Conference paper

Structural Features of TiNi-based Textile Materials and Their Biocompatibility with Cell Culture

V.N. Khodorenko[1*], S.G. Anikeev[1], O.V. Kokorev[1], M.R. Mukhamedov[2], E.B. Topolnitskiy[3], V.E. Gunther[1]

*Corresponding author:
V.N. Khodorenko,
email:
Hodor_val@mail.ru

[1] Tomsk State University, Research Institute of Medical Materials and Implants with Shape Memory, Tomsk, Russia

[2] Oncology Research Institute of Tomsk Scientific Research and Marketing Centre, Tomsk, Russia

[3] Siberian State Medical University, Tomsk, Russia

Received: 23 March 2017
Accepted: 9 April 2017
Published: 16 July 2017

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the SMBIM Conference Committee.

OPEN ACCESS

Abstract

This study investigates the structural features of TiNi-based textile materials. It is established that woven materials have a regular cellular structure, while knitted mesh materials are characterized by presence of cells with various sizes both in the longitudinal and cross directions. The surface oxide layer of threads has a microporous structure that provides the improved adaptation in organism tissues. It is shown that the fibroblast colonization rate of the knitted mesh implants depends on the cell size and quantity of mesh knots. Smaller cells are quicker colonized. Mesh knots are the centers of the cell cling. The feature of cell interaction with the tread surface of various sizes is analyzed.

1 Introduction

From a wide range of implant designs it should be distinguished textile implants by means of which complex surgical problems are solved. Properties of the textile implants are mainly determined by characteristics of chosen material and their manufacture technology [1-8]. Woven, knitted, crocheted, felted and other kinds of modern mesh materials are got using different methods. The woven materials are characterized by a regular structure with uniform properties in all directions. They are made of two thread systems perpendicular to each other by mutual interweaving. The knitted, crocheted materials have a high deformation and elastic characteristics that are determined by a special connection of thread loops. Various

types of textile materials are differ by executable functions and behavior in the organism tissues, the level of biochemical and biomechanical compatibility.

Currently in a medical practice the textile implants from synthetic materials (teflon, polycaproamide, polypropylene) and inorganic materials (platinum, gold, nichrom) are widely used. However, the use of such materials often leads to development of a significant reaction from the surrounding tissues, followed by complex incompatibility «implant – organism tissue». In most cases an application of polymeric materials reduces to their destruction and fragmentation. Despite the high strength properties the textile materials from metal threads have a number of disadvantages associated with their behavior in condition of dynamic loads. Based on it a search of new perspective materials answerring to higher medical and biological requirements is an urgent task.

The research institute of medical materials and implants with shape memory (Tomsk) developed TiNi-based alloys that correspond to all requirements for their application in medicine. Experience in the use of a porous permeable TiNi as cell culture incubators showed its high biocompatibility with different cell types [1, 9, 10]. The manufacture technology was developed of thin TiNi threads with a diameter of 30-150 mμ, which is the basis of mesh designs of various types. Application of mesh and woven implants from TiNi-based threads in medical practice requires a detailed investigation of their structural features, determination of the optimal dimensional characteristics and properties that are necessary for active integration processes into organism tissues. In this regard the objective of the present research is the research of structural features of woven and mesh implants and their effect on development of cell cultures (fibroblast).

2 Materials and methods

Textile materials were made from TiNiMoFe threads. The threads with diameters of 90 μm, 60 μm and 40 μm were obtained by means of multiple wire-drawing of the NiTi alloy. Such threads create an optimal influence on tissues, showing the following properties:

– thread rigidity has low and uniform value in all directions of alternating deformation;

– thread bending is realized without material fatigue, i.e. effect of the thread deformation cycloresistance is developed;

– suture tension decreases without affecting tissue elasticity;

– visualization of the suture state can be monitored by X-ray control.

The woven and knitted mesh implants were made from superelasticity threads on knitting and weaving machines (Rishikesh): woven implant (thread diameter d=60 μm), knitted mesh implants of various modifications (thread diameters d=90 μm, d=60 μm, d=40 μm).

The macro- and microstructures of the materials were investigated with the scanning electron microscopy PHILIPS SEM 515 and the energy-dispersive X-ray spectroscopy was carried out on the microprobe EDAX ECON IV. The study of effect of implant structure on cell cultures was carried out using 3T3 fibroblasts. Before testing the implants were sterilized at T = 180 °C for 60 minutes in a dry room. The obtained samples were examined under a Quanta 200 3D scanning electron microscope. The fibroblasts were enzymatically harvested from culture vials and reinoculate in Petri dishes with implant specimens (10x10 mm). The cell suspension was brought to a concentration of 4.106/ml with complete medium and inoculated on TiNi implants in 24-well plastic plates (Nunc). The cultivation was carried out in medium DMEM / F-12 ("PanEco", Russia) with 10% FBS («HyClone», USA), 40 μg/ml gentamicin ("PanEco", Russia), 250mg/l glutamine ("PanEco "the Russian Federation). The cells were incubated at 37 °C, 100% humidity with 5% CO_2. Lifetime microscopy of fibroblasts with implant specimens in culture plates (Nunc) was carried out under an Optica XDS-2 inverted phase contrast microscope. Specimens were fixed in 2.5% glutaraldehyde solution (SIGMA) for 1 hour, washed 3 times in PBS (15x3 min) and fixed for 1 hour in 1% osmium tetroxide (SIGMA). Thereafter they were again washed 3 times in PBS and dehydrated through a series of ethanol solutions (30%, 50%, 70%, 90%, 100%; 15 min each). The dehydrated samples were dried and examined with the scanning electron microscope 200 Quanta 3D.

3 Results and discussion

The properties of woven and knitted mesh implants are determined by the TiNi material type (TN-10, TN-20, TN-CE) and thread diameters. Microstructure studies of surface of TiNi fine threads showed that they are a composite material consisting of a metal matrix (TiNi alloy) and porous permeable sheath (Fig. 1, a). The surface layer of TiNi-based threads has a microporous structure (Fig. 1, b). The large number of micropores on the surface increases a cell adhesion and lead to a high wettability.

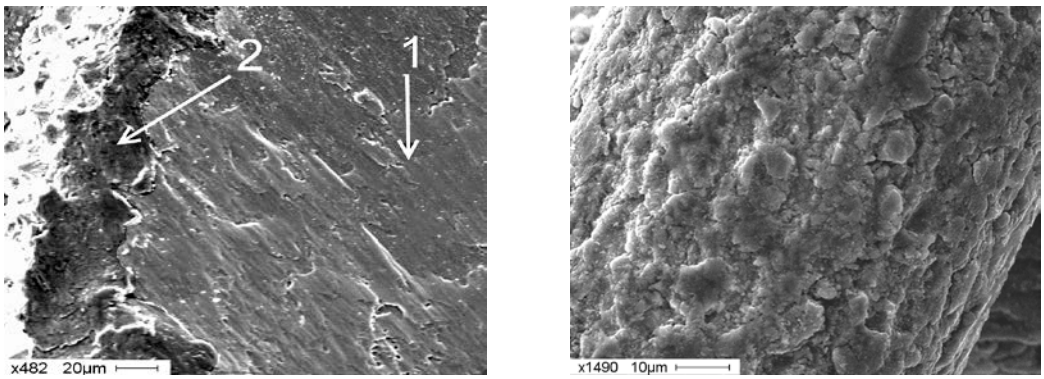

Fig. 1. The surface structure of TiNi-based thread: a – cross-section of the thread: 1 – metal matrix TiNi, 2 – oxide layer; b – the microporous surface of the thread

A principal constituent of wire metal matrix is an intermetallic compound TiNi in the two-phase state B2 and B19'. It was found a large number of Ti-rich Ti2Ni and Ni-rich TiNi3 phases with different sizes, which are arranged non-uniformly. Ti2Ni are incoherent particles with large sizes and various geometric shapes. TiNi3 are coherent, fine, they usually have circular and plate shape, rarely in the mesh form.

The oxide layer provides special biointegration properties of TiNi-based implants and it is formed during the drawing at repeated intermediate annealing. It is known that Ti segregates on free TiNi surfaces under heat treatment therefore the oxide layer is formed [1]. It is established that the oxide layer size decreases with decreasing of wire diameter. Thickness of layer is 15-25 mμ for specimens with a diameter of 1 mm, the layer thickness is 2-3 mμ for 60 mμ threads.

The energy-dispersive spectra analysis of wire material surfaces showed that the oxygen fraction is decreased in the surface layer with reduction of a wire diameter. It is connected with manufacture technology features of wire materials with different sizes. The oxide layer thinning occurs during the drawing of threads with smaller diameters and the intermediate annealing is carried out at lower temperatures, which naturally reduces the titanium activity to oxygen.

The oxide layer of material has a longitudinal - textured structure. Its surface is characterized by cellular microporous structure (Fig. 1, b). A rough structure of surface is clearly shown on threads with a diameter of 90 μm (Fig. 2, a). Decreasing of thread diameter leads to morphology changing of a surface layer and surface smoothing (Fig. 2, b, c). This structure is the result of multiple significant deformation and intermediate heat treatments at the wire drawing.

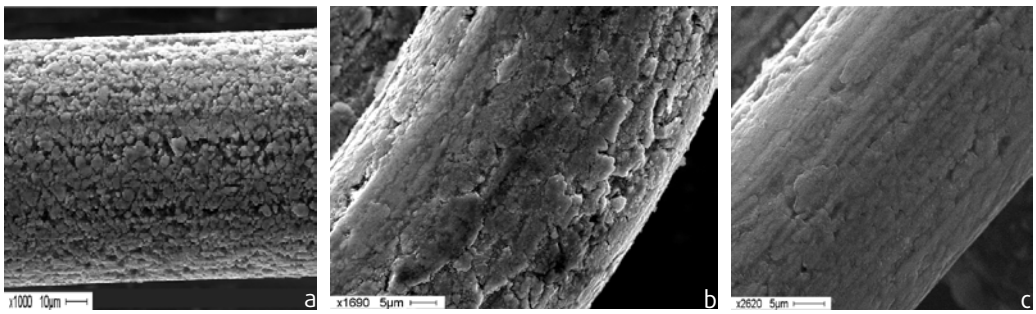

Fig. 2. Macrostructure of TiNi thread surface with diameters: a – 90 μm, b – 60 μm, c – 40 μm

The textile technology is used for production of knitted mesh and woven implants with important physical characteristics and properties which enable their medical application:

- a high biomechanical compatibility with body tissues. The optimal textile implant is similar to living tissue: it has a high elastic properties, preset hysteresis on the deformation diagram in conditions of loading-unloading;
- like tissues, the ability of elastic deformation in all directions;
- superelasticity effect consisting in shape return under the unloading;
- deformationcyclostability effect characterizing an ability of a material to retain initial properties after alternating deformation;
- a high elasticity and strength determining an ability of a material to be subjected to deformation in a preset range without destruction;
- wear resistance that are characterized the shape and size retention under long functioning;

- reliable sterilization of the implants by the available standard methods; - the implants should retain their characteristics upon long storage.

Woven implants are characterized by a high degree of structure regularity unlike other. Cell sizes in the longitudinal and cross directions are the same and make 200 μm. Woven material has a slight and identical stretching in various directions. Woven material is not rumple and keeps an initial shape. Due to the microporous surface the threads hold each other and the implant is not unraveled in the rags (Fig. 3, a).

Structural features of mesh implants considerably depend on thread properties. As previously shown, the oxide film morphology changes with decreasing of thread diameter. It is established that the implant from 90-μm wire has loops of regular structure with accurate contours. The loop sizes in the longitudinal and cross

directions make 750-1000 μm. The mesh structure of knitted implant from thread with a diameter of 40 μm is extremely non-uniform and it is characterized by a wide dimensional interval: in the longitudinal direction to 2500 μm, in the cross direction to 1500 μm.

The knitted mesh implants on a basis TiNi thread with a diameter of 60 μm have an optimum ratio of structural and functional properties (Fig. 3, b). The homogeneous, pronounced loopy structure is obtained from these threads in all the material volume. Loops have elongated shape to tops with longitudinal sizes up to 1500 μm and in a cross section to 1000 μm. Knitted material on the basis of thread with a diameter of 60 μm is the most perspective mesh implant due to high structural and morphological properties.

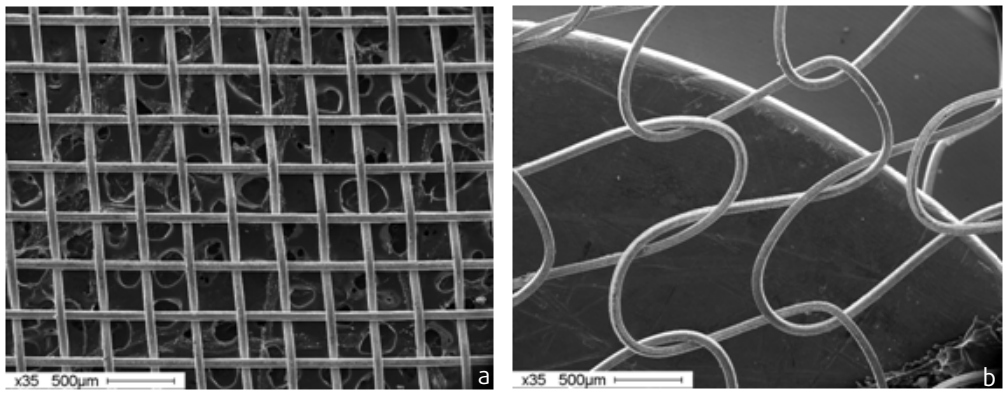

Fig. 3. TiNi-based textile materials: a – wove implant from thread with a diameter of 60 μm, b – knitted mesh implant from thread with a diameter of 60 μm

Researches of fibroblast cell interaction with the knitted mesh implants showed that a rough, porous and permeable hydrophilic surface of TiNi threads favors to fibroblast adhesion. Such surface structure promotes to rapid integration of the knitted mesh material into organism tissues, unlike hydrophobic polymeric materials.

The most rapid cell colonization was observed in implant with woven structure (thread diameter 60 μm). The cell size of this implant is 2.5 times smaller in comparison with other mesh specimens, consequently it has a larger effective adhesive area. Cells cling to the microporous surface of thread, often in mesh knots. The growth tissue vector was directed from the periphery to the cell center

(Fig. 4); the colonization rate in free space of cell is high. The colonized cell/total specimen area coefficient on day 17 was maximal in these specimens (45%).

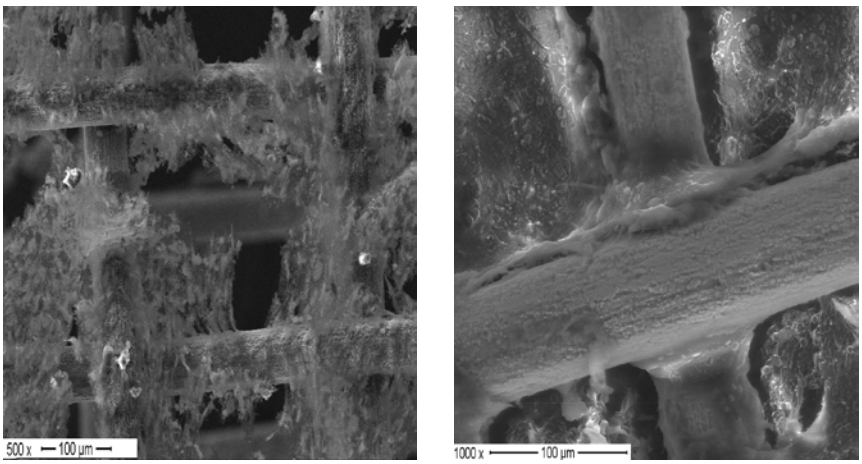

Fig. 4. Colonization of woven implant from 60 µm threads by fibroblast culture (day 17)

The knitted mesh implants from 60-µm threads by fibroblasts showed slower colonization in contrast with woven implant because of irregular cell distribution with large sizes. The colonization started from the mesh knots and the growth vector was directed from the cell periphery to its center (Fig. 5). A complete colonization of the mesh implant was much slower than the fabric implants.

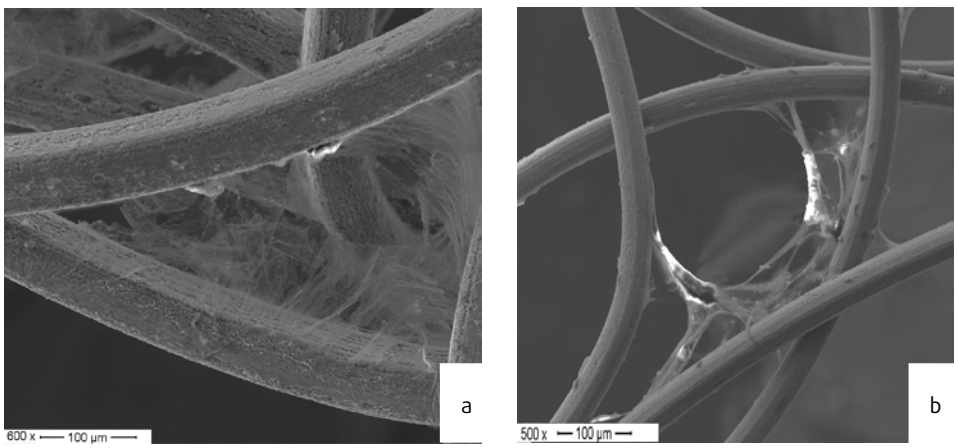

Fig. 5. Colonization of knitted mesh implant by fibroblast culture (day 17): a – from 90 µm threads, b – from 60 µm threads

The colonization rate of knitted mesh implant from 40-µm thread with fibroblasts was lower compared to the implants from 60-µm and 90-µm threads. The nature of fibroblast development had its own features. Fibroblast grew and tissue developed mainly along thread (Fig. 6). Fibroblasts first colonized the thread surface forming a monolayer, after they colonized free space of cells. Such feature

of fibroblast development is caused with a microstructure of threads. Probably, the decrease in thread thickness leads to adequate cell interaction with the material and exhibition of their "smart" potential to modifying the space structure with their size.

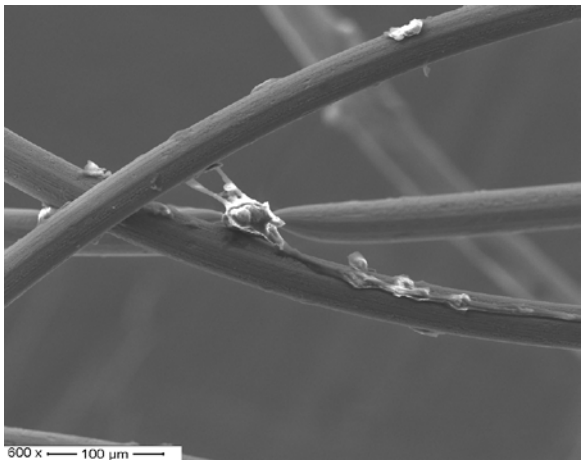

Fig. 6. The fibroblasts growth on surface of knitted mesh implant from 40 µm threads (day 17)

4 Summary

Thus, fibroblast cell interactions with the woven and knitted mesh implants from the same type of TiNi alloy are determined by thread microstructure and the size factor of cell implant. The porous-permeable cellular structure of the oxide layer promotes to cell adhesion and their evolution on the surface. The rate of knitted mesh implant colonization by the main connective tissue cells (fibroblasts) depends on the cell size and the number of knots. It is shown that the fibroblasts more rapidly colonized smaller cells, and mesh knots ensured better cell adhesion. The tissue grows from the thread to the cell center. The thread size affects on the fibroblasts colonization character. An interaction feature of fibroblasts with the surface of ultrathin filaments (40 µm) was revealed. The fibroblasts growth and the tissue development in the implants from ultrathin threads occur mainly along the threads, followed by colonization of cell space.

5 Acknowledgments

This study was supported by Government Assignment No HY 3.6492.2017/BY

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