Study of the structure of 3-D composites based on carbon nanotubes in bovine serum albumin matrix by X-ray microtomography

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Abstract. 3-D composites are widely used in tissue engineering. A comprehensive analysis by X-ray microtomography was conducted to study the structure of the 3-D composites. Comprehensive analysis of the structure of the 3-D composites consisted of scanning, image reconstruction of shadow projections, two-dimensional and three-dimensional visualization of the reconstructed images and quantitative analysis of the samples. Experimental samples of composites were formed by laser vaporization of the aqueous dispersion BSA and single-walled (SWCNTs) and multi-layer (MWCNTs) carbon nanotubes. The samples have a homogeneous structure over the entire volume, the percentage of porosity of 3-D composites based on SWCNTs and MWCNTs - 16.44%, 28.31%, respectively. An average pore diameter of 3-D composites based on SWCNTs and MWCNTs - 45 μm 93 μm. 3-D composites based on carbon nanotubes in bovine serum albumin matrix can be used in tissue engineering of bone and cartilage, providing cell proliferation and blood vessel sprouting.

1. Introduction
The 3-D composites are widely used in tissue engineering. [1]. To replace lost tissues composites must have a certain porosity (pore size, pore volume, the ratio of voids to solid part of the material). The porous structure of composites provides sprouting of blood. This requires the selection of optimal porosity characteristics [2].

3-D composites were formed by laser ablation of an aqueous dispersion of carbon nanotubes in the protein matrix. Most photostability type of protein has been selected – bovine serum albumin (BSA). Carbon nanotubes (CNTs) are allotropic form of carbon filamentary structure. CNTs have a high mechanical strength, excellent thermal and electrical properties. The mechanical properties similar to the properties of protein structures.

Comprehensive analysis of the structure of the 3-D composites based on carbon nanotubes in the BSA matrix was conducted by X-ray microtomography. This method allows to obtain high-resolution visualization of structure without requiring preliminary preparation of samples.

2. Materials and methods
X-ray Microtomograph Scyscan 1174 was used to study the structure of the 3-D composites based on CNTs in BSA matrix. The voltage and power of the X-ray tube current is 20-30 kV and 400 mA,
respectively. 14-bit X-ray camera with a resolution 2000 ms (projection ratio 1304x1024 pixels) was used to detect X-ray radiation. While scanning a 3-D composite is rotated around its axis (an angle of rotation 0.1 degrees ~ 3500 projections). Samples were placed on a special specimen stage of a transparent material to X-rays, printed on a 3-D printer. Scan time of one sample was 300 minutes.

After scanning composites, shadow projections were reconstructed using multi-threaded modified algorithm of Feldkamp. Image enhancement is achieved by averaging the several shadow projections in one angular position. Also used:

- misalignment compensation of the image projection to compensate for possible displacements during scanning;
- beam-hardening correction through a linear transformation;
- ring artifacts reduction to smooth the projections by masking pixel defect in the specified area.

Various palettes and settings is selected to create a model when visualization of the reconstructed images: volumetric model, the extent of absorption, the rendering mode. Maximum intensity projection used in the three-dimensional visualization.

Quantitative analysis of composites allows to estimate composites porosity. Area (region) of interest was allocated in such a way that it contains the most homogeneous area of 3-D composite. After the allocation of the zone of interest, the image binarization was performed. In these images, the white color represents areas with the brightness within the binary threshold "solid", and areas outside this interval corresponds to the black "void".

Experimental samples of composites were formed by laser vaporization of the aqueous dispersion BSA and single-walled (SWCNTs) and multi-layer (MWCNT) carbon nanotubes. Samples 1 and 2 based on SWCNTs and MWCNTs, respectively, formed by continuous semiconductor laser (Table 1). The wavelength and the laser power was 810 W and 2 nm, respectively.

Protein concentration in the aqueous dispersion of nanotubes and BSA was ~ 25% and CNTs concentration was ~ 1 g/l. It is subjected to mechanical stirring and ultrasonic dispersion to obtain a homogeneous dispersion. Further, the irradiation of dispersion was carried out until the solid phase state of the sample. When laser irradiation of an aqueous dispersion of CNTs and BSA, intense absorption of radiation occurs, followed by heating of individual CNTs and their conglomerates. Further, the local boiling of the BSA matrix around the CNTs and their conglomerates was occurred, causing denaturation of BSA. The degree of dispersion (the size of carbon nanotubes and their conglomerates) affect the size of the formed internal pores and cavities. Thus, if size of CNTs conglomerates in the initial solution standardized, the internal structure of the 3-D composite is a more homogeneous. 3-D composites based on SWCNTs (a) and MWCNTs (b) is shown in Figure 1.

![a](image1.png) ![b](image2.png)

**Figure 1.** The 3-D composites based on SWCNTs (a) and MWCNT (b)

### 3. Results

Figure 2, 3 show the results of the processing of the reconstructed images of 3-D composites. Figure 2a, 3a show the three-dimensional visualization of the reconstructed images of 3-D composites based
on SWCNTs and MWCNTs, respectively. Figure 2b, 3b show the reconstructed image of the 3-D composites based on SWCNTs and MWCNTs, respectively. The samples have a homogeneous structure throughout its volume. Large pores, which could prevent ingrowth of blood vessels, are not detected. Figure 2c, 3c show a two-dimensional visualization (enlarged section) of reconstructed images of 3-D composites based on SWCNTs and MWCNTs (the lighter portion, the greater the size of the object). There is a small pore system throughout the volume of the samples.

Figure 2. Two-dimensional and three-dimensional visualization of the structure of the 3-D composites based on SWCNTs. a - three-dimensional visualization, b - a reconstructed image of a 3-D composite in - a two-dimensional visualization of 3-D composite

Figure 3. Two-dimensional and three-dimensional visualization of the structure of the 3-D composites based on MWCNTs. a - three-dimensional visualization, b - a reconstructed image of a 3-D composite in - a two-dimensional visualization of 3-D composite

Table 1 provides a quantitative analysis of 3-D composites based on the reconstructed images. The average pore diameter of the composite 3-D based on SWCNTs - 45 μm, on MWCNT - 93 μm. 3-D composite based on SWCNTs has 16.44% porosity, on MWCNT - 28.31 %. The proliferation and the germination of blood vessels accelerates with an average pore diameter – 1-300 μm and porosity rate – 10% - 30% [3]. The difference between the parameters of the quantitative analysis of 3-D composites based on SWCNTs and MWCNTs can be explained by the different structure of SWCNTs and MWCNTs. The average SWCNTs diameter was ~ 1.4-1.6 nm, its length was — 0.5-1.5 μm, its surface area was ~ 400 m²/g. The MWCNTs diameter was ~ 50-60 nm, length — 3-7 μm, the wall thickness — 15-20 nm, the core space diameter — 10-20 nm, the specific surface area — 90-120 m²/g [4].

The intense radiation absorption followed by heating of single CNTs and its conglomerates occurs during the laser radiation of the water-albumin CNTs dispersion. Next the local boiling of water-albumin matrix happens around CNTs and its conglomerates that causes BSA denaturation. The dispersion level (size of CNTs and its conglomerates) affect the size of the developed inner pores and cavities. So, if the size of CNTs conglomerates in the initial solution is normalized then the inner structure of 3-D bioconstructions become more homogenous.
Table 1. Quantitative analysis of 3-D composites based on carbon nanotubes. Sample 1 based on SWCNTs; sample 2 based on MWCNTs.

| Parameters                          | Sample 1 | Sample 2 |
|-------------------------------------|----------|----------|
| Minimum pore diameter (μm)          | 13       | 40       |
| Maximum pore diameter (mm)          | 0,14     | 0,31     |
| The average pore size (μm)          | 45       | 93       |
| Porosity (%)                        | 16,44    | 28,31    |
| The percentage of open pores (%)    | 0,02     | 0,02     |
| The percentage of closed cells (%)  | 16,42    | 28,29    |

4. Conclusion

Comprehensive analysis of bulk composites by X-ray microtomography was carried out: scanning of 3-D composites, image reconstruction of the shadow projections, two-dimensional and three-dimensional visualization of the reconstructed images, quantitative analysis of the samples. It was found that 3-D composites based on SWCNTs and MWCNTs are homogeneous throughout the volume. 3-D composite based on MWCNT has greater porosity (28.31%) than 3-D composite based on SWCNTs (16.44%). The average pore size of the 3-D composites based on SWCNT and MWCNT - 45 μm and 93 μm, respectively. Such parameters: percentage porosity and average pore diameter provide cell proliferation and blood vessel sprouting. Therefore, 3-D composites can be used in tissue engineering of bone and cartilage. This work was provided by the Ministry of Education and Science of the Russian Federation (14.578.21.0221, RFMEFI57816X0221).

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