Conference paper

Evaluation of Porous TiNi-based Alloy as a Scaffold for Liver Tissue Engineering

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Abstract

Complex analysis of development of cells of liver – hepatocytes in the biocompatible scaffold, fabricated of porous-permeable TiNi-based alloy has been performed. Specific stages of development of cellular population in pores of scaffold are displayed in the present investigation. The stepwise characteristic of developing of hepatocytes in porous-permeable structure from TiNi-based alloy is demonstrated. Nanoporous surface of pores in incubator permit cells to be reliably fixed and produce growth to cellular colonies, and porous-permeable structure of scaffold allows penetrating to nutrients from environment during all time of investigation in vivo. Examination on CCl_4-induced hepatitis has displayed effective antitoxic activity of population of hepatocytes in scaffold from TiNi-based alloy and their prolonged effect.

1 Introduction

New discoveries in cellular biology and regeneration of organs was basis for development of new treatments for liver diseases. Steel is actively developing new biotechnological methods, the effect of which is aimed at filling the outside of the damaged liver tissue, as well as to stimulate the regeneration of remaining liver tissue [1]. Currently, the scope of liver diseases treatable cell transplantation is constantly expanding but acquired disorders (cirrhosis and hepatitis), genetic and hematopoietic system disorders, metabolic deficit conditions [2, 3].

Now most of researchers are unanimous about the lack of effectiveness of use transplantation suspension of donor cells in organism. The transplantation of isolated cell does not provide stable therapeutic effect, characterized by low levels of cells engraftment and eliminates the possibility of long-term exposure to the introduced cells regeneration process. It is believed that main reason for low efficiency of suspension of liver cells is the lack of conditions for allogeneic organism attachment and contact interaction of cells with the formation of structural and functional units of organ [4-7].

In this regard, one of the directions of regenerative cellular therapy was the use of isolated liver cells in tissue-engineering intracorporeal structures [8-10]. Many works are devoted to the cultivation of various cells in 3D matrices - scaffold (currently there are made commercial products) [11]. They differ in their composition, structure, surface topography, time degradation etc. The main problem in the application of such technology - the choice of adequate biological material to replace the extracellular matrix in hepatocytes. All this gives many criteria and parameters for structure biomaterials and methods for culturing in comparison with the existing methodology previously isolated cells grown in monolayer [12]. The most promising for use as biomaterial for incubator of liver cells cultures are porous TiNi-based alloy. Created by the Research Institute of Medical Materials and Implants with Shape Memory (Tomsk) porous permeable cellular scaffolds TiNi-based alloy have unique properties: have open porous-permeable structure, have high degree of wettability by tissues fluids, comply with the biomechanical and biochemical compatibility with tissues of organism [13-15].

This study aims to look into the applicability of porous TiNi-based alloy (SMA) scaffold as an incubator for hepatocytes in vivo and impact this scaffold on organism at experimental hepatitis.

2 Experimental

Porous TiNi-based SMA

A porous TiNi-based SMA was fabricated using a self-propagating high-temperature synthesis (SHS) 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 (4×4×20 mm) of porous TiNi-based SMA were prepared by electric-discharge (ED) wire-cut. An analysis of the pore

structure of the scaff old 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.

Laboratory animals

All procedures on animals were carefully carried out, with strict adherence to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986), and with the European Communities Council Directive 86/609/EEC. CBA/j inbred Wistar rats were used (10-week-old, males).

The hepatocytes growth in scaffolds (in vivo)

Hepatocytes were isolated from livers of Wistar rats by modified Seglen's method (Shen et al. 2012). Isolated hepatocytes were seeded at final concentration of 15 x 10^6 cells/ml in porous TiNi-based SMA scaffold, and cultured at 37° C in a 100% humidified atmosphere of 5% CO_2 for 24 h. Then, the scaffolds were implanted into the abdominal cavity of Wistar rats under ketamine anesthesia (1mg/10g dose). The implanted scaffolds were harvested respectively at days 7, 14, 21 and 28 post-implantation.

Rats within 10 days were administered CCl_4intragastrically at dose of 50 mg / kg in oil solution.

The animals were distributed 5 groups:

- Group 1 "Control"—intact rats;
- Group 2 "CCl_4"— animals with toxic hepatitis induced by administration of CCl_4;
- Group 3 "TiNi"— animals with introduction of CCl_4 and scaffold from TiNi-based alloy implanted without cells;
- Group 4 "Hep"— animals with CCl_4 administration and injection of hepatocytes;
- Group 5 "Hep + TiNi"— animals with introduction of CCl_4 and implanted scaffold from TiNi-based alloy with hepatocytes.

Biochemical methods of assessment.

In serum of animals was measured dynamics liver transaminases: ALT, AST, and alkaline phosphatase using veterinary biochemical analyzer "RFE-90 WET» during the 45 days .

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. The 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.

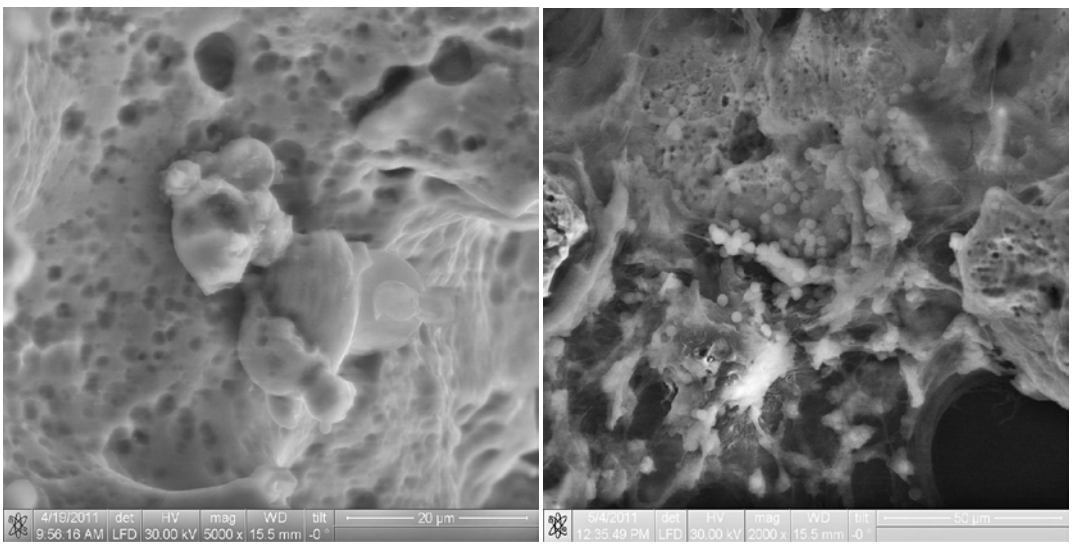
3 Results and discussion

The structure of the porous-permeable cellular incubator of TiNi-based alloy is three-dimensional pore space, morphological structure which is typical of highly porous materials with liquid phase. The porous material has large specific surface due to the presence in it a system of open and interconnected pores. Due to the open pores (90%) and the hydrophilic surface of the material has a high degree of permeability, due to method of producing a porous material - self-propagating high-temperature synthesis (SHS) [13]. The samples had porosity of about 70%. The walls have been a relief and nanoporous surface. Using different schemes SHS method - changing temperature conditions of process, initial parameters of powders and etc., can receive different structure porous permeable material with defined pore size and determined pore size distribution and also that the especially important in tissue engineering. with particular topography and surface condition of pore space.

In our experiment, the viability of hepatocytes suspension obtained, determined according to ISO 109 935 was 85-88%. When cultured on plastic for 7-8 days, cells exhibited typical hepatocyte morphology with signs of dividing cells.

In the pre-implanted scaffold at day-0, cells were in the process of active attachment and proliferation. Along the network of interconnected pores, cells were fully spread and fixed on scaffold walls, which has a lot of small pores measuring less than 5 μm in size (Figure 1a).

At day-7 post-implantation, cells were in the process of further proliferation, with synthesizing extracellular matrix and forming spatial incrustations of various shapes and sizes. Cells were observed to spread across the pores. Relatively small pores were fully filled with cells and extracellular matrix (Figure 1b). Beginning from day-7 post-implantation, the cellular ingrowth gradually invaded the inner porous structure, from the periphery towards the center. At day-28 post-implantation, all pores were closed and completely filled with cells and extracellular matrix.

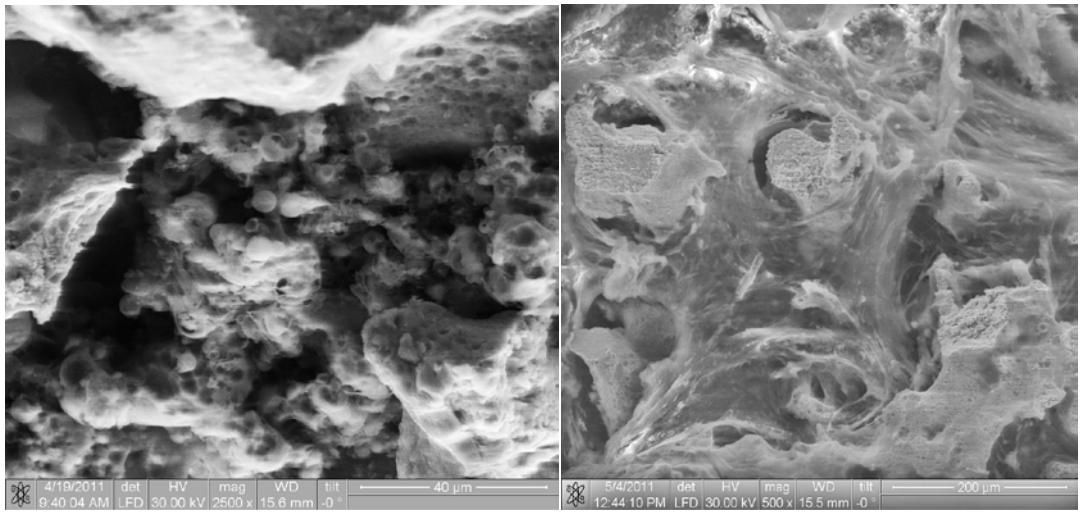


a b

Fig. 1. The attachment, growth and reproduction hepatocytes on inner surface of TiNi-based alloy scaffold

Further detailed analysis of the interaction of hepatocytes with an inner surface of the porous TiNi incubator showed that on day 21 reproduction and synthesis of extracellular matrix were amplified (Fig. 2a). The increase in growth going by achieving required critical level of cell mass and corresponding increase in concentration gradient of growth and nutritional factors in three-dimensional pore volume. Tissue gradually fills the inner surface of pores and then fills pore space.

At day-28 post-implantation, all pores were closed and filled by 80% with cells and extracellular matrix (Fig. 2b).

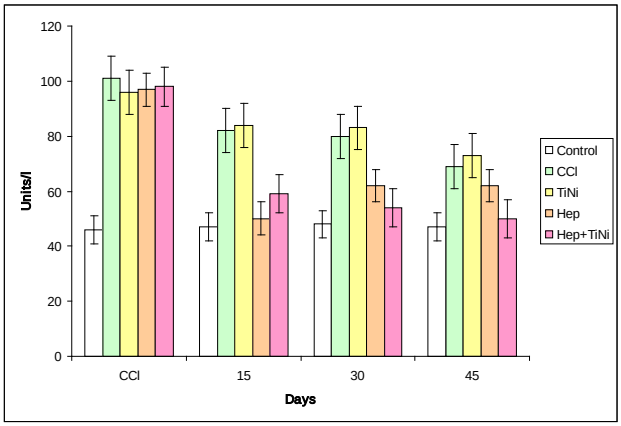


a b

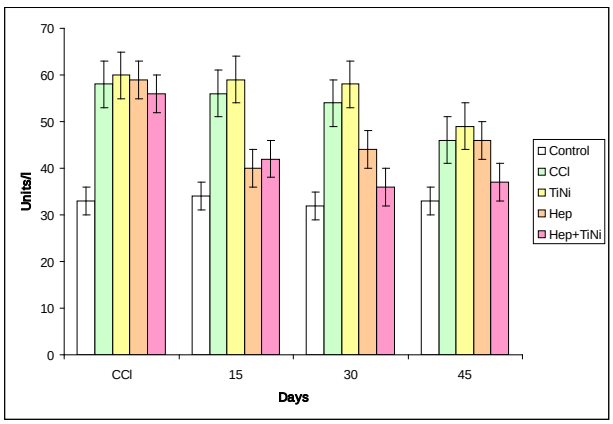
Fig. 2. Generation of cells and extracellular matrix synthesis fibers at day 14 (a), the phase of rapid fill the pores of cell population at days 28 (b)

Thus, based on the studies we can conclude that porous-permeable incubator of TiNi-based alloy is biocompatible with liver cells - hepatocytes. Cells actively attach, grow, reproduce and form the corresponding tissue in allogenic environment.

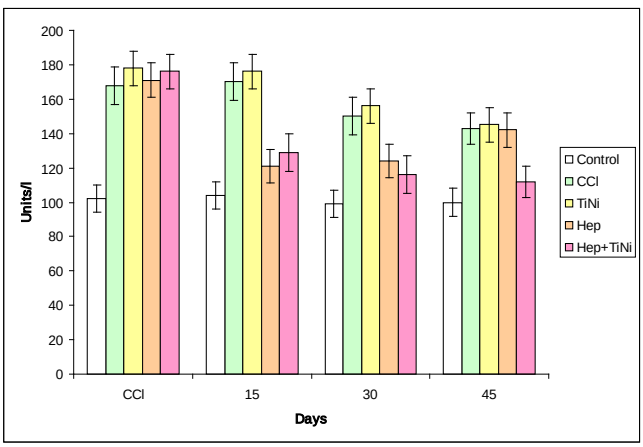
Also, functional characteristics of hybrid liver with Cl-induced hepatitis were tested. The development of acute CCl_4-induced hepatitis is accompanied by emergence of necrotic foci liver tissue and degeneration of hepatocytes. At the same time there is increase aspartattransferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) enzymes (group «CCl»). Transferase activity (Fig. 4 (a-c)) in the groups «CCl» and «TiNi» increased on 0-30-th day after the administration of CCl_4, and 45 days have stabilized. Injecting hepatocytes resulted in rapid decrease in cytolytic enzymes for 15 days, but then there is increase of enzymes. Implantation of scaffolds with immobilized cells of liver leveled increase transferase activity in the 30th and 45th day after CCl_4 administration, and ALT - during the entire observation period ($p \leq 0,05$ as compared to the group, where hepatocytes were administered by injection). In the experimental group of animals after the administration of hepatocytes in porous-permeable incubator of TiNi-based alloy biochemical blood parameters (ALT, AST, alkaline phosphatase), close to original values intact healthy animals, indicating that inhibition degenerative processes and necrosis in the liver and restore its functional activity.



a



b



c

Fig. 4. Indicators of enzyme (U/L) in Vistar rats with CCl_4-induced hepatitis after implantation of porous TiNi-based alloy scaffold with allogeneic hepatocytes (a - AST; b-ALT; c-alkaline phosphatase)

It is noted that life span for 1.5 months in the group with toxic hepatitis was 20%. In the group of animals with injection of hepatocytes was 40%. The most effective

were groupe «Hep+TiNi»: under similar experimental conditions, life span of animals with implanted cells on scaffold of TiNi-based alloy is increased to 60%.

This investigation shows complex analysis of the development of liver cells - hepatocytes - in biocompatible scaffolds made of highly porous TiNi-based alloy. Displaying phased development of hepatocytes in porous-permeable structure of scaffold of TiNi-based alloy. Nanoporous surface creates conditions in incubatosr for safeguard and growth of hepatic colonies in permeable structure allows penetration of nutrients from environment. Adhered to the surface of hepatocytes then build liver tissue inside the incubator. Research on model CCl_4-induced hepatitis showed effective antitoxic effect of hepatocyte populations in scaffold and their prolonged effect. At the same functional characteristics of liver tissue-engineering hybrid design as an example CCl_4-induced hepatitis proves operability of liver tissue that develops in scaffold. The results show that porous TiNi-based SMA is unique biocompatible incubator for hepatocytes and can be successfully used for tissue bioengineering and artificial organs.

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