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Biocompatibility of NiTi Alloy Implants *in vivo*

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***Abstract*—**In this study, the powders of Ni and Ti with 50.5 at.% Ni for 12 h were blended and cold pressed at the different pressures (50, 75 and100 MPa).The porous product obtained after Ni-Ti compacts were synthesized by SHS (self-propagating high-temperature synthesis) in the different preheating temperatures (200, 250 and 300oC) and heating rates (30, 60 and 90oC/min). The effects of the pressure, preheating temperature and heating rate were investigated on biocompatibility in vivo. The porosity in the synthesized products was in the range of 50.7–59.7 vol. %. The pressure, preheating temperature and heating rate were found to have an important effect on the biocompatibility in-vivo of the synthesized products. Max. fibrotic tissue within the porous implant was found in vivo periods (6 months), in which compacting pressure 100MPa.

***Keywords*—**NiTi, biomaterial, SHS, biocompatibility.

I. Introduction

NITI shape memory alloys (SMAs) are materials widely used in numerous biomedical applications (orthodontics, cardiovascular, orthopedics, urology, etc.) due to their good biocompatibility, unique shape memory properties, mechanical properties, superior damping capability, excellent corrosion resistance and wear resistance [1-8]. Since their mechanical properties are closer to those of cortical bones than stainless steels and titanium alloys [9], porous NiTi alloys show promising potential in the application of bone implantation. The porous structure also allows the ingrowths of new bone tissue along with the transport of body fluids, thus ensuring a harmonious bond between the implant and the body [4]. Measurements in literature suggests that NiTi alloy is safe and biocompatible due to its surface oxide film which is composed mainly of titanium oxide and acts as a barrier to prevent nickel from corrosion and ion leaching [10]. However, some negative side effects have been pointed out [9]. For example, the osteogenesis process and osteonectin synthesis activity in NiTi alloys are unfavorable compared to stainless steels and titanium alloys, Cell death rate is severe on NiTi alloys and proliferation of human gingival fibroblasts on NiTi samples with rough surface is slow compared to stainless steels and Ti alloys with the same surface roughness. The short-term biocompatibility of porous NiTi was determined to be comparable to that of dense NiTi. Moreover, in vivo standard allergy potential evaluation showed that porous NiTi has no potential to produce irritation, systemic toxicity reactions, or sensitization in animal models [11].

Porous NiTi SMAs have been fabricated with powder metallurgy (PM) processes such as self-propagating high-temperature synthesis (SHS), metal injection molding (MIP), hot isostatic pressing (HIP) and spark plasma sintering (SPS) [4, 5, 12, 13]. These processes can avoid the problems associated with casting, like segregation or extensive grain growth and have the added advantages of precise control of composition and easy realization of complex part shapes [13]. The occurrence or amount of fibrosis inside the porous NiTi implant in bone tissue has not been assessed before. Rhalmi et al. [14] reported some fibroplasia and bone marrow within the pores of the porous NiTi implant.

The aim of this study was to find out the possible cytotoxic effects of NiTi implant material in vivo. NiTi implants with 50.5 at. % Ni was fabricated by SHS at the different preheating temperatures, heating rates and pressures. The effects of the pressure and duration in vivo were investigated for the biocompatibility of implant material.

II. Material and Method

A. Experimental Procedure for Production

Titanium and nickel powders were used to produce porous NiTi alloy implants. The raw materials were 325 mesh Titanium (99,5 % in wt.) and Nickel (99,8% in wt.) powders. Characteristic features of powders used are given in Table I.

TABLE I
Characteristics of Ti and Ni Powders
[http://www.alfa-chemcat.com, 2007]

Feature of Material	Nickel	Titanium
Purity (%)	99.8	99.5
Spesific gravitiy (g/mol)	58,71	47,9
Powder dimension (mesh)	-325	-325
Melting heat (^oC)	1453	1680
Spesific weight (g/cm^3)	8,9	4,507
Boiling heat (^oC)	2832	3260

The mixed powders of Ni and Ti with 50.5 at.% Ni were blended for 12 hours and then cold pressed in a cylindrical die with 10 mm diameter under different compaction pressures using a hydraulic press. The cold compacted porous samples were heated under different heating rates with the protection of high purity argon gas (99.9%) of about 0.1 MPa in a furnace. The samples were ignited under different preheating temperatures using electrical discharge pulse (14 kV and 30 mA). Once ignited, combustion waves could self-propagate along the axis to the other end of the compact in a very short time, and then porous NiTi implants were obtained by

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synthesizing. The procedure was repeated at different compaction pressures, preheating temperatures and heating rates to investigate the effects of these parameters on biocompatibility. The parameters used in the experiments are shown in Table II.

TABLE II
PARAMETERS USED AS VARIATION IN INVESTIGATE

Parameters	Values
The compaction pressures (MPa)	50, 75, 100
Preheating temperatures (^oC)	200, 250, 300
Heating rates (^oC/min)	30, 60, 90
Service period in vivo of the implants (month)	2, 4, 6

Thus biomaterial, both with high porosity and close to mechanical properties of the bone, was produced. The porosity (average void volume) of the implants was determined according to the formula was calculated by the following formulae:

$$\varepsilon = \left(1 - \frac{\rho}{\rho_0}\right) x100 \quad (1)$$

where ρ and ρ_0 are the density of the specimen and its corresponding theoretical density, respectively. The density of the specimen was determined by measuring its weight and dimension. The theoretical density of Ti–50.5 at.% Ni alloy is 6.21 g/cm^3.

B. Surgical Procedure

The protocol of this study was reviewed and approved by the local ethics committee, in accordance with the Helsinki Declaration. The animal tests were performed after approval by the ethical committee of the University of Firat. Implantation of produced implants was performed in Firat University Veterinary Faculty Surgery Clinic.

Nine adult mongrel female clinically healthy dogs aged between 2 and 3-year-old (between 24-32 kg weight) were used in this study. The dogs were provided by Elazig Municipality, Turkey. The dogs were treated against antiparasite and vaccined. The animals were housed freely in three separate rooms, all cases were left free walk in the room and had free access to water and standard feed throughout follow up periods. Preoperatively the dogs were left hungry for 12 hours. General anaesthesia was induced in animals by intramuscular administration of combination of Xylazine hydrochloride 2 mg/kg (Rompun, Bayer, 23.32 mg/ml), and ketamine hydrochlorur 15 mg/kg (Ketalar, Parke-Davis, 50 mg/ml).

Skin was incised along the medial side of the tibia. The skin incision extended from just proximal to the tibia to the distal end of the tibial crest. Subcutaneous tissues were incised on the same line and mobilized with the skin. Diaphysis of the radius was exposured by the conventional incision of subcutaneous fascia and muscles [15]. A 10 x 15 mm defect was created in the metaphysis of the tibia.

The dogs were divided into three groups randomly and produced NiTi implants were implanted into the defect area. The first, second and third groups were transplanted produced by 50 MPa, 75 MPa and 100 MPa implants respectively. Operation wound was closed by the conventional operational techniques. Postoperative medications included procaine peniciline G (22.000 IU/kg, IM, q 12 h) administered for 5 days. Skin sutures were removed 10 days after the surgery.

Clinical evaluations were carried out at 30 days intervals. One dog in each group was sacrificed by overdose sodium penthobarbital at 2nd, 4th, and 6th months, and one dog in each group were harvested a piece of bone including implanted regions for histopathological examinations.

Histopathologic examinations were performed in the Pathology Laboratuary of Elazıg Harput Research and Application Hospital. The samples were placed in 10% neutral buffered formalin immediately after reoperation. The section was fixed in 10% neutral buffered formalin for 5 days and decalcifed in 1% formic acide solution. The samples were cut into 5 μm sections after complete decalcification and stained with hematoxylin and eosin (H&E) and examined by light microscopy (Olympus BX51).

III. RESULTS AND DISCUSSION

NiTi alloys are used as biomaterials. Endurance and life of nitinol implants in vivo is still being studied and risk factor in utilization of this alloy in vivo is also unclear [16]. In this study, implant was produced at different preheating temperature, heating rate and pressure values.

It is seen that porosity is significant in bone-implant inerface at 250^{o}C preheating temperautre and 60^{o}C/min heating rate (53-59 %) where the highest porosity among these implants is obtained [17]. This means that the best (threshold) production conditions were achieved by a preheating temperature of 250^{o}C, and a heating rate of 60^{o}C /min. Therefore only the implants produced under these conditions and different compacting pressures (50, 75 and 100 MPa) were implanted to the dogs and effort was paid to determine a relation between porosity and biocompatibility.

In the study conducted by Kujala et. al., the histological evaluation showed that the implants were well tolerated and the hard tissue preparations revealed, at the level of light microscopy, that bone grew into direct contact with the implant surface and into the void spaces inside the implant with all porosities [16].

Bone growth into porous metal surfaces depends on several factors, including the porosity of the surface, the stability and degree of micromotion between the implant and bone, whether the host bone is trabecular or cortical, and the presence of gaps between the implant and the bone surface [16]. In this paper, deformation of cells and infection was not observed in implants of all animals. This shows that the implant is well tolerated. The proper surgical procedure minimized the gapping and micromotion of the implant.

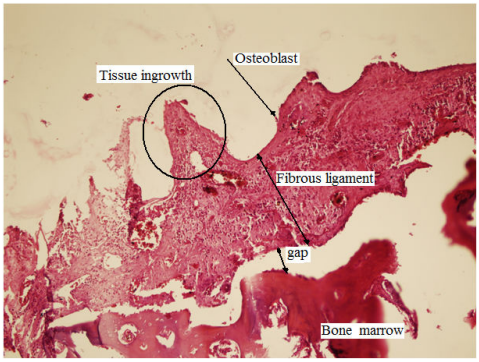
When two-month cases are examined,

In samples produced under 50MPa pressure, locally organized bone lamellae in this tissue were observed together

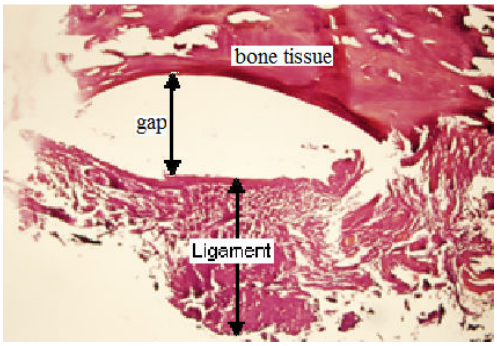
with the formation of widespread granulation tissue. The surface of the implant is directly surrounded with new bone [18]. Direct bone connection is very important depending on the placement of the prosthesis for implant [19]. The expected bone response is the formation of fibrosis/fibroplasia followed by bone formation and maturation [18]. Increasing porosity helps bone ingress, which also helps body fluid flow through the pores with cappilary effect [19].

The formation of fibrous tissue (bone tissue) next to the young callus formation is observed in samples produced under 75 MPa pressure (Fig. 1 (a)). The thickness of occurred fibrous tissue was measured as 0.1 cm. Osteoblasts appears normal. In addition, active transformation of bone and osseointegration inside the pores of the implants was witnessed.

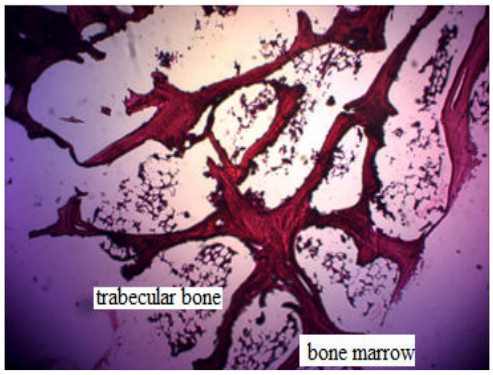
Bone tissue did not grow inside the pores and fibrosis capsule did not form in the implant fabricated by a compact pressure of 100 MPa. In addition, it was observed that the metal parts phagocytosed between trabecular bones and in free state. It was seen that bone tissue didn't have enough time to grow inside the pores because of low porosity.



(a)



(b)



(c)

Fig. 1 Histopathologic appearence of a case of the 75MPa group HE x10 a) At postoperative 2nd month b) At postoperative 4nd month c) At postoperative 6nd month

When the vivo period of implant is increased as 4 months, it was observed that fibrous tissue was occurred at 50MPa produced samples with high porosity (Fig. 1 (b)). Metal residues were observed at 75MPa produced samples with lower porosity. The young callus formation was occurred at 100 MPa produced samples with lowest porosity.

When six-month cases are examined, neither repair reactions were observed nor primary callus formed at 50MPa pressure produced samples. The granulation tissue (primary callus) formation wasn't observed at 75MPa pressure produced samples (Fig. 1 (c)). In addition, inflammatory reactions were not observed between the implant and the tissue. Common young granulation tissue and newly formed bone tissue were observed at 100MPa pressure produced samples.

Bone formation was only observed at 100MPa produced samples for 6 month in vivo period.

IV. Conclusion

In this study biocompatibility of the implant from NiTi alloy was investigated. As a result the followings were concluded.

In 2 and 4-month period of in vivo implants made of pressure 50MPa, 2-month period of in vivo implants made of pressure75MPa and 4 and 6-month period of in vivo implants made of pressure 100MPa, The developing reaction against implant in animal and reformed of bone tissue indicates that a good way of repair.

Especially for 2 months in vivo of implants made 75 MPa pressure and for 4 months in vivo of implants made 50MPa was obtained best data that observed the fibrous tissue together with repair in implants.

6 months in vivo of implants made 50MPa and 75 Mpa were occurred a bad way of repair.

Being in the cytoplasm of osteoblasts to have been phagocytosed and free in region of the metal particles on implant in animals associated with the duration of the experiment at 4-month in vivo period of implants made pressure of 75 MPa and 2-month in vivo period implants made pressure 100MPa pressure.

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