

ADVANCED NANOCERAMICS FOR BONE TISSUE ENGINEERING

S. M. Barinov, V. S. Komlev, V. V. Smirnov, I. V. Fadeeva, A. S. Fomin

Abstract

Bone tissue engineering is a new concept for repairing damaged hard tissue through induced osteosynthesis. The key problem in this technology is a cell-supporting scaffold which should be biocompatible, biodegradable and of special architecture to provide for biological flaws inside, including neovascularization. Some recent achievements in scaffolding ceramic materials are outlined. Results in the field of calcium phosphate nanoceramics to be used as scaffolding materials are presented. Some data on mechanical properties are given.

Keywords: *bone tissue engineering, scaffolds, nanoceramics*

INTRODUCTION

Tissue-defect management relies on auto- or allografting techniques. Tissue substitutes have been developed to avoid the problems posed by the surgically-induced morbidity of autologous grafts and by the inherent immunogenicity of allografts. The first generation of the materials used was biologically inert, the goal being the achievement of the best possible imitation of the physical properties of the replaced tissue(s), minimising the toxic response in the host. The next generation of synthetic biomaterials is aimed at a controlled interaction with the physiological environment.

However, survival analyses of implanted devices have shown a high risk of failure and a high frequency of revision surgeries. This is mainly due to the fact that synthetic materials cannot respond to changing physiological loads or biochemical stimuli occurring in the patient. As a consequence, tissue engineering represents a feasible and productive approach to repair and reconstitute bone by combining knowledge and technology of the biomaterial field with knowledge and technology derived from cell biology. A new, third generation of biomaterials is under development, aiming at a better integration with the host tissue in the initial post-operative period and at a complete substitution with newly formed tissue in the long run [1-5].

Among different biomaterials considered as scaffolds for bone tissue engineering, calcium phosphate-based materials have proved to be of great interest, given their osteoconductivity and ability to "integrate" with the bone tissue [6-10]. Despite recent advances toward the development of ceramic materials for tissue engineering applications, several challenges still remain, including, e.g. ceramics tend to be too brittle and weak to be used in load-bearing applications, ceramics must have a controlled mass transport (that is, permeability and diffusion) properties, nutrition, as well as the neovascularisation of living tissue in a 3D structure, providing a sequential transition in which the regenerated tissue assumes function as the scaffold degrades.

The recent trend in calcium phosphate bioceramics research is shifting towards nanosized powders and nanocrystalline sintered ceramics. Nanohydroxyapatite (nano-HA) is a constituent of bone tissue, which is a natural composite of nano-HA with collagen fibers. The use of nano-HA in tissue engineering is considered to be promising, owing to its dimensional similarity with the hydroxyapatite (HA) crystals in bone tissue. Specific behaviour of nanoceramics in tissue engineering applications is believed to result from features of their initial interaction with proteins that control subsequent cell function [11]. Apart from the bone/cartilage tissue engineering, selected examples of application of nano-HA-based ceramic materials are orthopedic implants and drug carriers for various bone diseases [11]. Nanosized HA powders are also used as an initial highly active component of bone cement: the reaction of nano-HA with phosphoric acid leads to the crystallization of brushite and *in situ* hardening of the cement paste [12]. Nano-HA is introduced as a reinforcing phase into polymer-base composite materials, thus ensuring their biocompatibility and enhanced mechanical properties [13-15]. Nano-HA particulate is expected to be a promising as drug carrier, especially for intravenous administration and cell therapy [16]. The nanosized HA has an enhanced solubility, which can be used in fabrication of resorbable scaffolds.

EXPERIMENTAL PROCEDURE

In our work the sintering of nanocrystalline HA ceramics was studied with use of a high-pressure treatment of powder prior to sintering. The initial HA powder was synthesized in the presence of the high-molecular weight compound, polyvinylalcohol (PVA). A classical method based on the reaction of calcium nitrate and diammonium phosphate was applied. The precipitating agent was a 25% ammonia solution. The temperature in the course of the process was maintained constant (25°C). The process was performed for 2 h with stirring. The solutions were added in the following order: 330 ml of 1 M calcium nitrate solution, then 250 ml of ammonia solution, then 100 ml of PVA solution. After 15-min stirring for homogenizing the mixture, 300 ml of 0.6 M diammonium phosphate solution was added dropwise over 10 min, with the pH of the solution being maintained constant (10.5) by adding ammonia. Then the solution was kept for a day for ageing. The precipitate was filtered off in a Büchner funnel and transferred to an ethanol medium. The suspension obtained was dried in a drying cabinet at 120°C.

RESULTS AND DISCUSSION

Figure 1 shows a TEM image of the powder with the maximal specific surface area (79 m²/g), which was synthesized in the solution containing 0.25 g/L PVA. A dark-field analysis in the totality of microbeams determined that the apatite nanocrystal size does not exceed 20 nm. The specific surface area corresponds to a crystal size less than 20 nm, as it was estimated using a spherical model. Thus, synthesis from a solution containing PVA allowed the precipitation of nanocrystalline apatite powders. The powder was compacted in a metal mould using a PM 140.50 small-scale hand hydraulic press with a maximal moulding force of 140 tons, resulting in 3.5 GPa pressure, to obtain pellets 12 mm in diameter. The pellets were ground to form powder, the powder was granulated, and the grains were compacted again. Thereby, we succeeded in obtaining compact pellets of nanodisperse powder without compacting cracks, which usually arise in compaction of fine powders under high pressure. The pellets were sintered in a muffle furnace at temperatures in the range from 640 to 1150°C for 2 h at a heating rate of 5 K/min.

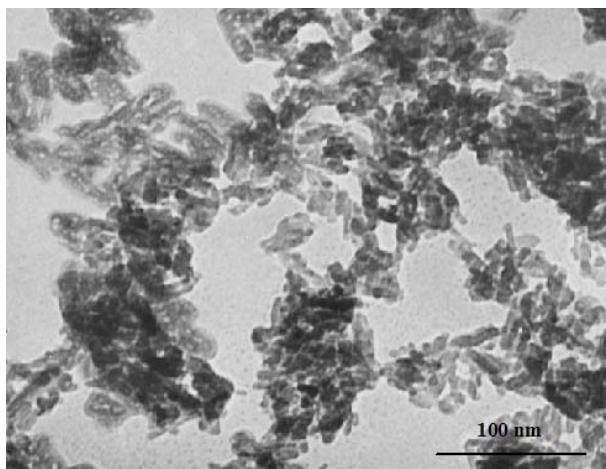


Fig.1. TEM micrograph of initial HA powder.

Figure 2 presents TEM microscopy images and microdiffraction patterns of the initial compacted pellet of nanodisperse powder and the ceramic body produced by sintering at 640°C, respectively. The material obtained retains the compact crystal structure of apatite with the initial nanoparticle size. Accumulative recrystallization caused by heat treatment leads to the growth of crystallites. The average size is about 30 nm; meanwhile, some grains may grow up to 50 nm. Thus, the sintered ceramic is nanocrystalline. An increase in the sintering temperature over 750°C leads to an increase of grains size up to more than 100 nm. Hydrostatic weighing showed that the density of the ceramic obtained by sintering at 640°C is close to, but somewhat less than, the theoretical density of hydroxyapatite. At the sintering temperature of 750°C measured density becomes equal to the density of HA (3.16 g/cm³).

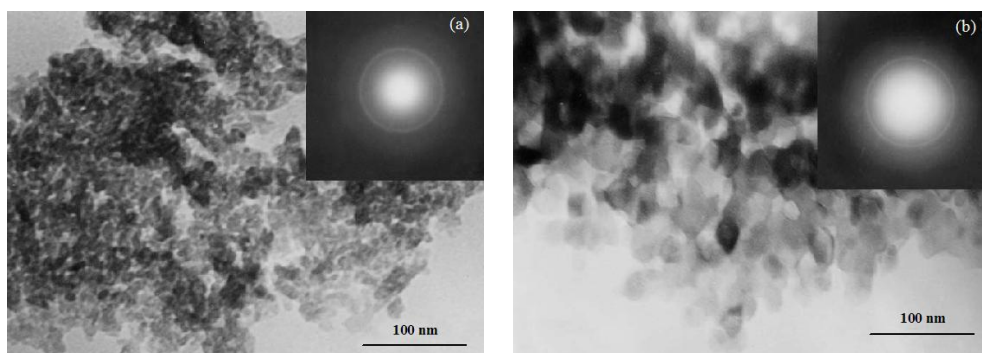


Fig.2. TEM micrographs of green pressed (a) and sintered at 640°C (b) bodies.

Figure 3 shows microhardness of ceramics versus sintering temperature. Initial rise is due to decreasing porosity, while further grain growth leads to slightly decreased microhardness values. The microhardness of nanocrystalline ceramics, 5.8 ± 0.6 GPa, is higher than that of microstructured HA-ceramics, 4.6 GPa [17], due to smaller grain size.

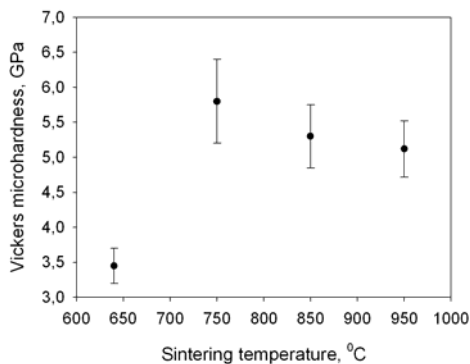


Fig.3. Vickers microhardness versus sintering temperature of ceramics.

Thus, the use of high pressure in compaction results in decreasing the sintering temperature by about 600°C in comparison to the temperature required for sintering of micron-sized hydroxyapatite powders. High pressure significantly increases the contact area and activates the interaction of nanoparticles with high specific surface area and, correspondingly, with increased specific interfacial energy.

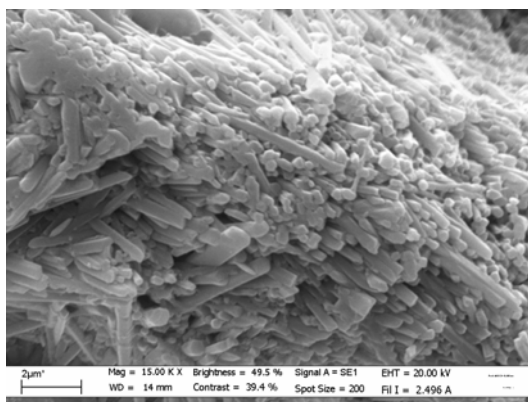


Fig.4. Rod-like grains crystallized at a liquid-phase assisted sintering of HA nanopowders.

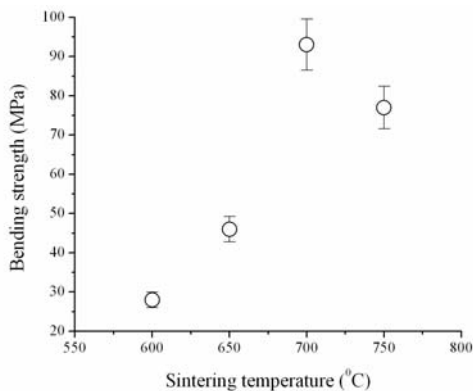


Fig.5. Bending strength of ceramics versus the sintering temperature.

Alternative techniques to activate the densification of nanopowders are needed. It should be, however, noted that sintering with a liquid phase-forming additive, probably, cannot be employed, just because the interaction of liquid and major phases results in secondary crystallization and significant grain growth. Figure 4 demonstrates an example of this phenomenon, where the fragments of microstructure of ceramics sintered at 750°C using the batch consisting of HA nanopowders (specific area 90 m²/g) and a carbonate low-melting additive (5 wt.%) are shown. Coarse grains were crystallized from the melt, part of them being of a rod-like morphology that effects on mechanical properties of ceramics. Figure 5 shows bending strength of ceramics versus the sintering temperature.

CONCLUSION

In this work nanocrystalline HA ceramics were fabricated with the use of a high-pressure treatment of powder prior to sintering and with a liquid phase forming additive. The obtained nanocrystalline HA ceramics seem to be promising materials for bone tissue engineering and for both low and high load bearing applications where sufficient mechanical properties are required.

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