Conference paper

Features of Cells Integration on TiNi-based Porous Scaffold

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Abstract

Developing of standard population of cells — line fibroblasts 3T3 in porous structure of incubator having fixed composition, porousness and pore size distribution has been studied. The volume of scaffold space was shown to determine the potential cell division and its function. The characteristic integration of fibroblasts in porous incubators from TiNi-based alloy having different volumes was analyzed. The relation of colonization by cells and growth of tissues in porous samples from titanium nickel depending on depth of penetration is determined.

1 Introduction

The main goal of tissue engineering is to provide anatomical and functional substitutes for tissues, three-dimensional structure of which corresponds to damaged organs. A necessary component for implementation of this strategy become scaffolds for cells - the three-dimensional structure of biomaterials, which mimic the natural extracellular environment of tissue [1].

The physiological purpose extracellular matrix - the establishment of structural support (providing adhesion and cell integration), vascularization and remodeling capacity of regenerating tissue, optimal conditions for metabolism and cell differentiation. This matrix must match the biomechanical properties of the tissue in which the implant design (elasticity, strength), as the main of this - biomechanical skeleton for cells [2]. Matrix must have number of options that could help createa better environment for donor cells. Such properties are: optimum pore size for the spatial distribution of cells, vascularisation, nutrient

diffusion and removal of waste products; the presence of the adhesive, tissuegenic surface promoting cell proliferation and differentiation; the absence of immunological rejection and others. [3]. The structure of matrix must perform the elimination of metabolic products, active transport and delivery of nutrients, oxygen, growth factors and for preventing ischemic damage and cell necrosis. The fabrication technologiesfor tissue engineering scaffoldshould allow to produce the appropriate shape and structure design [4].

Scaffolds must have certain requirementsfor biochemical as well as chemical and physical properties: surface for initial cell-surface attachment, high degree of porosity, interconnectivity, adequate pore size and pore size distribution for cellsto attach, migrate and proliferate [5-7]. Tissue engineering creates large-scale biological tissuesrequires appropriate porous implant cells saturation technique. Scaffolds must be permeable with interconnecting pores to facilitate cell growth and cell free penetration of aqueous suspensions. Accordingly, the problem delivery of oxygen and nutrients in tissue-engineering structures and cellular metabolites output is important task. In addition porous materials permeable pores are necessary for blood vessel ingrowth. Currently no defined optimal parameters for scaffolds: volume, porosity, pore size distribution, their interconnectedness and configuration (scaffolds) necessary for development of different tissues [8-10]. One of the key stages of the creation of the necessary tissue-equivalent is the selection of optimal porous scaffold for transplanted cells.

Among large number of biomaterials porous TiNi-based alloys are obtained by self-propagating high-temperature synthesis (SHS) meet the required parameters [11, 12]. This advanced technique enables control over porosity and pore size in the porous TiNi-based SMA. This material has unique properties: permeable porous structure by open interconnected pores, characterized by high degree of wettability with tissue media and nanoporous inner surface of the pore walls, exhibits high adhesion to various cell types, so all mentioned meet requirements of the biochemical and biomechanical compatibility. The use porous-permeable implants from TiNi-based aloy in practical surgery necessitates detailed studies of structural characteristics of the implants, evaluation of the optimal size and properties essential for active integration into body tissues after implantation. Experience gained with the use of porous permeable TiNi as cell culture scaffolds have demonstrated high biocompatibility of this material with various cells [11-13]. Porous TiNi-based scaffolds allow continually to keep the functionality cells and

prolong their action [14, 15]. In this regard, this study to look into determine the optimum size characteristics of the porous incubator of TiNi-based alloy necessary for best development of 3T3 cells. 3T3-fibroblasts have mesenchymal stem cell properties (adhesion, the rapid growth in culture), but purity lines 3T3 allow to obtain uniform and reliable results than multipotentmesenchymal cells.

2 Experimental

Porous TiNi-based SMA

A porous TiNi-based SMA was fabricated using a self-propagating high-temperature synthesis technique, at the Research Institute of Medical Materials. The pore size in the porous TiNi-based SMA was controlled by adjusting the fabrication conditions. Scaffold blocks 0,4x10x10mm and 1x10x10mm with permeable porosity of 70% of porous TiNi-based SMA were prepared by electric-discharge (ED) wire-cut. An analysis of the pore structure of the scaffold was performed using Hg-porosimetry and the Quanta 200 3D scanning electron microscope (SEM). Prior to soaking into culture medium, the scaffolds were degreased with 70% ethanol, washed in an ultrasonic bath, and autoclaved at 180° C for 1 h.

Biocompatibility scaffolds tested.

Biocompatibility of porous scaffolds was tested on fibroblast 3T3 line. The fibroblasts were enzymatically harvested from culture vials and reinoculated in Petri dishes with implant specimens (10x10 mm). The cell suspension was brought to a concentration of $0{,}25x10^6$ cells/ml with complete medium and inoculated on TiNi samples in 12-well plastic plates (Nunc). Culturing was carried out in DMEM/F-12 (PanEco) with 10% fetal calf serum (HyClone), 40 μg/ml gentamicin (PanEco), and 250 mg/liter glutamine (PanEco). The cells were incubated at 37°C and 100% humidity with 5% CO_2 at days 9. The medium was replenished twice a week.

SEM imaging

SEM imaging was performed, with the preimplanted scaffold at day-0 and harvested scaffolds respectively at day 7, 14, 21 and 28 post-implantation. Th e scaffolds were washed with phosphate-buffered saline (PBS) and fixed with 2.5% glutaraldehyde solution for an hour, after which the scaffolds were triply flushed with PBS for 15 min and fixed in 1% osmium tetroxide solution (SIGMA) for an hour, followed by triple flushes with PBS for 15 min. Finally, after dehydration by a serial

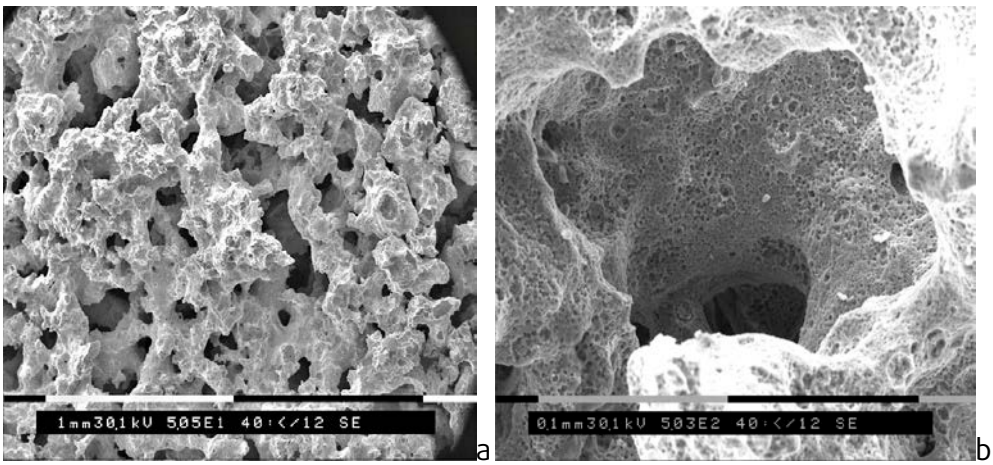
change of ethanol concentration (30, 50, 70, 90 and 100% strength) for 15 min in each solution, each sample was dried. SEM imaging was performed in a Quanta 200 3D SEM (FEI Co. Japan), under a 30 kV operating voltage.

Statistical analysis

The results are expressed as mean ± standard deviation, with $n \pm 10$. Error bars in figures represent standard deviations. Differences between experimental groups were analyzed according to a paired nonparametric Mann-Whitney's U-test, with $p \leq 0.05$ considered statistically significant.

3 Results and discussion

The structure of the porous TiNi-based SMA scaffold The SEM examination indicated that the porous TiNi-based SMA samples has interconnected pores (Fig. 1, a). Due to the open pores (90%) and the hydrophilic surface of the material has high permeability and rough pore walls with a nanoporous surface (Figure 1b). The samples surface was smooth, with the exception of the cut edge. Actual pore size was determined by measuring the maximum and minimum diameters of a pore on the SEM photos of the surface in several areas of each implant.



a b

Fig. 1. The structure of the porous permeable samples of TiNi: a - macrostructure sample; b - the microstructure of pore walls

The measured mean pore sizes of the structure of porous TiNi-based SMA were 150 ± 89 μm (n = 15) and pore sizes in the range of 10^{-1} - 10^{3} microns. The mean porosity (evaluated by Hg-porosimetry) was 70,3 ± 6.7% (n = 37).

In comparative studies of thin (0.4 mm) and thickened (1 mm) porous samples showed that the growth of fibroblasts in thin porous samples during cultivation

period is more active. This is due to increase in initial cell concentration in thin sample. It was noted that the large pores in thin samples overgrown slower than the thicker samples. This indicates failure of fibroblasts in short time to envelop large open pores of thin sample or inability to create the necessary concentration of growth factors in large open pores. In comparison with thicker samples where space in such pores closed and limited, there is possibilityto create local growth factors required concentration (Fig. 2).

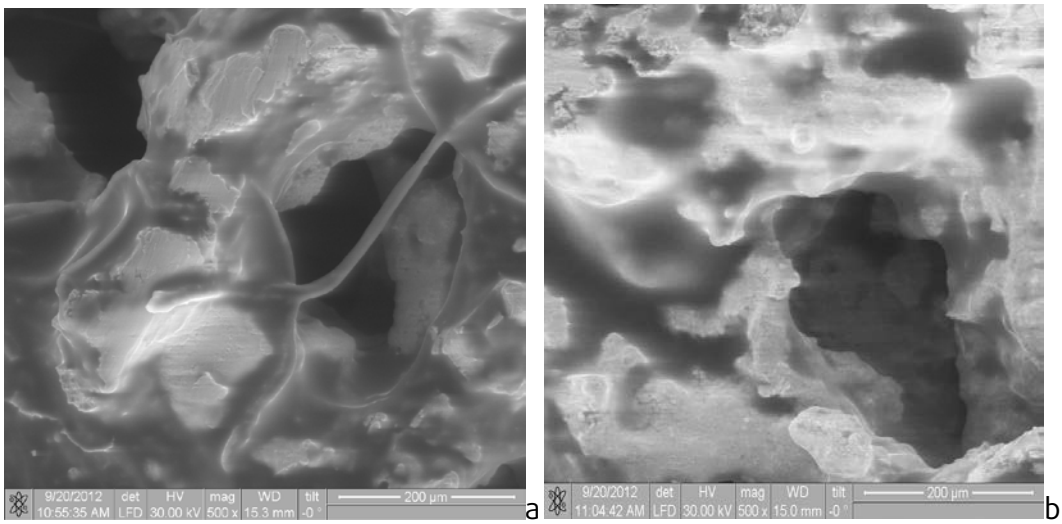


Fig. 2. The growth of fibroblast cells: a - thin samples (0.4 mm), b - thickened samples (1 mm), (at day 9 post-incubation)

Figure 3a shows the development of fibroblast tissue structure at depth of 1 mm from surface on the 9th day after inoculation of cell suspension per sample. As the depth of penetration into the porous structure of the incubator as marked reduction in the growth rate of fibroblasts and extracellular matrix and reduce the amount of cell mass. At depth of 2 mm can be seen final stages of newly formed tissue layer (Fig.3b).

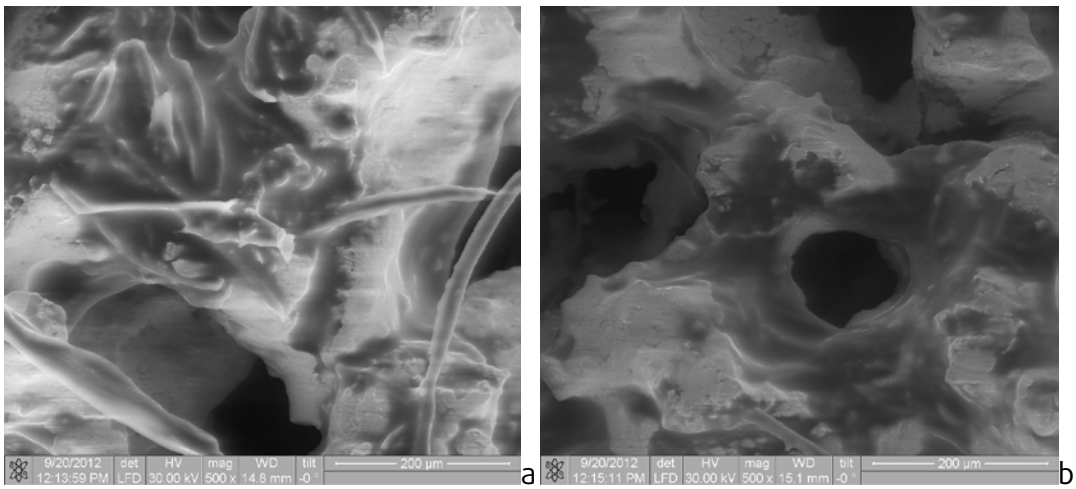


Fig. 3. Fibroblast tissue structure at depth of 1 mm (a), final stages formation of cells monolayer at depth of 2 mm (b) (at day 9 post-incubation)

At depth of 3 mm from sample surface was observed isolated clusters of cells (Fig. 4, a), at depth 4 mm from surface – only single adherent cells (Fig. 4, b.).

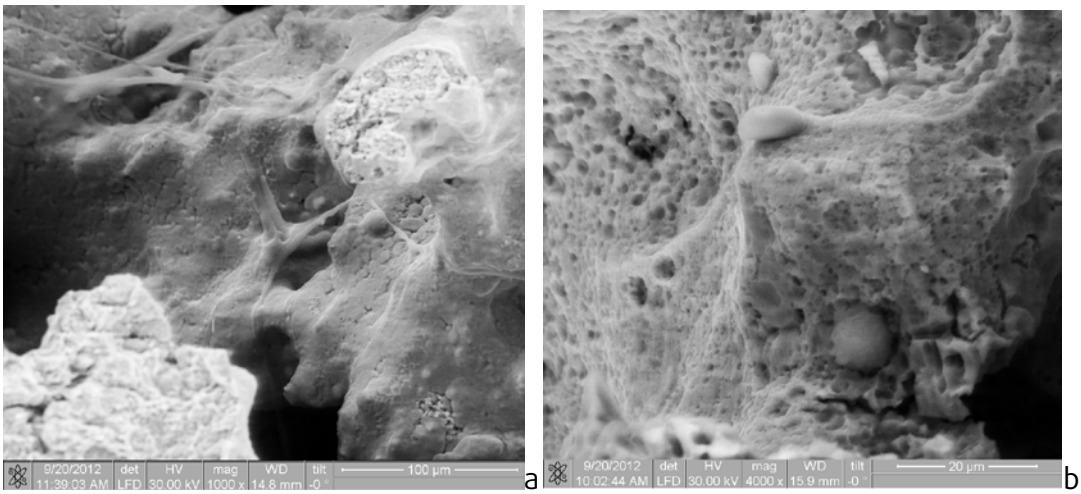


Fig. 4. Populations of cells at depth of 3 mm (a), single fibroblasts at depth of 4 mm (b) (at day 9 post-incubation)

It must be emphasized, that it is technically difficult task is to adjust distribution of cells around pore space of incubator. The distribution of cellular suspension is uneven in porous scaffold: large amount of cells is deposited on upper surface of sample under action of gravity. Accordingly, most intense tissue growth process will be in the upper surface layer of scaffold. Thus, highest cell concentration is at depth of 1 or 2 mm from top surface and corresponding development of tissues is enhanced. Deeper than 2 mm from surface ofinitialcell-seedingdensity of fibroblasts decreases and development of tissue structures is very slowly.

When using small tissue-engineering constructs cell density ratio in volume is increased and consequently increase initialization growth process is much higher. However, large penetrate pores of thin samples are populated with fibroblasts slower than closed large pores in thick samples. Thin porous materials may find use for emergency replacement of differentiated tissues, as large-scale structures may be used in pathological conditions that require prolonged action. It was established that during the spontaneous saturation of porous TiNi-based alloy scaffold cell size should be limited by 3 mm from surface of scaffold. Using scaffold structures having large dimensions, therapeutically ineffective, as it leads to prolonged colonization of internal pore space cells or these pores of cellular scaffold enables colonize nonspecific cells of host organism.

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