **6**, No 4, 04039 (2014).
5. E.B. Hunziker, K. Lippuner, M.J. B. Keel, N. Shintani, *Osteoarthritis and Cartilage* **23**, 334 (2015).
6. D. Wu, P. Isaksson, S.J. Ferguson, C. Persson, *Acta Biomater.* **73**, 1 (2018).
7. L.F. Sukhodub, *Biopolymers. Cell.* **32**, No 2, 83 (2016).
8. L.F. Sukhodub, L.B. Sukhodub, O. Litsis, Yu. Prylutskyy, *Mater. Chem. Phys.* **217**, 228 (2018).
9. L.B. Sukhodub, L.F. Sukhodub, Yu.I. Prylutskyy, N.Yu. Strutynska, L.L. Vovchenko, V.M. Soroca, N.S. Slobodyanik, N.G. Tsierkezos, U. Ritter, *Mater. Sci. Engin. C* **93** No 1, 606 (2018).
10. S. Kubinova *Neural Regen. Res.* **12**, No 9, 1430 (2017).
11. Y. Hu, J. Ran, Z. Zheng, Z. Jin, X. Chen, Z. Yin, C. Tang, *Acta Biomaterialia* **15**, No 71, 168 (2018).
12. A.E. Jakus, N.R. Geisendorfer, P.L. Lewis, R.N. Shah, *Acta Biomaterialia* **72**, 94 (2018).
13. S. Derakhshanfar, R. Mbeleck, K. Xu, X. Zhang, W. Zhong, M. Xing, *Bioactive Mater.* **3**, 144 (2018).
14. R.D. Farahani, M. Dube, D. Therriault, *Adv. Mater.* **28**, 5794 (2016).
15. Q.L. Loh, C. Choong, *Tissue Eng. Part B Rev.* **19** No 6, 485 (2013).
16. D.J. Kelly, P.J. Prendergast, *Tissue Eng.* **12** No 9, 2509 (2006).
17. G. Chen, C. Dong, Li Yang, Y. Lv, *Appl. Mater. Interf.* **7** No 29, 15790 (2015).
18. X. Han, S. Huang, Y. Wang, D. Shi, *Mater. Sci. Eng. C* **64**, 87 (2016).
19. L. A. Rasskazova, *Theor. Found. Chemic. Engin.* **48** No 5, 682 (2014).
20. N. Eliaz, N. Metoki, *Mater. (Basel)* **10** No 4, 334 (2017).
21. Azadeh Rezakhani, M.M. Kashani Motlagh, *Int. J. Phys. Sci.* **7** No 20, 2768 (2012).
22. D. Zhang, X. Wu, J. Chen, K. Lin, *Bioactive Mater.* **3**, 129 (2018).
23. S. Shima, N. Leila, A.M. Seifalian, Gordon W. Blunn, *Open. Orthop. J.* **10**, 900 (2016).
24. S. Geetika P. Gopinatha, P. Jeevanandam, *Colloids Surf. B* **103**, 441 (2013).
25. L. Rasskazova, N. Korotchenko, G. Zeer, *Russ. J. Appl. Chem.* **86** No 5, 691 (2013).
26. S. Gupta, A. Bissoyi, A. Bit, *Bio. Nano. Sci.* **8**, 868 (2018).
27. H. Li, J. Chang, *Acta. Biomater.* **9** No 6, 6981 (2013).
28. M.J. Olszta, X.G. Cheng, S.S. Jee, R. Kumar, Y.Y. Kim, M.J. Kaufman, E.P. Douglas, L.B. Gower, *Mater. Sci. Eng. R. Rep.* **58** No 3-5, 77 (2007).
29. Z. Saidak, P.J. Marie, *Pharmacol. Ther.* **136** No 2, 216 (2012).
30. F. Yang, D. Yang, J. Tu, Q. Zheng, L. Cai, L. Wang, *Stem. Cells* **29** No 6, 981 (2011).
31. K. Lin, Y. Zhou, Y. Zhou, H. Qu, F. Chen, Y. Zhu, *J. Mater. Chem. B.* **21** No 41, 16558 (2011).
32. V. Aina, L. Bergandi, G. Lusvardi, G. Malavasi, F.E. Imrie, I.R. Gibson, G. Cerrato, D. Ghigo, *Mater. Sci. Engin. C* **33** No 3, 1132 (2013).
33. A. Laskus, J. Kolmas, *Int. J. Mol. Sci.* **18** No 12, 2542 (2017).
34. H. Li, J. Chang, *Acta Biomater.* **9** No 6, 6981 (2013).
35. M.J. Olszta, X.G. Cheng, S.S. Jee, R. Kumar, Y.Y. Kim, M.J. Kaufman, E.P. Douglas, L.B. Gower, *Mater. Sci. Eng. R. Rep.* **58** No 3-5, 77 (2007).
36. P. He, S. Sahoo, K.S. Ng, K. Chen, S.L. Toh, J.C. Goh, *J. Biomed. Mater. Res. A* **101**, 555 (2013).
37. Z. Sheikh, S. Najeeb, Z. Khurshid, V. Verma, H. Rashid, M. Glogauer, *Mater. (Basel)* **8** No 9, 5744 (2015).
38. T. Kokubo, H. M. Kim, M. Kawashita, *Biomater.* **24** No 13, 2161 (2003).
39. Q. Chen, C. Zhu, G.A. Thouas, *Prog. Biomater.* **1** No 1, 2 (2012).
40. T. Zhang, H. Zhang, L. Zhang, S. Jia, J. Liu, Z. Xiong, W. Sun, *Biofabrication*, **9** No 2, 025021 (2017).
41. H. Zhou, J. Lee, *Acta. Biomater.* **7** No 7, 2769 (2011).
42. M. Supova, *J. Mater. Sci. Mater. Med.* **20** No 6, 1201 (2009).
43. M. Bhattacharya, *Mater.* **9**, 262 (2016).