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Structure and Biochemical Study of Nanocomposite Bioconstruction for Restoration of Bone-cartilaginous Defects

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Abstract

Porous and strong nanocomposite bioconstructions were formed by laser evaporation of an aqueous dispersion of carbon nanotubes in a protein matrix. The homogeneous dispersion was exposed to laser irradiation to create solid constructions. Continuous laser radiation with a wavelength of 970 nm and a power of 5-7 W was used. The porosity of nanocomposite bioconstructions was studied by the method of low-temperature nitrogen porosimetry and X-ray microtomography, the tensile strength and relative elongation of bioconstructions were evaluated, and their biocompatibility was tested in vitro. It was found that with an increase of the carbon nanotube's concentration, a slight decrease in strength (3-15 %), a decrease in the pore size (20-40 %), and an increase in the degree of deformation (10-12 %) were observed. At the same time, the mechanical parameters of the bioconstructions met the requirements for the materials for the restoration of bone-cartilaginous defects. Using optical microscopy and the MTT-test, proliferative activity and structural features of bone tissue cells on the surface of nanocomposite bioconstructions were evaluated. Studies have shown no toxic or inhibitory effect on cells. The results of the studies can talk about the advantage of nanocomposite bioconstructions using as an implant material for improving the growth of biological cells and regenerating damaged biotissues.

Keywords: Nanocomposites, laser radiation, mechanical properties, porosity, X-ray microtomography, biocompatibility

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1. Introduction

Today various bioconstructions are widely used in tissue engineering as scaffolds for growth and regeneration of damaged tissues of the body [1]. To replace lost or damaged tissues, bioconstructions must have a certain porosity, strength, adequate to the strength of native tissues, and high biocompatibility [2].

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The new implantable material based on carbon nanotubes was created [3-5]. This material was obtained by laser evaporation of water from nanocomposite dispersion of carbon nanotubes and bovine serum albumin. The result was a rubber-like black or dark-grey material. To improve the elasticity, strength and biocompatibility of this material the suspension of bovine collagen was added to nanocomposite dispersion.

To prove the possibility of using nanocomposite bioconstructions to restore bone-cartilage defects, the structure and biocompatibility of bioconstructions were studied by various methods: methods of low-temperature porosimetry, X-ray microtomography method.

2. Materials and Methods

The nanocomposite bioconstructions consisted of two types of single-walled carbon nanotubes (SWCNT): SWCNT90A made by Carbon-ChG (Russia) and SWCNT95TA made by OCSiAl (Russia) and purified by Carbon-ChG. The first type of SWCNT has a 98 % of purity, and the second one – about 90 %. Also all these nanotubes had carboxyl groups on their surface, promoting the homogeneity of aqueous dispersions of nanotubes. To provide a biocompatible matrix for carbon nanotubes the powder of bovine serum albumin (BioClot GmbH) and suspension of bovine collagen (Belkozin-Pro, Russia) were used. The concentrations of both types of carbon nanotubes were 0.01 and 0.1 %, of bovine serum albumin – 25 %, of collagen – 1 %. The laser method of nanocomposite bioconstruction's creation described in [3]. We used continuous laser radiation with wavelength of 810 nm and power of 4 W.

In this study we evaluated the porosity and mechanical properties of experimental samples of nanocomposite bioconstructions.

The nanostructure of nanocomposite bioconstructions was studied by methods of low-temperature porosimetry using analyzer of specific surface "Sorbtometer-M" ("Katakon", Russia). The sample was placed in a glass ampoule and weighed on an electronic balance. The mass of the sample was determined as the difference between the masses of the ampoule with the sample and the empty ampoule. Then the ampoule with the sample was placed on the sample preparation station of the specific surface analyzer, where the sample was annealed to remove previously adsorbed substances at a temperature of 50 °C for 1 hour. Analysis of specific surface area was based on the method of Brunauer-Emmett-Teller. The specific pore volume was estimated from the value of adsorption at a relative pressure close to the saturated vapor pressure. The pore size distribution was calculated by Barrett-Joyner-Halenda method.

The microstructure of nanocomposite bioconstructions was studied by X-ray microtomography method. The following parameters of X-ray microtomography were selected: the voltage at the cathode of the X-ray tube was 26 kV; the current at the cathode of the X-ray tube is 400 mA; the current at the cathode of the X-ray tube is 9 W; the rotation step is 0.15 (~ 4000 shadow projections); the spatial resolution is 12 μm.

To study the mechanical properties we prepared the samples with a dumbbell shape and a constriction of the same size. The constriction had the following dimensions: 3.5 mm length, 3.5 mm width, and 1.4 mm thickness. The distance between the grippers was 2.5 mm, the loading rate was 1 mm/min, and the maximum load was 50 N. Samples were examined on the universal testing machine Instron 3343 (load cell 2519-102, 50 H).

Bone tissue cells – osteoblasts, were incubated with experimental samples for 3, 24, 48 and 72 h and then fixed with glutaraldehyde. The cells life cycle during cells incubation with the 3-D nanocomposites was studied using optical and atomic force (AFM) microscopy. For optical microscopy we used the optical microscope Biolam M-1 (Russia), and Bruker (USA) for AFM.

3. Results

The results of a porosity study of nanocomposite bioconstructions are presented in Table 1. The ingredients and its concentrations are described in column 1.

Method of low-temperature nitrogen porosimetry showed that with an increase of the carbon nanotubes concentration a decrease in the pore size (20-40%) was observed. With an increase in the concentration from 0.01 to 0.1 %, porosity in general was increased from 16 to 28 %. The percentage of open pores was the same for all samples and was 0.02.

A comprehensive analysis of nanocomposite bioconstructions was carried out, including: 2D and 3D visualization nanocomposite bioconstructions (Figure 1) Sample SWCNT 90A (0,01 %), BSA (25 %), collagen (1 %) has a homogeneous structure with a series of small pore spaces with a diameter of about 0.4 mm and micropores with a diameter of 65 μm (Figure 1 A). Sample SWCNT 90A (0.1 %), BSA (25 %), collagen (1 %) has a homogeneous structure with micropores with a diameter of 43 μm (Figure 1 B). Sample SWCNT 95TA (0,01 %), BSA (25 %), collagen (1 %) has a series of micropores with a diameter of 37 μm. (Figure 1 C). Sample SWCNT 95TA (0.1 %), BSA (25 %), collagen (1 %) has a series of micropores with a diameter of 49 μm (Figure 1 D).

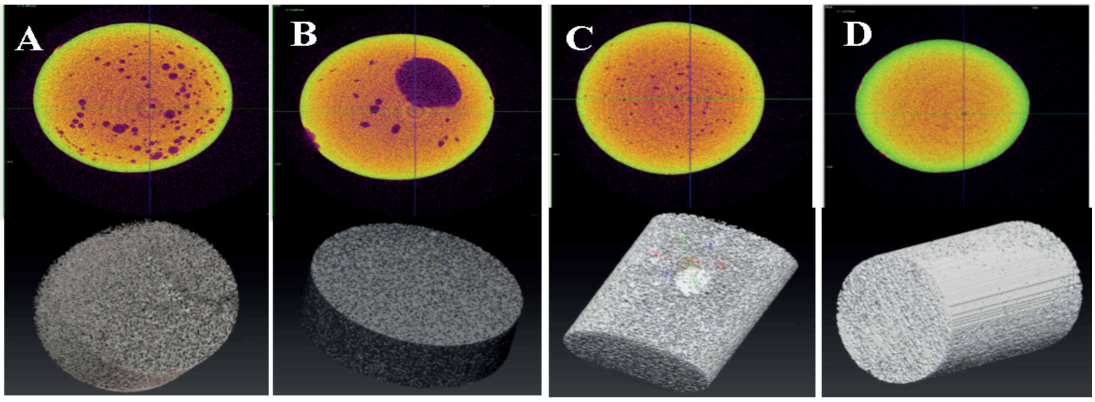

Figure 1: 2D and 3D visualization of nanocomposite bioconstructions: A — Sample made of SWCNT 90A (0,01 %), BSA (25 %), collagen (1 %), B — SWCNT 90A (0,1 %), BSA (25 %), collagen (1 %), C – SWCNT 95TA (0,01 %), BSA (25 %), collagen (1 %), D — SWCNT 95TA (0,1 %), BSA (25%), collagen (1 %).

Table 1: Porosity of nanocomposite bioconstructions.

Sample	Porosity, measured by method of low-temperature nitrogen porosimetry			Porosity, measured by method of X-ray microtomography	
	Specific surface area, m^2/g	Specific pore volume, ml/g	Average pore diameter, nm	Average pore diameter, μm	Porosity (%)
I. SWCNT 90A (0,01 %), BSA (25 %), collagen (1 %)	0.476	0.008	31.96	65	16.44
II. SWCNT 90A (0,1 %), BSA (25 %), collagen (1 %)	1.124	0.068	26.63	43	28.31
III. SWCNT 95TA (0,01 %), BSA (25 %), collagen (1 %)	1.089	0.008	46.88	37	24.33
IV. SWCNT 95TA (0,1 %), BSA (25 %), collagen (1 %)	0.583	0.001	27.94	49	26.67

The results of the tensile strength and relative extension's study are presented in Table 2. Thus, it was found that with an increase of the carbon nanotube's concentration, a slight decrease in strength (3-15 %), and an increase in the degree of deformation (10-12 %) was observed. Type of nanotubes had no significant effect to the mechanical properties of nanocomposite bioconstructions.

Table 2: Mechanical parameters of nanocomposite bioconstructions.

Sample	Average tensile strength of native cartilage, MPa	Average tensile strength, MPa	Average relative extension, %
I. SWCNT 90A (0,01 %), BSA (25 %), collagen (1 %)	3	3.7	12.2
II. SWCNT 90A (0,1 %), BSA (25 %), collagen (1 %)		3.6	13.7
III. SWCNT 95TA (0,01 %), BSA (25 %), collagen (1 %)		3.9	11.9
IV. SWCNT 95TA (0,1 %), BSA (25 %), collagen (1 %)		3.4	13.1

The process of cell attachment and growth is illustrated by Pic. 1. In the above row the optical microscopic images are presented, and in below row – the AFM ones.

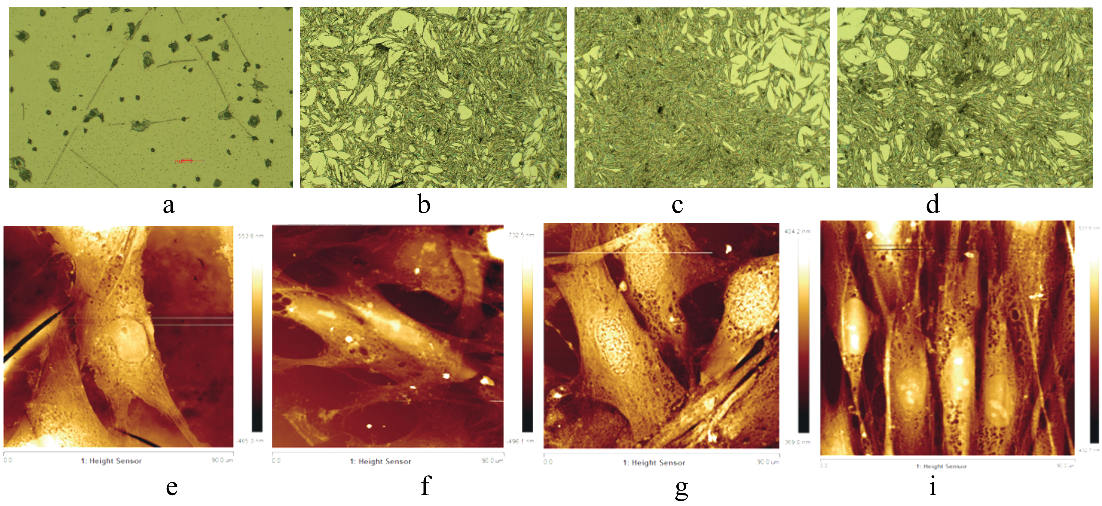

Figure 2: Optical (a-d) and AFM (e-i) microscopic images of osteoblasts incubated on the surface of nanocomposite bioconstructions during 3 (a, e), 24 (b, f), 48 (c, g) and 72 (d, i) hours.

There has been a process of cell adhesion and fixation to the samples, further spreading and breeding. Cells were found in the division phase after 24 h (pic.2 g). 3-D nanocomposite showed no toxicity in contact with living cells, and showed the ability to stimulate cell growth.

4. Conclusion

As a result, it was found that nanocomposite bioconstructions based on dispersion of BSA – 25 %, collagen – 1 %, single-walled carbon nanotubes - 0.01 %t have a highest homogeneity (16,4 %), the average pore size — 37 μm. This sample has excellent mechanical properties: hardness nanoscale reached to 370 MPa, the elastic modulus to 4.2 GPa, and elastic recovery previous form after indenter puncture to 40%. It is known that, with an average pore diameter of 1-300 μm, and a percentage porosity of 10-30 % of implantation materials, the proliferation and germination of blood vessels is accelerated. The results of the study show that the structural and mechanical parameters of nanocomposite bioconstructions satisfy the requirements of biomedicine. It has also been shown that nanocomposite bioconstructions do not inhibit cell growth and can act as a scaffold for the regeneration of damaged tissues of bone-cartilage compounds.

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