

# Chapter 1

## Bacterial Cellulose/Hydroxyapatite Printed Scaffolds for Bone Engineering



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**Abstract** The bone tissue recovery after serious injuries has been a goal of regenerative medicine for more than a decades. So the issue of recovering the acetabular bone deficit has not been resolved. The typical practice of “deficient areas” filling is metal porous augments using, which is a short-term solution to this issue. It should be noted that the use of augments is not entirely justified, since for 5 years and more, bone tissue lysis also occurs due to the inevitable macrophage reaction of the body to friction products (debridement). One of the solutions, competing with auto- and/or alloplastic, is the use of composite biomimetic structures obtained by 3D printing. The paper describes the method of inkjet printing of a composite material based on bacterial cellulose(BC)/hydroxyapatite (HA). Printing inks was obtained by mixing the BC nanoparticles in an aqueous solution of  $\text{CaCl}_2$  and  $\text{Na}_2\text{HPO}_4$  with different proportions. Drying the resulting structures was carried out by freeze drying. The samples with different architecture were obtained. The samples’ microstructure, functional composition were studied. It was found that obtained composite have homogeneous structure of HA paricle’s spreading among BC matrix.

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## 1.1 Introduction

Hydroxyapatite (HA) opens broad possibilities in bone engineering application due to being an inorganic component of natural bone. Despite to its high biocompatibility and osteoconductivity, low range of mechanical properties makes restricts its spreading in bone engineering. The permissible way to solve this problem is a developing HA based composite materials. The authors of [1–4] suggested to incorporate HA nanocrystals into organic matrix, which has high biocompatibility and mechanical strength.

Bacterial cellulose (BC) has high level of biocompatibility along with high mechanical properties comparable with aramids. It makes actual producing hybrid composite materials based on BC/HA. Such composites were investigated in [5–11], where was showed that the BC/HA nanocomposites are promising for applications in various biomedical fields: tissue engineering and drug delivery.

In case of bone tissue recovery after serious injuries it becomes important, a providing appropriate conditions for fast osteoregeneration. In spite to the great progress in that area, there is lack of techniques for the acetabular bone deficit solving. The typical practice is a filling “deficient areas” with metal porous augments, which is a short-term solution to this issue. It should be noted that the use of augments is not entirely justified, since for 5 years and more, bone tissue lysis also occurs due to the inevitable macrophage reaction of the body to friction products (debridement) [12–16]. One of the solutions, competing auto- and /or alloplastic, is the use of composite biomimetic structures obtained by 3D printing [14, 15, 17]. The paper describes the method of preparation composite based on BC/HA inks for inkjet printing.

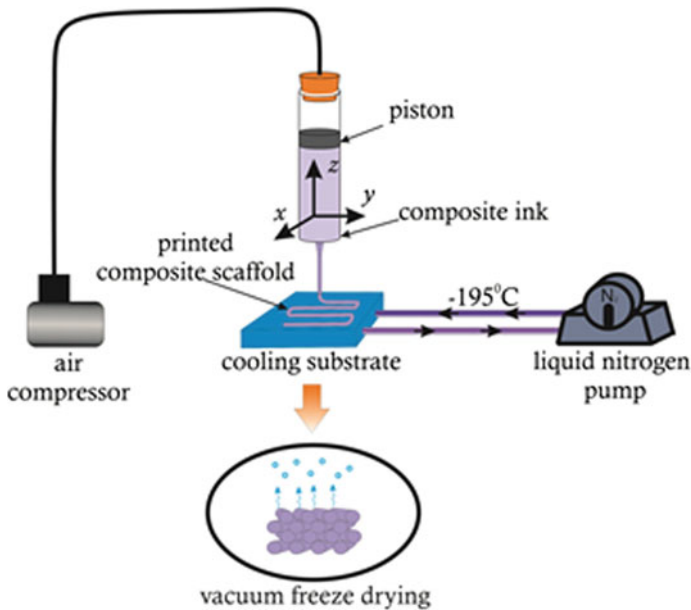
## 1.2 Method and Materials

The printing ink of composite material was obtained by mixing in an aqueous solution two different proportion of BC powder,  $\text{CaCl}_2$  and  $\text{Na}_2\text{HPO}_4$ . The managing of BC content explains by its viscous properties. The molar ratio of Ca/P was 1.67 as in stoichiometric HA. The two BC/HA biocomposites were prepared by follows:

2.94 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 4.29 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  were dissolve in 40 ml of bi-distilled water and mixed. After 0.15 g of BC was put into the solution

1.47 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 2.15 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  were dissolve in 40 ml of bi-distilled water and mixed. After 0.3 g of BC was put into the solution.

The resulting materials' suspensions were milled by planetary milling machine for 3 h. The milled suspensions were washed up by distilled water and were filtered. The composite ink was put into 3D printer's cylinder. The work principle of 3D printing setup is schematically illustrated in Fig. 1.1 The biocomposite ink presses



**Fig. 1.1** The BC/HA scaffolds printing process

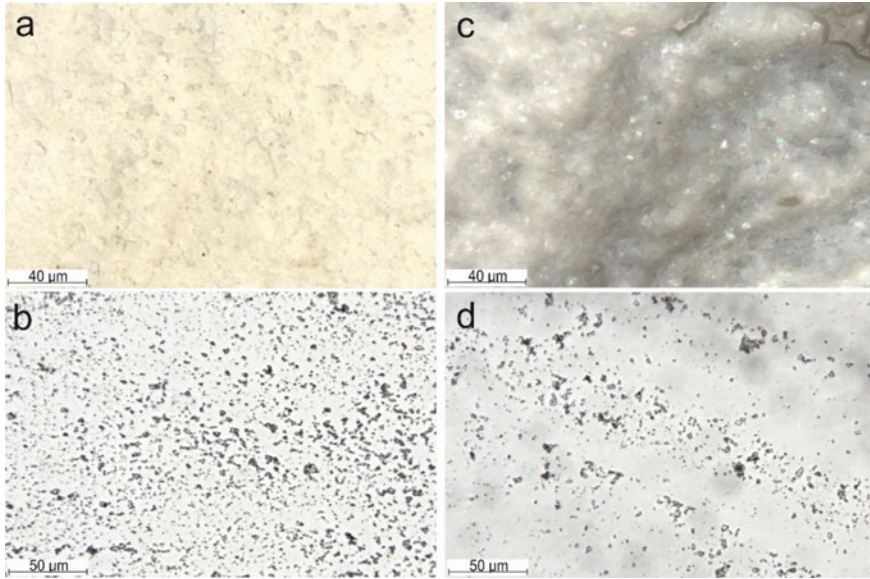
through needle filler by piston under pressure of air. The 3D moving noodles of biocomposite put onto cooled substrate and immediately freeze.

The resulting structures dried by freeze drying method. The microstructure of the samples was studied on optical microscope Olympus BX-51, a digital images were processed by SIAMS program. The functional composition was determined by FTIR spectrometry. It was found that obtained composite have homogeneous structure of HA paricle spreading among BC matrix.

### 1.3 Results and Discussion

The microstructure of BC/HA composites with different proportions are shown in Fig. 1.2. The suspension is homogenous. The particles are evenly spaced, Fig. 1.2a. At suspension that was obtained by second method (where BC content increased for 4 times) the HA microparticles are incorporated into BC matrix, Fig. 1.2c. It also makes some problems at printing, due to high viscosity of ink. We solved it by increasing fluidity of water solution. It is notable that increasing of BC content enlarges average microparticles size from  $2.98$  to  $3.36\ \mu\text{m}$ , according to Table 1.1.

Also, the maximum square of particles increased from  $9.51$  to  $12.25\ \mu\text{m}^2$ . We suppose that particles' sizes increasing caused by viscosity of BC material, which blocks suspension fluidity at milling process (Fig. 1.3).



**Fig. 1.2** The BC/HA biocomposites microstructure. **a, b**—for lower content of BC; **c, d**—for BC increased content (4 times); **a, c**—light reflected images; **b, d**—light transmitted images

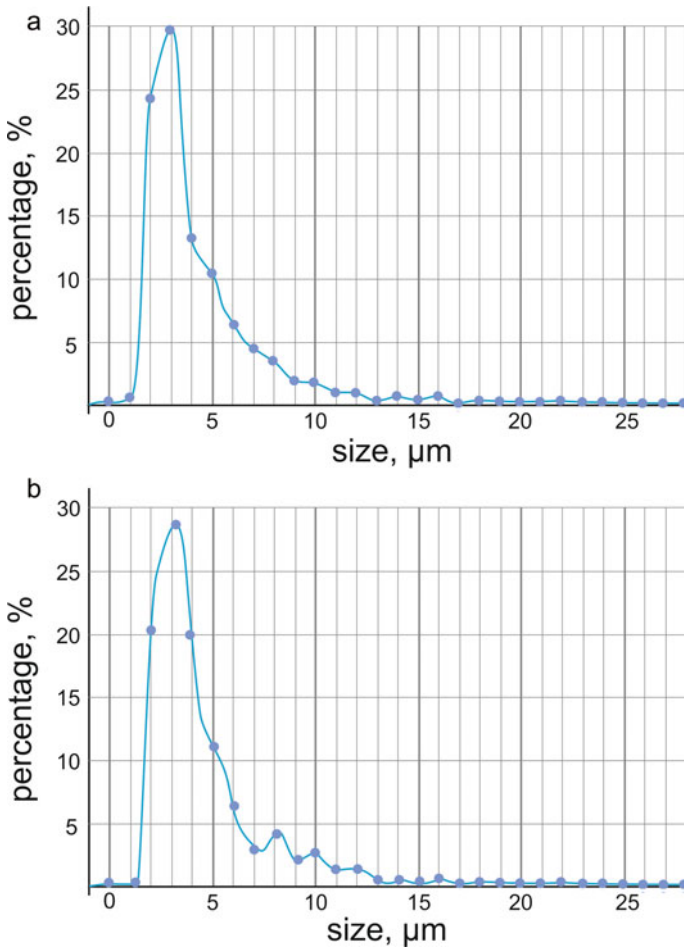
**Table 1.1** Statistical values of biocomposite particles' dimensions

Parameters	Statistical values					
	BC/HA—1			BC/HA—2		
	Min.	Max.	Ave.	Min.	Max.	Ave.
$S \mu\text{m}^2$	1.01	132.51	9.51	1.01	344.47	12.25
$D \mu\text{m}$	1.13	13.58	2.98	1.13	22.04	3.36

The Fourier transform infrared spectroscopy was used to evaluate the HA/BC interactions. All obtained IR spectra were referenced on HA spectra, (Fig. 1.4c). The 562 and 602  $\text{cm}^{-1}$  bands in HA spectra belong to the O-P-O groups ( $\nu_4$  bending mode) [18, 19]. The highest band at 1026  $\text{cm}^{-1}$  refers to asymmetric stretching ( $\nu_3$ ) of P-O vibrations. The carbonate groups enhancing at 1456 to 1716  $\text{cm}^{-1}$  caused by BC addition and indicates the bonding of BC with HA.

## 1.4 Conclusion

The BC/HA biocomposite materials with different BC content for use in 3D printing were obtained. The microstructure, functional groups composition were investigated.

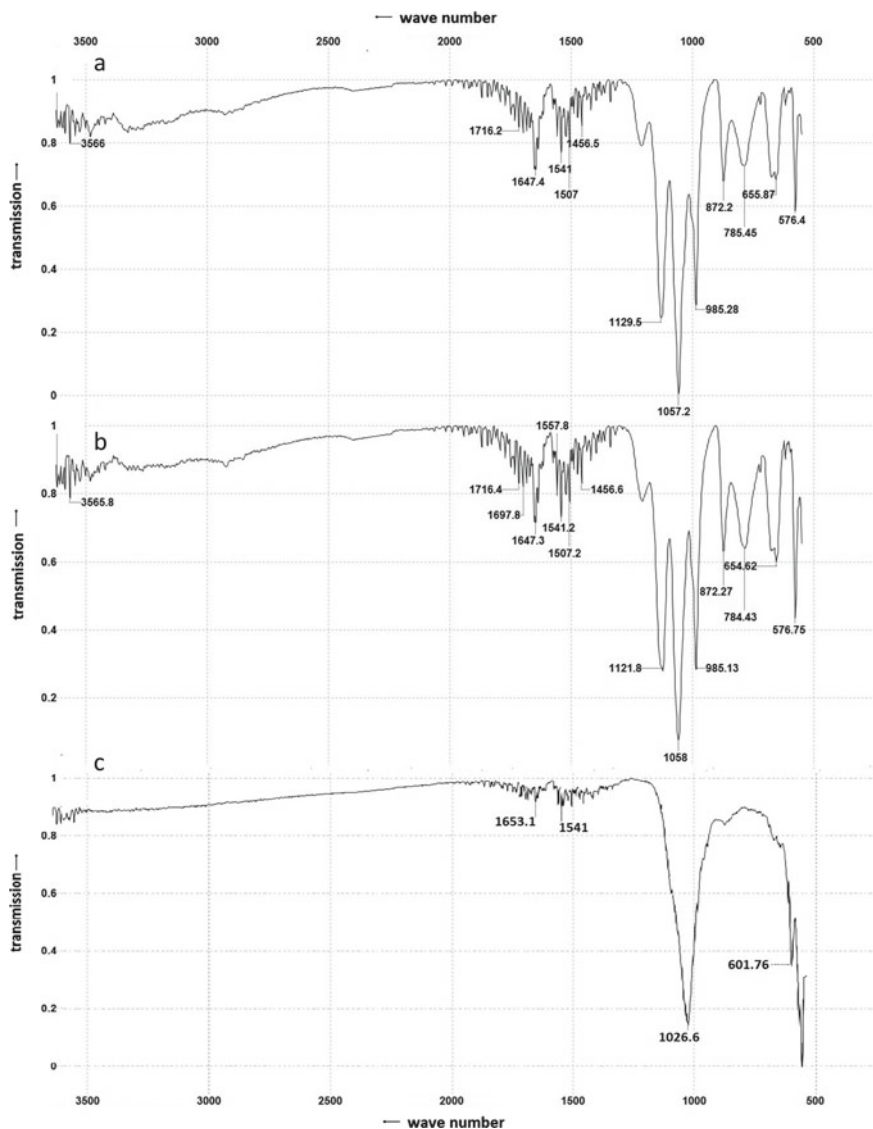


**Fig. 1.3** Measurement of the size and shape of particles. Differential numerical particle size distribution

It was shown, that increasing of BC content has direct influence on particles' size at suspension. The method of BC/HA biocomposite 3D printing is suggested.

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**Fig. 1.4** FTIR spectra of the synthesized samples: BC/HA—1 (curve a), BC/HA—2 (curve b) and HA (curve c)

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