

Biodegradable composites based on nanocrystalline calcium phosphate and bioresorbable polymers

N. Ignjatović and D. Uskoković*

Nanocrystalline calcium phosphates (NC CP) are suitable for the synthesis of CP/bioresorbable polymer composite biomaterials for the application in bone engineering. There are different forms of NC CP/bioresorbable polymers composite biomaterials. Calcium phosphate was synthesised in highly NC form. A NC calcium phosphate/poly-DL-lactide-co-glycolide (CP/DLPLG) composite biomaterial in filler forms was synthesised in the shape of spherical granules (150–200 μm in diameter) and nanoparticles (30–40 nm in diameter). Each CP granule or nanoparticle was coated with amorphous DLPLG polymer. Using NC and nanosized CP/DLPLG composite materials, an injectable composite biomaterial was prepared. By hot pressing of highly porous NC CP/bioresorbable polymer composite biomaterials at the polymer's melting temperature, blocks were obtained with relatively uniform distribution of phases. Designing of the properties of CP/bioresorbable polymers composite biomaterial block was provided by combining high pressure, temperature and time.

Keywords: Composite biomaterials, Injectable paste, Nanocrystalline calcium phosphate, Resorbable polymer

Introduction

Human bone tissue is a prime example of a nanocrystalline (NC) composite system in the human body. Calcium phosphate (CP) crystals, typically a few nanometres in size, incorporated into the polymer matrix, are the key components of human bone tissue.¹ The use of calcium phosphate dates back to 1920.² Good osteoconductive characteristics of this biomaterial enabled development of a wide spectrum of CP based composite biomaterials.^{3,4} Nanocrystalline biphasic calcium phosphate (BCP), composed of hydroxyapatite (HAp) and tricalcium phosphate (TCP), can be a successful alternative to natural bone tissue due to its good osteoconductive characteristics.⁵ In dental application NC HAp was used for the treatment of tooth perforations and jaw cysts.^{6,7}

Mechanical properties of the composite biomaterials based on NC and nanosized HAp are the most similar to those of the natural bone tissue.⁸ The structure of HAp/bioresorbable polymer composite mostly resembles the natural bone tissue structure. Continuous matrix of the polymer, with HAp crystalline particles finely distributed within, is similar to the collagen component. The principal idea is that, after implantation into a bone defect, the polymer component reinforced with HAp becomes bioresorbed, and thus relinquish its place to newly formed tissues.^{9,10}

Nanocrystalline and nanosized calcium phosphate

Calcium phosphates were synthesised in the form of spherical granules of NC BCP and poorly crystalline nanosized particles (NPs) of calcium phosphate (CP). Nanocrystalline and nanosized forms are suitable for the synthesis of BCP (CP)/bioresorbable polymer composite biomaterials for the application in bone engineering.

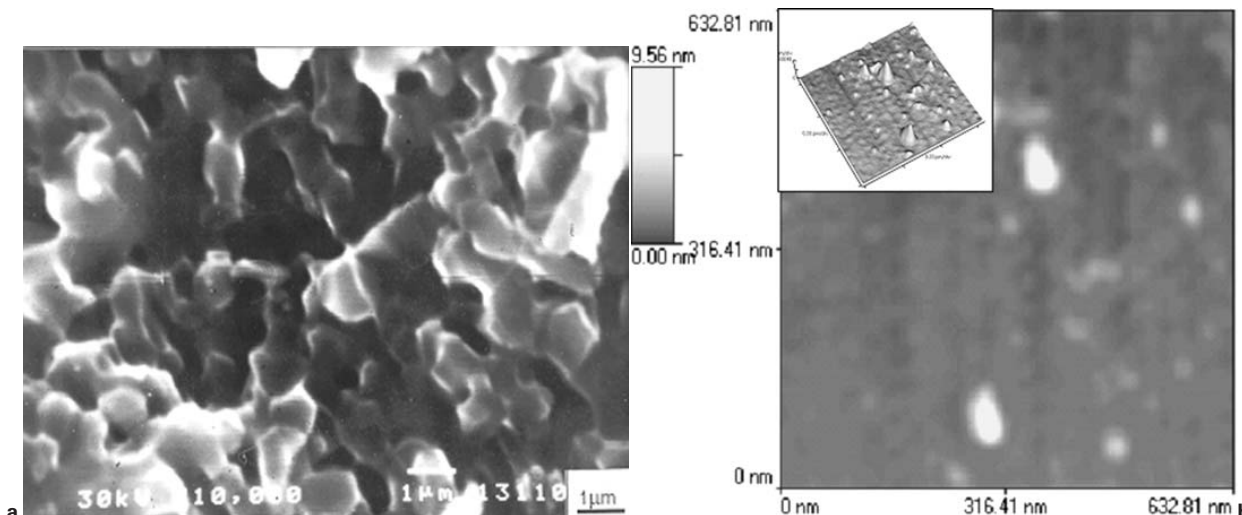
The microstructure of the ceramic phase (a BCP granule with the size of 1 mm) obtained by scanning electron microscopy (SEM) is presented in Fig. 1a. Biphasic calcium phosphate particles are arranged in rounded grainy clusters, which continuously grow and become bonded into agglomerates. These mutually bonded agglomerates, with voids of 0.01–0.3 mm in diameter between them, form the basis of the BCP structure. These voids represent the inner porosity of the HAp phase.¹¹ Figure 1b shows an atomic force micrograph (AFM) of NPs of CP obtained by precipitation in a solution via reaction between $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_3\text{PO}_4$.¹² The particles of CP powder are spherical in shape, with sizes ranging from 10 to 100 nm.

From micro to nanosized filler of nanocrystalline calcium phosphate (CP)/poly-DL-lactide-co-glycolide (DLPLG)

Calcium phosphate mixed with poly-DL-lactide-co-glycolide (DLPLG) polymer intensifies the activity of alkaline phosphatase, more than CP itself, which is

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a SEM of NC BCP granules; b AFM of NPs of CP"
1 Microstructure of calcium phosphates

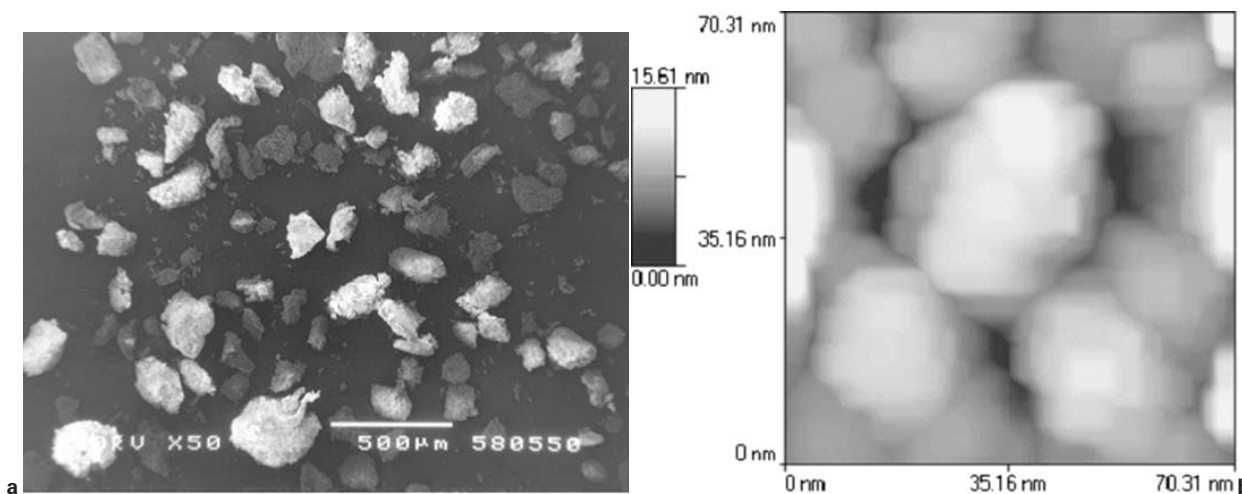
important for the differentiation of osteoblasts that dictates the regeneration process within the organism.¹³ Calcium phosphate/poly-DL-lactide-co-glycolide composite biomaterials, based on BCP or hydroxyapatite as a ceramic calcium phosphate component, exhibit good adhesion onto human cells indicating a high level of biocompatibility of these materials.¹⁴ Poly-DL-lactide-co-glycolide mixed with CP can be used as a carrier in tissue engineering.¹⁵ Biphasic calcium phosphate/DLPLG composites have been used as bone fillers, in which each BCP granule is approximately a micrometre in size and coated with DLPLG polymer. They proved successful in *in vitro* tests for the repair of bone defects.¹⁶ In the present studies, a possibility to synthesise NC CP/DLPLG composite biomaterial in form of granules with 150–200 μm in diameter was demonstrated,¹⁷ and indications of how to prepare the same material, but in the form of NPs of up to 100 nm in size were also given.¹² Spherical particles of composite biomaterials could be suitable for obtaining filler forms optimal for bone reconstruction.¹⁸

Figure 2 shows the morphology of CP/DLPLG composite biomaterial composed of microcrystalline particles and NPs, prepared according to emulsion

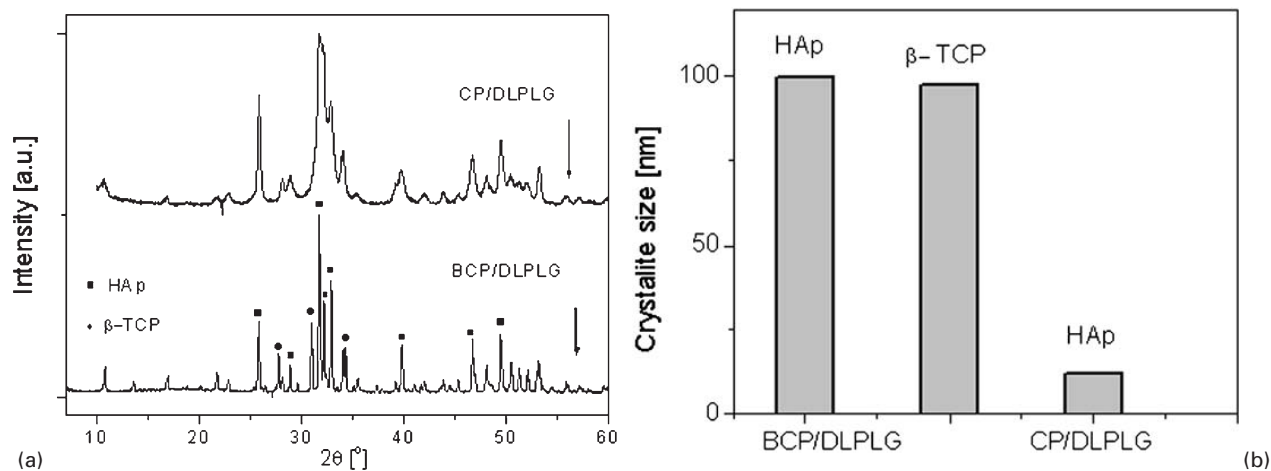
solvent evaporation method.^{12,17} Each CP particle was coated with DLPLG, and the average particle diameter was between 100 and 200 μm (Fig. 2a). Using CP gel, spherical granules of CP/DLPLG composite biomaterial, having 30–40 nm diameter, were also obtained (Fig. 2b). Microgranules or NPs of CP are encapsulated in DLPLG micro/nano spheres, and are suitable for osteoporotic alveolar bone repair.¹⁸ Because of the small size of thermosensitive NPs, the instability of which comes from the thin layer of DLPLG that coats CP, non-contact AFM method was used to analyse the surface morphology instead of SEM analysis.¹²

Dry powder composed of NPs with the average CP/DLPLG particle size of 20–40 nm, in form of agglomerates, was obtained by emulsion solvent evaporation method.¹² Dynamic light scattering measurements showed that agglomerates of 1.6 μm diameter made the largest part of it. Successful deagglomeration could be achieved by various techniques, including sonochemical processing, ball milling, etc.

Nanosized particles have several advantages over the micro ones in interactions of the biomaterial with the organism. Usually smaller than 100 nm, they are made by forming nanocrystals or drug polymer systems, or by



a SEM of NC BCP encapsulated in DLPLG; b AFM of NPs of CP encapsulated in DLPLG
2 Microstructure of composite biomaterials



a XRD patterns of BCP/DLPLG and CP/DLPLG composite biomaterials; **b** crystallite size of HAp and β -TCP in CP/DLPLG composite biomaterials

3 X-ray diffraction analysis of composite biomaterials

creating nanoscale shells that entrap drug molecules. Because of their size, they are often taken up by cells, whereas larger particles would be excluded or cleared from the body. Small molecules, proteins and nucleic acids can be loaded into NPs that are not recognised by the immune systems.¹⁹

The diffraction patterns of NC BCP/DLPLG and NPs of CP/DLPLG composite are shown in Fig. 3. The size of the crystallites of HAp and β -TCP were determined from the half width of the diffraction line. Figure 3b shows the crystallite size of CP in BCP/DLPLG and CP/DLPLG composite biomaterials.

Based on an earlier described methodology,²⁰ weight content of HAp and β -TCP, 80 and 20% respectively, were calculated, confirming the biphasic nature of the calcium phosphate. X-ray diffraction patterns show no peaks for DLPLG polymer, because the latter is known to be of amorphous character.²¹ The diffraction peaks of CP/DLPLG sample indicate poorly crystalline HAp.²¹

Figure 3b represents the sizes of HAp crystallites from CP/DLPLG and BCP/DLPLG obtained from the XRD patterns using Rietveld method.²² Other authors also suggested Rietveld method as suitable for the determination of crystallite sizes of bone apatites.²³

As of today, injectable composite biomaterials based on CP and bioresorbable/non-bioresorbable polymers have been the subject of investigations aimed to obtain injectable biomaterials with satisfactory mechanical and biocompatible characteristics.^{24–26} Of special interest is their application in substitution of bone tissue both in dentistry and medicine due to easy and fast formation of new bone tissue.²⁷ *In vivo* investigations performed have confirmed an easy and suitable application of injectable biomaterials based on CP in soft tissue environments as well.²⁸ Injectable biomaterials based on BCP or bioresorbable polymers are of special interest in bone tissue engineering. Their advanced characteristics come from good mechanical properties, biocompatibility and convenience in practical regeneration of defects.²⁹

In the present recent studies, a possibility to synthesise an injectable paste composed of NPs CP/DLPLG composite biomaterial was demonstrated.³⁰ Nanosized particles of CP/DLPLG powder were dispersed in physiological solutions to prepare pastes with different contents of solids (50, 55, 60 and 65 wt-% of the

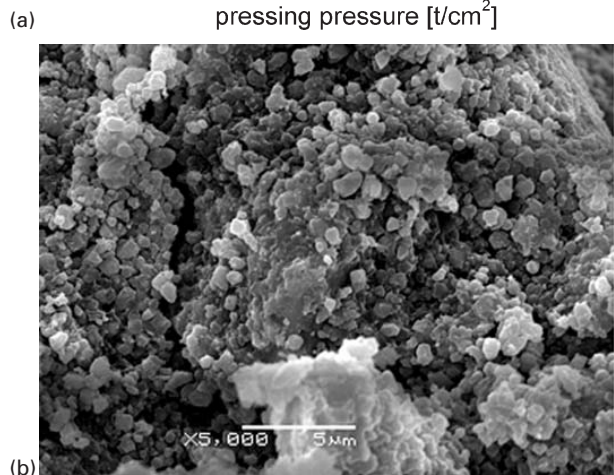
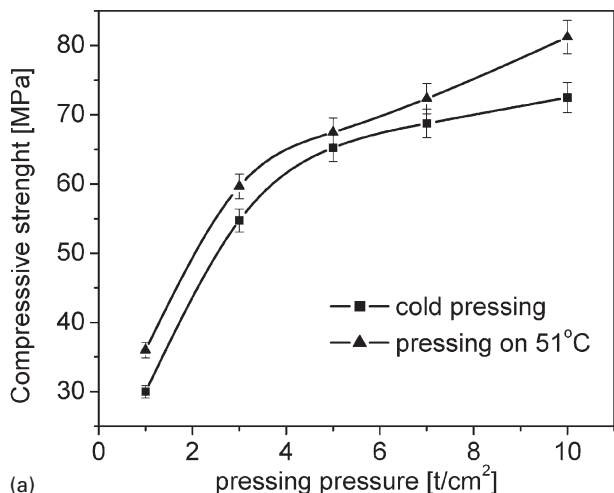
powder). Complex dynamic viscosity decreases with lowering the powder content and increasing the frequency in the case of injectable nanosystem as well.³¹ Similar dependencies were obtained in the case of 50–60% content of the NPs of CP/DLPLG. However, the curve for the content of 65% deviates from this family of curves due to somewhat higher η^* values for the given frequency interval. These values might suggest a possible formation of aggregates at higher concentrations of NPs of CP/DLPLG composite. A higher content of nanopowder is expected to increase the content of potential clusters for agglomeration.³²

The use of such calcium phosphate/bioresorbable polymer materials as the basis of implantable drug delivery systems for local antibiotic treatments of bone infections is of great interest.^{33,34} The association of CP or CP/DLPLG and fibrin sealants may develop clinical applications of composite bone substitutes.³⁵

Different NPs spheres were prepared from CP and DLPLG using a solvent/non-solvent emulsion technique to successfully adsorb autologous plasma and fibrin on the surface of the particles of composite biomaterials.²⁹ Histological analysis of the experimental group of animals with and without NC BCP/DLPLG and the given biostimulative agent implanted into osteoporotic bone tissues revealed recuperation of the alveolar bone previously diminished by osteoporosis, starting from the sixth week of repairation.²⁹ The application of BCP/DLPLG facilitated overgrowth of newly formed vascular tissue and fibroblasts, and intensified the activity and adherence of osteoblasts. Nanocrystalline BCP/DLPLG composite biomaterials loaded with biostimulative agents (autologous plasma or fibrin) showed the highest reparatory results and the highest intensity of osteogenesis followed by a regular bone structure formation, as compared to the same composite without biostimulative agents.¹⁷

Blocks of NC CP/bioresorbable polymers composite biomaterials

In vitro research on cellular cultures of human (MRC-5) and mouse (L-929) showed good adherence of fibroblast cells of both cellular cultures to the blocks of NC BCP/DLPLG composite biomaterial surface. The existence of



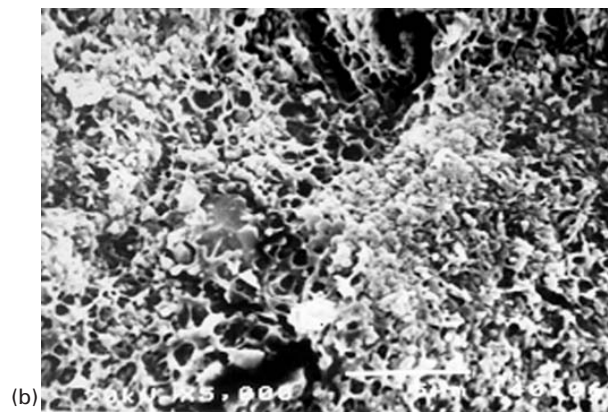
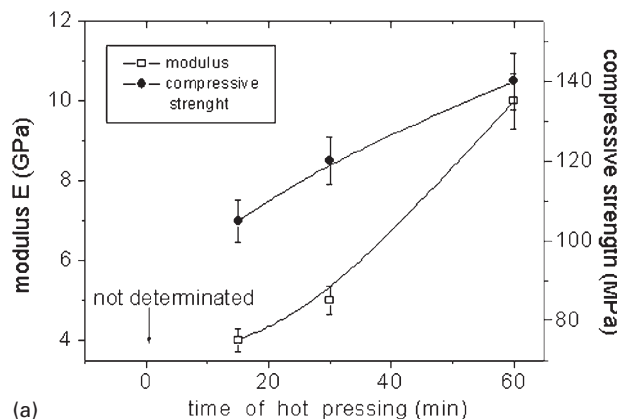
a dependence of compressive strength on processing pressure and temperature; b SEM of fracture surface after 15 min of hot pressing

4 Properties of blocks of NC BCP/DLPLG composite biomaterials

peripheral fibroblast extensions, extending towards the composite surface and adhering thereto, indicates an intimate contact at the interface of BCP/DLPLG/fibroblast. Biphasic calcium phosphate/DLPLG composite biomaterial does not induce inhibition of fibroblast growth in cellular cultures. Cellular survival is high (90% for MRC-5) and cytotoxic effects, therefore, do not exist.¹⁶

The blocks of the theoretical density of 97% and the compressive strength of 82 MPa were produced by cold and hot pressing of BCP/DLPLG microparticles at the polymer glass transition temperature (324 K).³⁶ Figure 4a shows the values of compressive strength of the blocks pressed at different pressures and two temperatures: 291 K for cold and 324 K for hot pressing. The maximum value of compressive strength (82 MPa) was achieved when blocks were pressed at 1 GPa and 324 K, while at the same pressure but different temperature (291 K), the obtained blocks had a lower value of compressive strength (73 MPa). Figure 4b shows the microstructure of the blocks obtained after pressing at 324 K for 15 min.

Bioresorbable poly(lactides) (PLA) and their copolymers belong to the group of nontoxic polymers, because the final products of their degradation (CO₂ and H₂O) enter without difficulties the 3-carboxylic acid cycle, not

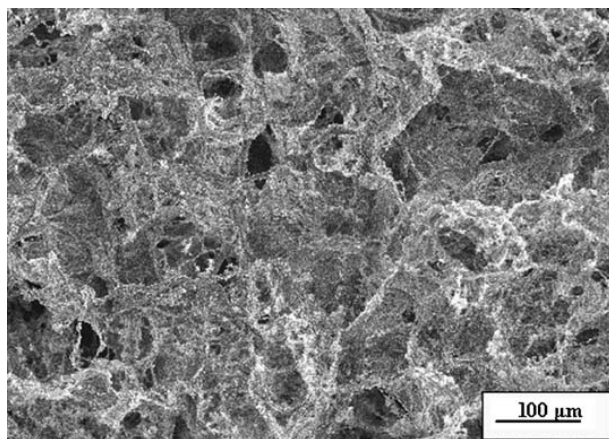


a dependence of compressive modulus and compressive strength of blocks on hot pressing times; b SEM of fracture surface after 15 min of hot pressing

5 Properties of NC HAp/PLLA composite biomaterials

disturbing the metabolism of the surrounding tissue in the process.³⁷ Compared with pure hydroxyapatite, composite biomaterials of HAp/PLLA type induce formation of a large number of surface osteoblasts, necessary for the repair of bone defects. The concentration of alkaline phosphates, required for the differentiation of osteoblasts, is higher near HAp/PLLA composite biomaterial than near pure PLLA.¹³

Response of the organism to the implanted composite biomaterial depends on numerous factors. The most important among them are biocompatibility, porosity (size and distribution of pores), surface microstructure, etc. In addition, the behaviour of NC HAp/PLLA composite biomaterial depends on the structural features of both NC HAp and PLLA. Bioresorption time of PLLA depends on the ratio and distribution of amorphous/crystalline phase in the polymer, their molecular weights, polymer porosity, etc. Generally, a decrease in the polymer crystallinity decreases the bioresorption time.³⁸ HAp/PLLA composite biomaterial was obtained by the synthesis and subsequent polymerisation or forging of HAp and PLLA mixtures.^{39,40} The blocks of NC HAp/PLLA composite material of appropriate densities and mechanical characteristics were obtained by hot pressing at the PLLA melting temperature.⁴¹⁻⁴³ These blocks exhibited superior mechanical characteristics.⁴⁴ During the hot pressing of highly porous NC HAp/PLLA composite biomaterial, and its transformation into a nonporous one, physicochemical changes occurred in the PLLA phase, while the NC HAp phase remained stable. Figure 5a



6 Images (SEM) of collagen scaffolds with NPs of CP/DLPLG

shows the values of compressive modules and compressive strengths obtained at different hot pressing times.⁴² From the dependencies presented in Fig. 5a, it is clear that the blocks compacted with 60 min hot pressing have compressive strength of 140 MPa and elasticity module of 10 GPa.

Collagen scaffolds with NPs composite biomaterials CP/DLPLG

Tissue engineering offers an alternative approach to repair and regeneration of damaged human tissue. In bone tissue engineering, a scaffold serving as matrix of tissue formation plays a pivotal role and has to fulfill a few basic requirements, such as high porosity and proper pore size, specific surface properties that permit cell adhesion, differentiation and proliferation, desirable mechanical integrity to maintain the pre-designed tissue structure, non-cytotoxicity and osteoconductivity.⁴⁵ The interest of processing bone tissue-engineered scaffolds with both highly porous structure and desired mechanical strength drives the authors to focus on preparing scaffolds using polymers and nanosized CP.⁴⁶

The authors have successfully produced scaffolds of collagen and NC CP with complex internal morphology and macroscopic shape using a solid free form fabrication mould and critical point drying technique. Figure 6 shows the microstructure of collagen scaffolds with 1 wt-% of NPs of CP/DLPLG.

Conclusions

The bone tissue is an especially interesting subject of scientific research, due to both frequent diagnoses of osteoporosis, osteomyelitis etc., and the formative nature of living organisms. Natural bone is mostly composed of NC CP. Whether bone trauma was caused artificially or through illness, the number of reconstructions increases every year worldwide, which induces a continual increase in the monetary investments into this field as well. As of today, numerous kinds of composite biomaterials have been used for this purpose. The development of equipment and characterisation techniques has enabled an exponential progress in new and advanced biomaterials' synthesis. Many qualitative and quantitative characterisation concepts and organisation of biomaterials on all structural levels were taken from

nature. Composite biomaterials for reconstruction of bone tissue, very similar to human tissue, in the form of composite blocks, injectable cements, nanofillers, etc., were produced this way.

New ways of synthesis and processing will lead to various novel forms of calcium phosphates bioceramics suitable for bone engineering. Composite biomaterials based on NC and nanosized CP with drugs or bioactive agents will be important factor in the increase of the quality of life.

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