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A Study of the Biocompatibility of Carbon Nanotube-Based Nanocomposite Structures Implanted into Muscle Tissue

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A protein dispersion of carbon nanotubes and the nanocomposite structures obtained by laser irradiation of this dispersion were shown to be biocompatible and long-lived. The dispersion and the nanocomposite structures were implanted in laboratory birds, and the histologic pattern at the site of implantation was studied. It was shown that in 35 days after implantation a connective tissue capsule was formed, and tissue strands grew into the liquid and solid implanted materials. In 90 days after implantation, continuation of this process led to bioresorption of the materials, mainly as a solid structure. A normal local inflammatory response was observed. It manifested itself as an accumulation of white blood cells, mainly of the lymphoid series, and in the formation of muscle fibers at the implantation site. Gradual resorption of the nanomaterials was observed against the background of a chronic inflammation, in the absence of changes in the health status of the laboratory birds.

Introduction

The lack of donor organs, high labor intensity of implantation, and a high risk of implant rejection are the major problems of modern implantation surgery. A promising and safe solution is to develop and introduce artificial implantable materials providing fully functional substitution for damaged biological tissues. Such materials should have physical and chemical properties close to those of the substituted tissues. They should also be biocompatible and bioresorbable, so as to stimulate regeneration of biological tissue at the lesion site. Biodegradation rate is another important parameter of an implant. It should be comparable to the rate of tissue regeneration at the implantation site. The regeneration time of cardiovascular tissues can be rather long – up to 4 months or more – if internal walls of organs (for example, blood vessels or the heart) have to be regenerated.

Biomedical nanoengineering provides approaches for solving the problems listed above; namely, laser nanostructuring of carbon nanotubes in bio-organic matrices is used to form 3D cell- and tissue-engineering structures (nanocomposite structures). Nanotubes highly resemble components of the extracellular matrix and have been used since their discovery for developing new biomedical nanomaterials [1]. Laser nanostructuring allows composite nanomaterials based on nanotubes in biopolymer matrices (albumin, collagen, chitosan) to be obtained. Physical and chemical characteristics of such materials were studied in [2-4]. They exhibit good mechanical (hardness, ~ 300 MPa) and electrical (specific conductivity, ~ 1 S/m) properties. Nanocomposite structures were shown to have a positive effect on proliferation of cells distributed over their volume [3, 5].

In this work, we present the results of our studies of the biocompatibility and longevity of nanocomposite structures implanted into muscle tissue of laboratory birds. The histologic pattern at the site of implantation was also studied.

Experimental Studies

Two types of protein dispersion of carbon nanotubes and bulk nanocomposite samples were used in the exper-

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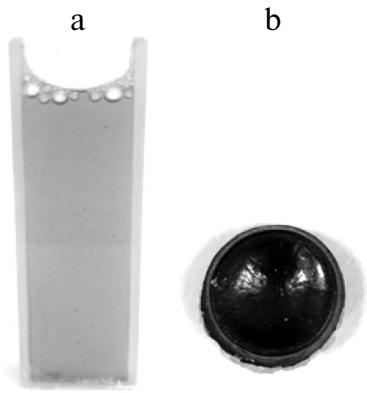

Fig. 1. Protein dispersion of carbon nanotubes (a) and nanocomposite structure (b): external view.

iments (Fig. 1). The concentration of single-walled carbon nanotubes was 0.01 wt%; albumin concentration, 25 wt%; the rest was deionized water. Nanocomposite structures were produced by laser solidification of the protein dispersion of carbon nanotubes using the technique described in [3]. The dispersion was exposed to a semiconductor laser radiation at 810 nm in continuous mode until solidification of the material.

Prior to implantation, the dispersion and the bulk samples were sterilized by ultraviolet light for 48 h. The biocompatibility studies were performed in laboratory birds (mature Moscow breed roosters). 0.5 mL protein dispersion of carbon nanotubes was introduced intramuscularly to the lateral shin previously treated with 70% ethanol. The birds were taken out of the experiment by groups of three on days 10, 14, 42, and 90. To implant the nanocomposite structures, 15-20-mm incisions up to 10 mm deep were made in the shin and chest muscles with a surgical scalpel. Structures 5 mm in size were implanted on both sides of the incisions. The surgical wounds were then closed with medical glue. The birds were taken out of the experiment on days 35 and 90.

Tissue was sampled from the implantation site for histological examination. Gelatin and paraffin slices were made and stained with hematoxylin and eosin. The health of the test birds did not deteriorate during the entire experiment. Their feeding and social behavior also did not change.

Results

Protein dispersion of carbon nanotubes. Ten days after introduction of the protein dispersion of carbon nanotubes, examination of histologic specimens revealed accumulation of nanotubes in the perimysium. Considerable blood cell infiltration was observed. The following blood cells were identified: red blood cells, acidophilic granulocytes (pseudoeosinophils and eosinophils), and agranulocytic cells in the specimen bulk and the surrounding connective tissue. Blood cells were observed both in the vessels and outside them, in the connective tissue. A large amount of white blood cells was accumulated in the connective tissue surrounding the specimen; a small amount of macrophages with phagocytized material in the cytoplasm was observed. The histologic pattern on the whole was typical of a late stage of acute inflammation (Fig. 2).

A well-pronounced capsule of connective tissue appeared two weeks after introduction. The concentration of the introduced material remained sufficiently high. White blood cells (mainly agranulocytic) started to consolidate into spherical structures distributed between accumulations of the introduced material. Macrophages englobing particles of the structure were observed in the connective tissue in and around the site.

After 42 days, the protein dispersion of carbon nanotubes was observed at the site of its introduction in the form of small separate conglomerates. Between them, white blood cells were distributed diffusely or in spherical accumulations. In addition, aggregates of precipitated red blood cells were identified outside blood vessels. Phagocytic white blood cells were rarer to observe. A connective tissue capsule surrounded the site where the dispersion was introduced. The observed pattern was typical of a chronic inflammation process.

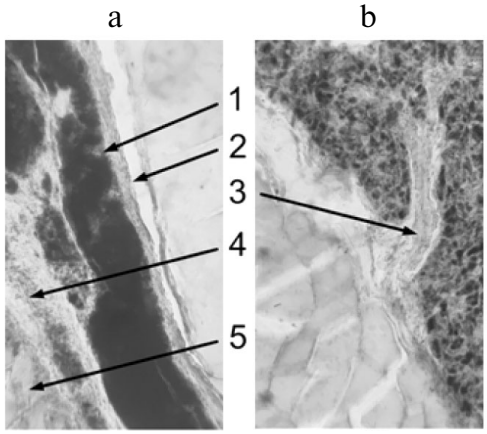

Fig. 2. Histologic pattern at the site of implantation of the protein dispersion of carbon nanotubes: a) on day 10, magnification ×300; b) day 90, magnification ×600 (1 – dispersion particles; 2 – connective tissue capsule; 3 – connective tissue strand; 4 – blood cells; 5 – muscle fiber).

Clumps of agglutinated red blood cells were observed at the end of the experiment. Thick strands of loose connective tissue started to grow actively from the connective tissue capsule into the introduced material (Fig. 3). Phagocytic cells were rare. Spherical structures composed of agranulocytic white blood cells and mononuclear macrophages were indicative of the inflammatory process transition into a chronic stage. It can be described as granulomatous inflammation with formation of non-infectious granulomas. According to the literature, such inflammation may eventuate in resorption of the granulomas or, in the case of overdevelopment of the connective tissue capsule, in sclerotization of the site [6, 7].

Bulk nanocomposite structures. In gelatin- and paraffin-fixed histologic specimens, the nanocomposite structure was identified as separate black accumulations. On day 35, histological examination of biological tissues of the shin revealed a ~40-50% decrease in the size of the introduced sample. The initial structure was divided into fragments surrounded and infiltrated with cells of various types, mainly agranulocytic white blood cells and other macrophages (Fig. 3a).

Growth of connective tissue and incapsulation of the nanomaterial was observed in tissues surrounding the implantation site. Fiber bundles and amorphous intercellular substance with cells typical of connective tissue (fibroblasts) were well seen in the specimens. Intensive vascularization of the connective tissue of the capsule was observed. Fibers (strands) of connective tissue began to be observed in the accumulations of white blood cells at the implantation site. Changes in individual muscle fibers of the surrounding muscle tissues can be seen in Fig. 3a. Such changes typically occur after implantation. They consist in transformation and substitution of sarcoplasm of some muscle fibers with connective tissue (in some cases, with packed white blood cells). There was also an increase in the white blood cell content in the endomysium (connective tissue layers separating individual muscle fibers). Among the white blood cells observed at the implantation site, phages that englobed particles of the structure were identified.

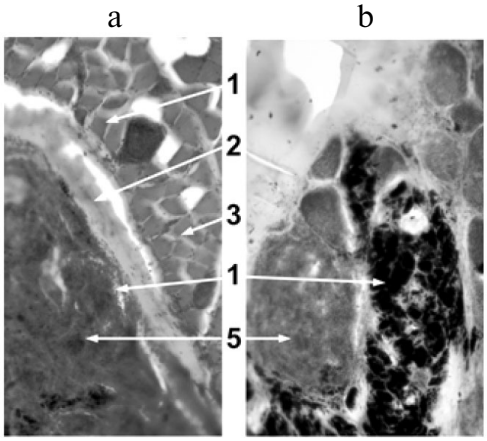

Fig. 3. Histologic pattern at the site of implantation of nanocomposite structures into laboratory bird shins on days 35 (a) and 90 (b). 1 – Small particles of the nanocomposite structure; 2 – connective tissue capsule; 3 – muscle fibers; 4 – transformed muscle fibers; 5 – accumulations of agranulocytes. Magnification ×100.

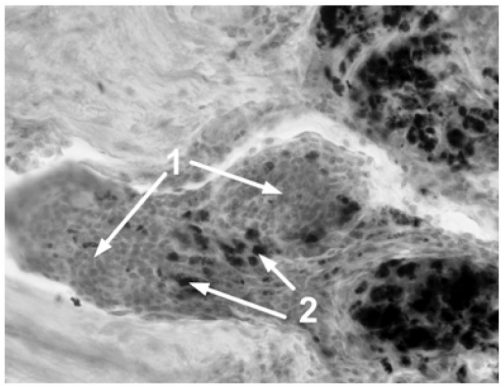

Fig. 4. White blood cells with phagocytized particles of the nanocomposite structure in the cytoplasm observed on day 90 at magnification ×400 (1 – accumulations of agranulocytes; 2 – white blood cells with phagocytized particles in the cytoplasm).

On day 90, a considerable reduction of the nanocomposite structure volume was observed. The connective tissue capsule was present around the implantation site. The number of connective tissue interlayers at the implantation site was considerably greater that in the specimens taken earlier; they were also much thicker (Fig. 3b). White blood cells with phagocytized particles in the cytoplasm were observed between the fragments of the structure (Fig. 4). The implant volume was found to decrease gradually during the three months due, probably, to its englobing by white blood cells and other macrophages. There was a gradual decrease in infiltration with lymphoid tissue. However, white blood cells consolidated into large spherical structures resembling lymphatic follicles.

The observed histologic pattern – prevalence of agranulocytic cells (lymphocytes and monocytes) and growth of connective tissue – is typical of the stage of chronic inflammation usually observed within a comparable time period after implantation of large and dense objects. If a foreign object remains implanted in the body, initial acute inflammation is followed by chronic inflam-

mation. Taking into account that the composite object implanted in the muscle is rather large and takes considerable time to resorb, it is probably inevitable that chronic inflammation should accompany the process of muscle tissue regeneration.

Conclusions

The study showed that the volume of implanted protein dispersion of carbon nanotubes decreased with time until only small fragments were left. A decrease in the size of implanted solid nanocomposite structures was also observed. There was a local inflammatory response at the site of implantation of the nanocomposite structures. A capsule of connective tissue was formed around the implantation site with further ingrowth of connective tissue strands into the site. Local chronic inflammation at the implantation site did not affect the health status of the laboratory birds. Thus, it was shown that nanocomposite structures facilitate muscle tissue bioresorption and can remain in the body for more than 90 days. Therefore, such structures can be used as cellular matrices for implementation of long-term therapy. In particular, they can be used in treating such cardiovascular diseases as myocardium infarction and aneurysm, as well as other disorders of biological tissue architectonics.

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