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Biodegradable ceramic matrix composites made from nanocrystalline hydroxyapatite and silk fibers via crymilling and uniaxial pressing

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ABSTRACT

The treatment of severe bone loss and resultant large bone gaps is a challenge due to the need to stimulate bone growth using a biomaterial that biodegrades slowly and has mechanical properties similar to the surrounding bone. This work proposes a novel biodegradable nanocomposite to address these needs. The first step of the technology is to prepare composite powders consisting of 5 wt% or 15 wt% silk fibers and nanocrystalline hydroxyapatite. Both are biodegradable materials. The composite powders are prepared using cryomilling. The second step is uniaxial pressing of the powders at a pressure up to 1 GPa and a temperature of 80 °C. This leads to a homogenous and dense ceramic matrix composite with good adhesion between the nano-hydroxyapatite and silk fibers. The nano-hydroxyapatite retains its nanocrystalline form.

The compressive strength is 276 MPa and the bending strength is 82 MPa, which are comparable to the strength of cortical bone.

1. Introduction

The stimulation of bone regrowth after severe bone loss remains a challenge, for example, when a cancer operation or complicated fracture results in critical bone defects that are too large to be filled by bone in the recovery process. Such a gap needs to be filled with a biocompatible, biodegradable implant material with mechanical properties similar to the surrounding bone [1] that will mechanically interact with the bone to stimulate bone regrowth. There are many metal, ceramic, composite, and bioresorbable implants on the market, yet there is still no satisfactory solution for tough bioresorbable implants [2]. The bone implants should have similar mechanical properties to those of the cortical bone: compressive strength between 100 and 230 MPa, bending strength between 50 and 160 MPa, and Young's modulus between 7 and 30 GPa [3].

The most promising material for use as a bone substitute is synthetic nano-hydroxyapatite (nHAP), which has a nanostructure similar to natural apatite, a mineral component of bone. Materials based on nHAP have greater biocompatibility than all other ceramics [4]. Because of the brittleness of hydroxyapatite ceramic, various polymers (PLA [5–8] or PE [9–10]) are added to improve its strength and fracture toughness [11].

Considerable research effort has focused on natural biocompatible materials [11–12]. Especially, silk seems to be promising because of its mechanical strength, chemical stability, slow biodegradability in a physiological environment, and good biological properties [13–16]. However, the majority of studies have examined HAP-silk composites, where silk is in the form of the fibroin (silk fiber protein) solution, and such composites have rather poor mechanical properties. HAP-silk composites assume the form of scaffolds, nanofibers, films, meshes, etc. [17–19].

There exist polymer matrix composites or fibers reinforced with calcium phosphates that have displayed increased strength and biological properties. However, to our knowledge, there has been no research on ceramic matrix composites of nano-hydroxyapatite reinforced with natural fiber. The study presented here describes a method of obtaining and forming a composite from bioresorbable materials, nHAP and silk fiber, resulting in mechanical properties similar to those of cortical bone.

2. Materials and methods

Nanocrystalline hydroxyapatite GoHAP^{TM}, synthesized in Laboratory of Nanostructures of IHPP PAS, was used as a ceramic

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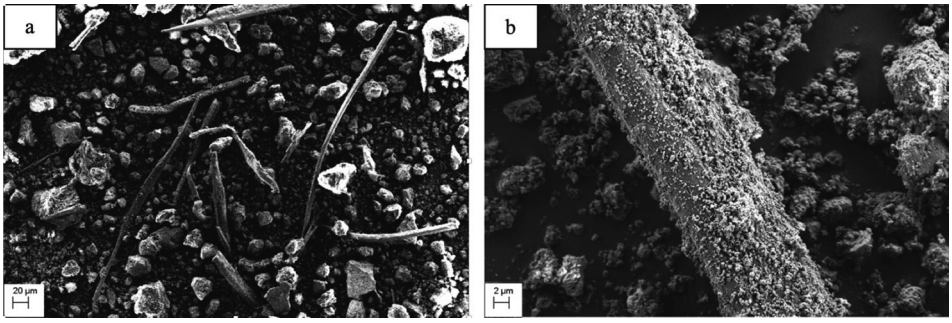

Fig. 1. SEM images: (a) the composite powder with 15 wt% silk and 85 wt% GoHAP^{TM} after cryomilling, (b) the surface of a single fiber.

Table 1
Density, relative densit, and porosity of the silk-GoHAP^{TM} composite samples.

Composition	Pressing temp. (T.K)	Density ($\rho \pm \sigma^*$.g/cm^3)	Relative density [$\rho_R \pm \sigma$%]	Porosity [P ± σ%]
5 wt% silk, 95 wt% GoHAP^{TM}	RT	2.35 ± 0.02	84.7 ± 0.6	15.3 ± 0.6
	353	2.47 ± 0.02	88.7 ± 0.5	11.3 ± 0.4
15 wt% silk, 85 wt% GoHAP^{TM}	RT	2.17 ± 0.02	83.2 ± 0.6	16.8 ± 0.6
	353	2.24 ± 0.01	85.9 ± 0.5	14.1 ± 0.5

matrix. It had a particle size of 8 ± 1 nm, a density of 2.86 g/cm^3 [5,20]. To reduce the brittleness of the GoHAP^{TM} matrix, silk fibers in the de-gummed form produced by *Bombyx Mori* moths were used.

The manufacturing process consists of two main steps: fabrication of a composite powder and its consolidation in a molder. The composite powder was obtained by cryomilling of the two components at liquid nitrogen temperature using a high-energy mill (model 6775 Freezer/Mill® produced by SPEX®SamplePrep). The cryomilling parameters are shown in Table 2. Two compositions were used: 5 wt% silk, 95 wt% GoHAP^{TM} and 15 wt% silk, 85 wt% GoHAP^{TM}. After milling, the powder was uniaxially compacted under a pressure of 1 GPa at room temperature or at 80 °C. Dense bars with dimensions 4 mm × 4 mm × 35 mm were obtained. The material's structure was examined by scanning electron microscopy using the Zeiss Ultra Plus microscope and by X-ray computed microtomography using SkyScan 1172 (Bruker). The density was analyzed by helium pycnometer AccuPyc II 1340 by Micromeritics and by the Archimedes method to obtain the density (ρ), relative density (ρ_R), and porosity (P). The three-points bending test was performed on MTS QTest/10 universal punch displacement with the velocity of 0.5 mm/min. The bending ultimate strength was calculated based on stress–strain curves. A compressive strength test was carried out using an MTS 858 dynamic machine with a punch displacement of 20 µm/min.

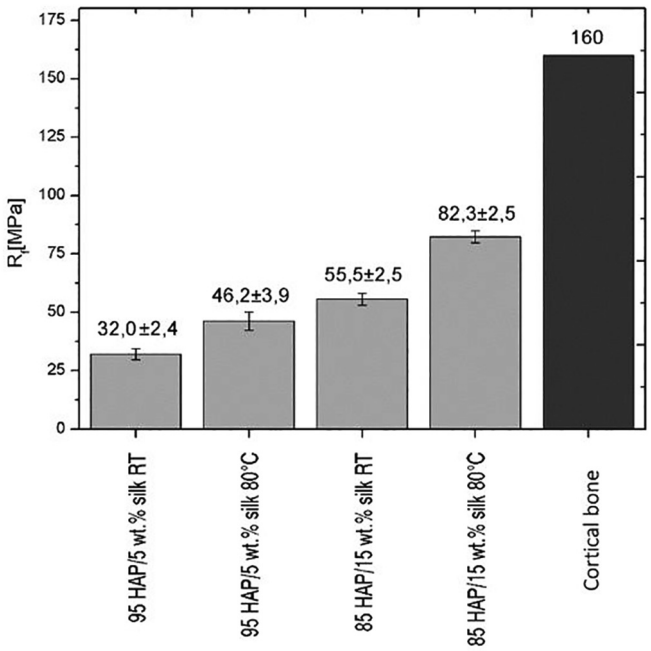

Fig. 2. Bending strength of the silk-GoHAP^{TM} composites compared to human cortical bone.

3. Results and discussion

Structure of the cryomilled composite powders. SEM images of the powders show that silk preserves its fibrous structure (Fig. 1a). The surface of the fibers is covered tightly with GoHAP^{TM} (Fig. 1b). This indicates good adhesion between the ceramic and organic part of the composite and is a prerequisite for its good mechanical properties. For instance, L. Wang et al. emphasized the lack of a physicochemical bond between HAP and the organic substance and thus suggested modifying the fibers to improve the mechanical properties of the composite [20].

As measured by helium pycnometry, the density of the cryomilled powders was 2.78 ± 0.04 g/cm^3 for 5 wt% silk and 95 wt % GoHAP^{TM}, and it was 2.61 ± 0.07 g/cm^3 for 15 wt% silk and 85 wt% GoHAP^{TM}. Also porosity and density of formed samples via uniaxial pressing were investigated and are shown in Table 1 (Fig. 2).

The bending strength (Rf) is shown in Fig. 2. The compressive strength (RC) and Young's modulus (E) for the composite with 15 wt% silk and 85 wt% GoHAP^{TM} pressed at 80 °C were RC = 276 ± 39 MPa and E = 8.4 ± 0.9 GPa, respectively. These values are similar to those of cortical bone, which are respectively RC up to 230 MPa, E from 7 GPa [2].

The composite with 15 wt% fiber shows the highest bending strength. The bending strength of the composites is two and a half times higher than that of pure GoHAP, and the bending strength of the sample with 15 wt% silk fibers is higher than that with 5 wt% silk, because natural polymer fibers prevent brittle fractures at low load.

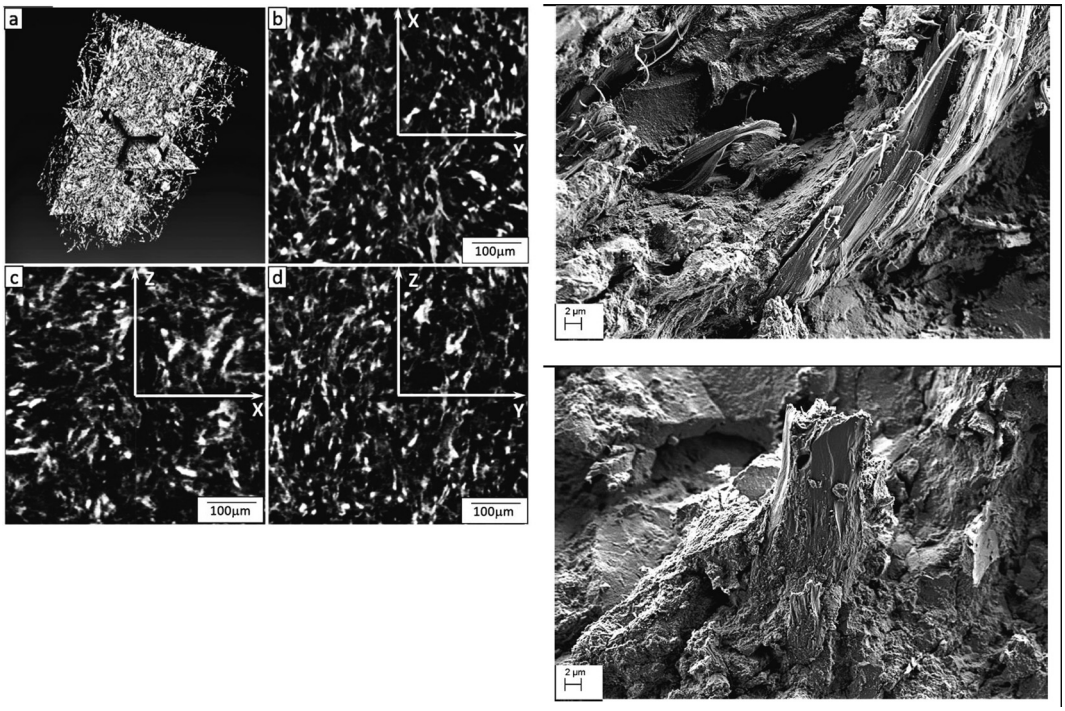

Fig. 3. Structure of the composite. (a-d) Computed microtomography scan of the sample with 15 wt% silk and 85 wt% GoHAP^{TM}. (e,f) SEM images of fracture of the samples pressed in RT after a bending test: (e) 5 wt% silk and 95 wt% GoHAP^{TM}, (f) 15 wt% silk and 85 wt% GoHAP^{TM}.

Fig. 3a and 3b show SEM images of the fracture surface after the bending tests. Silk fibers are observed to be broken for all samples, which confirms the good adhesion between the ceramic matrix and the silk fibers. Fig. 3c shows the computed microtomography scan of the sample containing 15 wt% silk and 85 wt% GoHAP^{TM} pressed at 80 °C. The structure of the sample is anisotropic, and the fiber distribution is homogenous in the volume of the sample.

4. Conclusions

The paper presents a promising solution to increasing the bending and compressive strength of a composite with a ceramic matrix. It is common knowledge that phosphate ceramic is a biocompatible material applied in bone loss treatment, but the brittleness of ceramics limits their application as implants.

The aim and novelty of this research is to obtain a composite with the matrix of nanohydroxyapatite, reinforced with silk fibers. The composite obtained with the nanometric structure is characterized by an increased bending strength in relation to pure ceramics. It has been proven using SEM imaging and CMT scanning techniques that the material is anisotropic and homogenous. Adhesion between these two components has been observed and the silk remains in its initial fibrous form (without chemical treatment).

It was possible to achieve such a structure thanks to a unique method of milling and uniaxial pressing at a high pressure of 1 GPa and a temp. of 80 °C. This method permits dense samples to be obtained without particle growth and preserves the silk fiber structure. This method allows avoiding chemical solvents to achieve a homogeneous composite as ones that are most often used for such types of composites.

The material obtained is characterized by a beneficial and unique structure, where the silk fibers are fixed in the ceramic matrix. These types of structures are desirable to obtain materials that are resistant to cracking. The paper examines the influence of the quantity of fibers and the temperature on the composite properties. The best bending and compressive strength, and Young's modulus comparable to the properties of the cortical bone, was achieved with the composition containing 15 wt% of silk and 85 wt% of GoHAP^{TM} pressed at 80 °C: R_f = 82.3 ± 2.6 MPa, R_C = 276 ± 39[1–20] MPa and E = 8.4 ± 0.9 GPa.

The material obtained has the potential for application in orthopedics for bone loss filling. The cryomilling and isostatic pressing method can be applied to other composites, which are characterized by a high compactness of the ceramic phase, and to fibers, especially materials that require processing at low temperatures.

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CRediT authorship contribution statement

E. Pietrzykowska: Conceptualization, Methodology, Writing - review & editing. **M. Małysa:** Investigation, Writing - original draft. **A. Chodara:** Resources. **B. Romelczyk-Baishya:** Investigation. **K. Szlązak:** Investigation. **W. Święszkowski:** Investigation. **Z. Pakieła:** Supervision. **W. Lojkowski:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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