Conference paper

Study of Correlation of Structural Features in Porous TiNi-based Materials Obtained by Sintering with the Integration Process of Bone Marrow Cell Populations

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Abstract

The structural features of porous-permeable TiNi-based materials obtained by sintering in depending on the temperature have been studied. It is shown that a material obtained at the sintering temperature of T_2=1250 °C and time t=40 min has an optimal degree of sintering and relates to finely porous materials with the porosity of 55 %. A positive dynamics of development of bone marrow cells in the TiNi-based sintered material was proved. It is noted that the finely porous macrostructure with developed rough surface of pore walls provides favorable conditions for development of cell populations. When the 21 day of cultivation a dense formed tissue on basis of bone marrow cells observes in the material pores.

1 Introduction

Porous TiNi alloys deserve a detailed study because of their extensive using in medical practice [1]. Self-propagating high-temperature synthesis (SHS) and sintering are the most frequently used methods among the many ways of obtaining a porous material [2]. The material obtained by these methods is characterized by a combination of structural and physical mechanical properties required for application in various fields of medicine.

Selecting the implant material has a fundamental importance in creating dental implants. One of advantages at using the sintering method is a possibility of

producing implants with needed sizes, it excludes a further machining. In this meaning a use of the sintering method of the TiNi powder to create a porous part of the dental implant is a perspective direction. At that the structural state of pore space has important meaning as it determines a dynamics of interaction with body cells [3]. Cells have to have an access to nutrients and a possibility of removal of metabolic products. All these processes can be realized most successfully in a volumetric porosity material. During development the cells form a three-dimensional structure of future tissue, which is also volumetric and consists of layers. Therefore, the most suitable material for an implant is material having a three-dimensional porous-permeable cluster.

The porous structure in the root portion of the implant has an important function, which consists in increasing the value of specific contact area with surrounding tissues in comparison with a standard screw thread on a monolithic surface with various modifications. Stepwise implant surgery features lead to necessity of acceleration of integration time into the jaw bone structure as the implant base survives from 4 to 6 months. During this period mechanical loads are not allowed because they can result in significant patient discomfort and high risk of complications. And so it is important to optimize the implant integration step. An additional contribution for acceleration of the integration processes can be get due to the surface condition of TiNi metal matrix.

Using various temperature conditions of the sintering method a porous material is received with various macro- and microstructure. And a material with a certain surface morphology of pore space and pore size distribution is obtained; it is particularly important in cellular engineering. Accordingly, the study of structural features effect on cell integration processes in the pore space of TiNi-based material, obtained by sintering, is a relevant investigation.

2 Experimental

Porous samples were prepared by sintering of the TiNi powder (PV–N55T45). The powder mass was filled in a carbon molding of 40 mm thickness, with holes of 3.5 mm diameter. Seal was made after powders filling. Sintering was carried out in two stages in the electric furnace at the pressure of 10^{-4} Pa. In order to produce a compact sample shapes the first sintering was made at the temperature T_1=1200 °C for 40 min. The second stage was carried out on molybdenum foil plate at temperatures of T_2=1220, 1240, 1250, and 1260 °C, respectively for 40 min. The

final cylindrical billets had sizes 3.5×30 mm. As-fabricated specimens in such a way are characterized by uniform surface and had no signs of cracking, delamination or chipping.

The macro- and microstructure of the samples were studied by means of metallographic specimens microsection prepared by a standard procedure [4]. Grinding is made with sandpaper (P493, P600, P3000) with abundant cooling water. The final step of grinding the samples includes using a diamond paste and a felt cloth. The metallographic examination was performed using an Axiovert-40 MAT microscope. The microstructure of the alloys, and topography of pore walls surfaces were examined under scanning electron microscopes (Philips SEM 515 and Quanta 200 3D). Phase composition was studied with using Shimadzu XRD-6000 diffractometer with CuKα-irradiation. The porosity and apparent density were determined by weighing technique using analytical balance A&D GH-200. The pore size and pore intersections were determined by a combination of the secant method and the method of inscribed spheres

We have made a quantitative description of the pore structure. The porosity was found by the weighting method and calculated by the following formula:

$$P = (1 - m_{pore}/m_{cast})*100\%, \quad (1)$$

where m_{pore} is a mass of a porous sample and m_{cast} is a mass of a cast sample.

The specific surface area was determined by random ΣS intersecting, based on the second stereometric ratio [5]

$$\Sigma S = 2m, \quad (2)$$

where m is an average number of intersections per millimeter.

In *in vitro* investigation bone marrow stem cells of hybrid mouse F1 CBA / j were used. Cultivation occurred in medium that consisted in: DMEM-F12 medium, 10 % fetal calf serum, gentamicin 40 g/ml glutamine and 250 mg/l. Incubators with cells were maintained at 37 °C with 100 % moisture and 5 % CO_2. Samples were taken for analysis on 7, 14, 21 days.

3 Results and discussion

Sintering powder system is carried out at temperature growth by a driving force, which tends to reduce a surface energy. Properties of the final sintered product mainly are determined by the temperature and time of sintering.

According to TiNi system state diagram, the first melt portion forms at the temperature of 955 °C at melting Ti_2Ni phase [6]. Contacts between powder particles during the first sintering at the temperature T_1=1200 °C form during the surface diffusion of atoms and local wetting powder particles contacts. The amount of melting secondary phases in NiTi powder is 2.7 wt. % according to the quality certificate. Consequently, the formed melt is enough only to create initial particle-particle contacts.

Primary sintering at higher temperatures leads to a large volume of melt. Its sources are TiNi dissolved grains in Ti_2Ni phase melt during the heating above the temperature of 1240 °C. This entails an activation of diffusion processes at the carbon atoms transfer from a graphite molding that leads to an inevitable contamination of TiNi alloy.

After the first sintering the samples that have a predetermined initial shape and geometric dimensions were received. Sintering samples to molding for this mode is not detected, and a shrinkage of the powder application phase is absent. By surface diffusion and wetting particles contact locations the fragile frame is formed in material volume. The processes of consolidation and pore formation have not yet began – the samples have a porosity that has developed as a result of coating and sealing (P=70 %). Morphology of the pore walls is characterized by pores powder particles structure which have a dual morphology. On their surface a lot of precipitates of various shapes were observed, which have been preserved from an original powder structure.

The analysis of structural-phase state material obtained by sintering at the temperature of T_2=1250 °C and the time t=40 min have indicated that this mode is the more optimum for creating TiNi-based porous permeable materials (Fig. 1, a). Sintering at the temperature of T_2=1220–1240 °C is incomplete in conequense of formation of small melt quantities and at the temperature of T_2=1260 °C processes of longitudinal and transverse shrinkage are observed, which results in a significant change of geometric dimensions and porosity.

X-ray analysis of the porous sintered alloy at different temperature regimes has showed a two-phase state of the metal matrix – B2 and B19' (Fig. 1, b). Secondary phases both Ti- (Ti_2Ni, $Ti_4Ni_2(O,N,C)$) and Ni-enriched ($TiNi_3$, Ti_3Ni_4) were found.

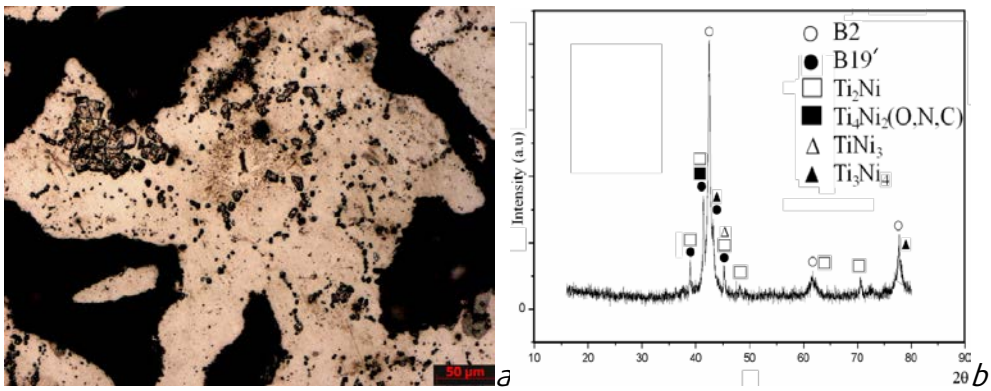
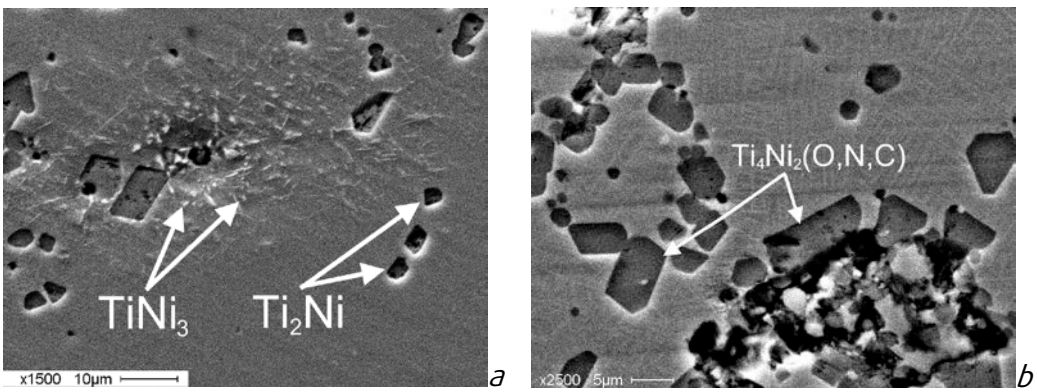
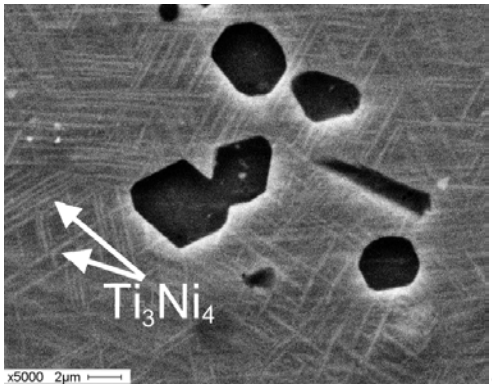


Fig. 1. Microstructure of porous permeable TiNi-based materials obtained by sintering at T_2=1250 °C, t=40 min: a – matrix of the TiNi-based materials; b – diffraction pattern of the porous matrix

A surface of the studying samples comprises a variety of single-particles and conglomerates, located mainly at the grain boundaries and pores and rarely within the grains themselves. Ti_2Ni phase particles have a rounded shape and size of not more than 5 m (Fig. 2, a), while Ti_4Ni_2(O,N C) have a form of rhombuses, trapezoids, parallelograms, triangles with larger sizes to 25 m (Fig. 2, b). Practically at high magnifications in all places a presence of finely dispersed Ni-rich phase is observed. $TiNi_3$ and Ti_3Ni_4 phases have size up to 0.2 m and round or platelet shape, respectively (Fig. 2, a, b).





c

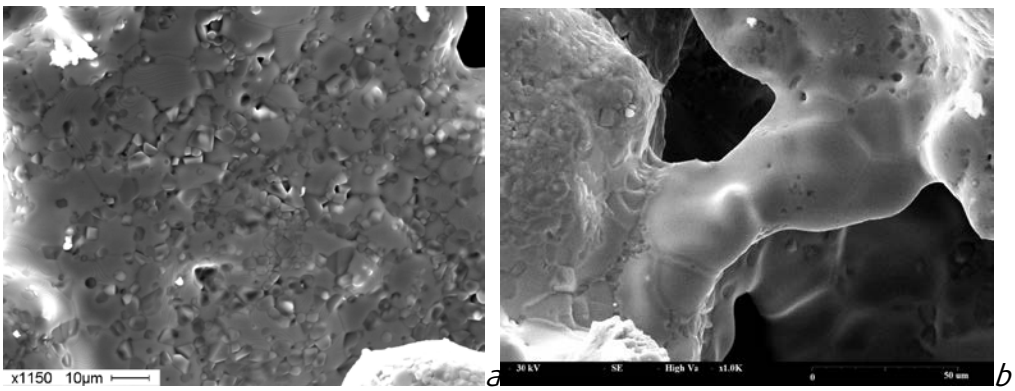
Fig. 2. Microstructure of porous permeable TiNi-based materials. Particles Ti_2Ni (*a*), $Ti_4Ni_2(O,N,C)$ (*b*), $TiNi_3$ (*a*) and Ti_3Ni_4 (*c*)

At sintering a more homogeneous microstructure of secondary phase's distribution in the alloy is shown than in the case of SHS materials because a ready TiNi powder undergoes the sintering. Sintering raw powders leads to their additional homogenization and a number of secondary phases significantly reduce due to an interaction with the melt.

Samples with a fine pore structure with an average pore size d_{por}=90 μm and porosity P=55 % were prepared by sintering at the temperature of T_2=1250 °C and time t=40 min. Unlike other modes of producing a material at lower sintering temperatures (T_2=1220–1240 °C) the high sintering quality is achieved due to a sufficient amount of melt, which wets the TiNi powder surface. Thus an excessive increase tn the sintering temperature to T_2=1260 °C leads to an over volume of eutectic liquid in the sintered system, that causes a decrease in porosity value to P=40 % at the expense of increasing the size of pore intersections d_{inters}=305 μm. There are signs of volume diffusion i.e. powder particles centers close together under the action of capillary contraction, causing the transverse and longitudinal shrinkage.

During sintering the powder system tends to minimize the surface energy. Protrusions and depressions smooth on surface of the precursor powders, structural defects are eliminated. This is confirmed by a decrease in the specific surface area, which is S_{sp}=31 mm^2/mm^3. The formation of a new surface under recrystallization processes is caused by a diffusion interaction of the melt with TiNi powder and its secondary phases. Clusters of secondary precipitates crystals form rough surfaces with a profile height of negative and positive signs (Fig. 3, a). In this case some surfaces are almost free of surface precipitates (Fig. 3, b). These

structures are defined by a double particle morphology of original TiNi powder. Compact powders have a plurality of secondary phase on its surface, while sponge-form powders are free from them.



a *b*

Fig. 3. The surface of pore walls of porous permeable TiNi-based materials produced by sintering at T_2=1250 °C, t=40 min: The macrostructure of pore wall inherited from a compact (a) and sponge-form (b) TiNi powder particles

Appearance of melt and wetting powder particles surface them is heterogeneous. Secondary phases complicate this phenomenon because several particles have a different ability to interact with the melt. Wetting contact angle of carbides and carbonitrides in vacuo with TiNi-melt is 18–25 °, while wetting of oxides and oxynitrides is very problematic [7]. Bright particles locate within or on a surface of crystallized melt (Fig. 4–highlighted rectangle) and have fewer interstitial elements of carbon, nitrogen, oxygen ($Ti_{23}Ni_{20}O_{17}N_{10}C_{30}$). Conversely dark particles are in recesses and (Fig. 4–highlighted circle) have not reacted with the melt. Oxynitrides dominate in their composition that prevent to wetting of particles ($Ti_{29}Ni_{25}O_{27}N_{10}C_9$).

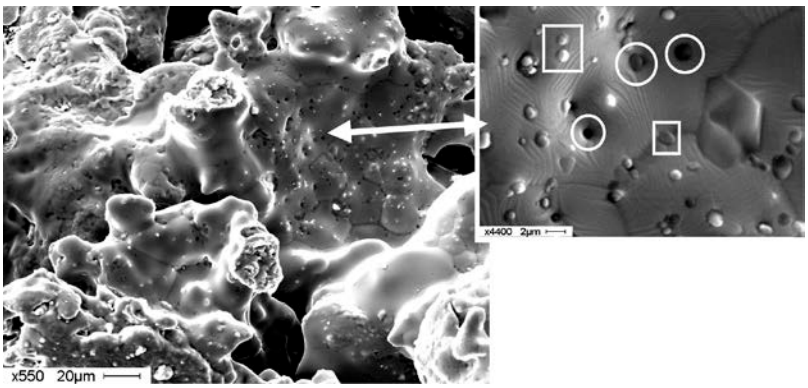


Fig. 4. Macrostructure of the surface of pore walls with secondary particles of different composition

The pore space of the obtained material is well adhered and biocompatible surface with bone marrow cells. The study of the interaction process of bone marrow cells

with the sintered material surface has revealed a number of features in a mechanism of their growth and reproduction. On the 7th day there is an active process of cell attachment and proliferation through creating a developed network of pseudopodies, which had a different degree of maturity by this time (Fig. 5, a). Mostly single cells have been noted there. Through cell mass the surface structure of pore walls are observed. Cells actively attached onto the roughened surface of the pore walls and in places where there are micropores.

When culturing for 14 days in the sintered samples an increase of cells number, their divisions, the synthesis of fibers of extracellular matrix were observed. Heterogeneity of cell mass development in pores was detected. In some pores there are cell groups with pseudopodies, whereas in other pores the process of tissue formation begins consisting in the generation of large fibers and extracellular matrix (Fig. 5, b). This phenomenon is connected with the size factor of pore space i.e. filling a pore with size of 100 microns is more active than in large pores.

On the 21 day the pores are filled with a tissue, in which cells, pseudopodies are virtually indistinguishable. The formed tissue is fully lines pore walls, at that the surface structure of pore walls does not observe (Fig. 5, c, d).

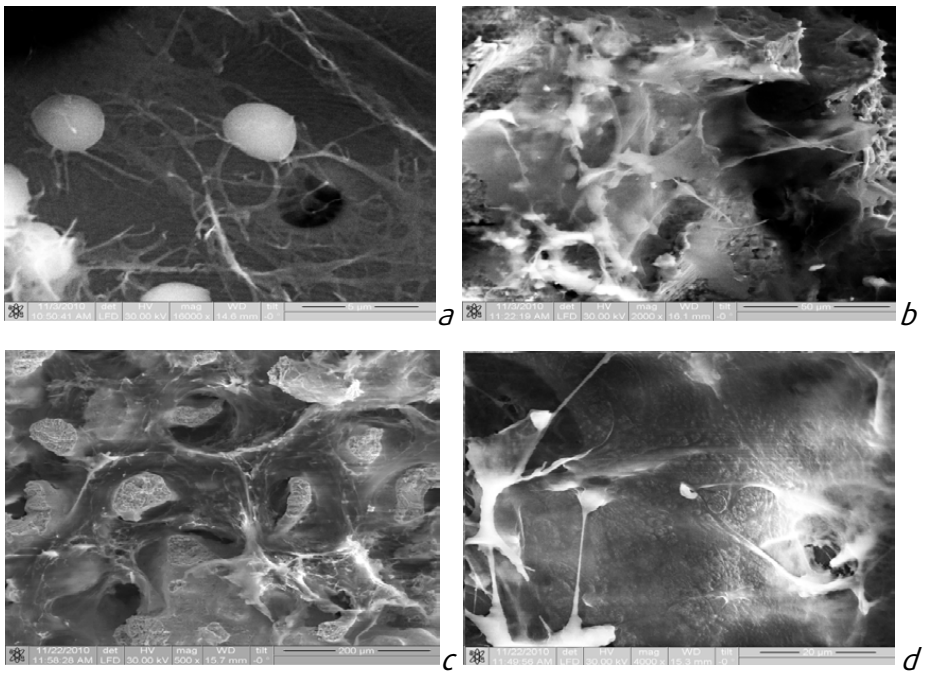


Fig. 5. Integration of bone marrow cells with porous TiNi-based materials, obtained by sintering: a – single cells and developed pseudopodias network (7 days), b – filling start of inner space with tissues (14 days), c – filled pore space with formed tissue (21 days) d – the microstructure of the resulting tissue (21 days)

4 Summary

Thus, as a result of the study it is established that the material obtained at the sintering temperature T_2=1250 °C and the time t=40 min is characterized as a small pore with porosity P=55 % and has an optimal degree of sintering. A positive dynamics of development of bone marrow cells in the TiNi-based sintered material was shown. It was found that the porous material structure satisfies to necessary conditions for the development, growth and reproduction of bone marrow cells. The resulting material is a good porous biocompatible material for using in a medical practice. The use of the sintered material allows creating favorable conditions for the development of cell populations that can significantly reduce integration times of dental implants.

5 Acknowledgments

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References

[1] V. E. Gunther et al., Medical materials and shape memory implants, first ed [in Russian]. MIC, Tomsk, 2011.

[2] V. N. Khodorenko et al.,Structural and strength properties of porous TiNi-base alloys obtained by self-propagating hightemperature synthesis and diffusion sintering, Russian Physics Journal, 57–6 (2014) 723–730.

[3] O.V. Kokorev et al., In vitro and in vivo evaluation of porous TiNi-based alloy as a scaffold for cell tissue engineering, Artificial Cells, 44–2 (2015) 1–6.

[4] ISO 3954:2007:Powders for powder metallurgical purposes–Sampling. International Standard Organization.

[5] S. A. Saltykov, The stereometric metallographic, Metallurgy, Moscow, 1976.

[6] K. Otsuka, Physical metallurgy of Ti–Ni–based shape memory alloys, Progress in materials science, 50–5(2005) 511–678.

[7] A.V. Tumanov, Study of the kinetics of wetting carbide and titanium carbonitride melts nickel intermetallic, Journal Physical Chemistry, 54–6 (1980) 14–34.