Biomaterials 32 (2011) 330–338

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Relationship between osseointegration and superelastic biomechanics in porous NiTi scaffolds

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ARTICLE INFO

Article history:
Received 19 August 2010
Accepted 29 August 2010
Available online 23 September 2010

Keywords:
Osseointegration
Biomechanics
Superelasticity
Porous NiTi shape memory alloy
Scaffolds

ABSTRACT

The superelastic nature of bones requires matching biomechanical properties from the ideal artificial biomedical implants in order to provide smooth load transfer and foster the growth of new bone tissues. In this work, we determine the biomechanical characteristics of porous NiTi implants and investigate bone ingrowth under actual load-bearing conditions *in vivo*. In this systematic and comparative study, porous NiTi, porous Ti, dense NiTi, and dense Ti are implanted into 5 mm diameter holes in the distal part of the femur/tibia of rabbits for 15 weeks. The bone ingrowth and interfacial bonding strength are evaluated by histological analysis and push-out test. The porous NiTi materials bond very well to newly formed bone tissues and the highest average strength of 357 N and best ductility are achieved from the porous NiTi materials. The bonding curve obtained from the NiTi scaffold shows similar superelasticity as natural bones with a deflection of 0.30–0.85 mm thus shielding new bone tissues from large load stress. This is believed to be the reason why new bone tissues can penetrate deeply into the porous NiTi scaffold compared to the one made of porous Ti. Histological analysis reveals that new bone tissues adhere and grow well on the external surfaces as well as exposed areas on the inner pores of the NiTi scaffold. The *in vitro* study indicates that the surface chemical composition and topography of the porous structure leads to good cytocompatibility. Consequently, osteoblasts proliferate smoothly on the entire implant including the flat surface, embossed region, exposed area of the pores, and interconnected channels. In conjunction with the good cytocompatibility, the superelastic biomechanical properties of the porous NiTi scaffold bodes well for fast formation and ingrowth of new bones, and porous NiTi scaffolds are thus suitable for clinical applications under load-bearing conditions.

1. Introduction

Bones provide the skeleton with the necessary rigidity to function as attachment and lever for muscles while supporting the body against gravity. Accidents, injuries, congenital defects, and degradation related to aging make bone repair and regeneration one of the biggest challenges in orthopedics and dentistry. Traditional methods used in bone reconstruction often utilize autografts and allografts on account of their good osteoinductive and osteoconductive properties, but the drawbacks include the limited supply of autografts as well as possible infection and immunological incompatibility caused by the allografts [1,2]. Synthetic porous scaffolds have been suggested as bone substitutes because they have a porous structure similar to that of natural bones and in principle allow bone tissue ingrowth and mineralization in the porous channels [3]. Although porous ceramics such as hydroxyapatite, tricalcium phosphate, and biphasic calcium phosphate as well as some natural polymers are potential bone scaffolds due to the similar chemical composition with bones or biodegradable properties [4,5], their application as bone substitutes under large load-bearing conditions, such as spinal interbody fusion, has been restricted because of their poor mechanical properties [6]. In comparison, metals such as titanium, titanium alloys, nickel–titanium, stainless steels, and tantalum are mechanically more suitable. However, the high stiffness of dense metals often leads to large stress-shielding from the surrounding bone tissues. This does

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doi:10.1016/j.biomaterials.2010.08.102

not favor the formation of new bone tissues consequently giving rise to poor fixation between the implant and tissues and possible eventual failure. Considering the excellent corrosion resistance and good biocompatibility [7,8], porous titanium produced by various powder metallurgical (PM) methods is a potential alternative [8–15]. Past studies have mainly focused on two aspects, namely the use of different PM methods to enhance the porous structure [8,9,11,14,15] and preparation of a bioactive surface layer to improve the osteoconductivity and osteointegration [10,12,13,16–19]. However, most studies have neglected the superelastic biomechanical behavior of bones under load-bearing. In this respect, porous titanium implants do not possess this property and in practical applications involving large loading, the mechanical properties of porous titanium and bone tissues do not match well despite a lower Young's modulus and a bioactive surface layer. As a result, large stress can occur in local areas and does not favor fast ingrowth of bone tissues thereby increasing the healing time and even causing implant failure. In fact, some dental implant failures have been observed to be predominantly caused by biomechanical complications [20,21].

Since the near-equiatomic NiTi shape memory alloy has similar superelastic biomechanical properties as some human hard tissues such as bones and tendons [22–24], the unique shape memory effect, excellent biocompatibility, low elastic modulus, high strength, and new bone tissue ingrowth ability and vascularization, porous nickel–titanium is a promising bone substitute [25–31]. Most of the research activities have hitherto focused on how to improve the surface biocompatibility, manufacturing processes, and general mechanical properties [27,29–42]. To our knowledge, there have been very few studies on the *in vivo* superelastic biomechanics and corresponding osteointegration effects. In this work, we investigate the superelastic biomechanical properties of porous NiTi scaffolds and influence on bone ingrowth *in vivo* and perform a systematic comparison to porous titanium produced by the same capsule-free hot isostatic pressing (CF-HIP) process. In order to unequivocally demonstrate the advantages of porous NiTi, a careful choice of the implantation site in the rabbit is needed to accurately simulate the real load-bearing conditions. Hence, the implant should first be tested under such conditions to assess whether the superelasticity has any effects on the formation and ingrowth of newly formed bones. Secondly, the implant should be exposed to both cortical and cancellous bones. Cortical bones represent the load-bearing portion of bone tissues. The properties measured from the bone-implant interface such as bonding strength and bone ingrowth can provide information about the suitability of porous NiTi as a load-bearing implant. Cancellous bones normally make up the bulk of the bone interior and the response of these bone tissues reflects the suitability of porous NiTi as a long-term bone substitute. The objectives of the present work are to: (1) demonstrate that CF-HIP synthesized NiTi does not hamper or even fosters bone ingrowth, (2) determine the bone penetration depth in the CF-HIP NiTi and compare the performance to that of porous titanium, (3) quantify the bonding strength at the bone-implant interface in comparison with porous CF-HIP titanium, dense NiTi, and dense Ti, and (4) study the effects of the superelastic properties of porous NiTi on osteointegration.

2. Materials and methods

2.1. Preparation of NiTi and Ti scaffolds

The porous NiTi shape memory alloy was prepared by capsule-free hot isostatic pressing (CF-HIP) [32]. Equiatomic nickel and titanium powders with NH_4HCO_3 as the space holder were thoroughly mixed in a horizontal universal ball mill and then pressed into green compacts using a hydraulic press. These green compacts were pre-heated at 200 °C in a tube furnace under a continuous flow of 99.995% argon to remove the space holders, put into the capsule-free stainless steel canisters, and finally sintered in the small ABB HIP unit under 150 MPa pure argon. The details of the CF-HIP process can be found elsewhere [32,43]. The porous samples were prepared by linear cutting. The porous titanium samples as control were also fabricated by the same CF-HIP process except using titanium powders as starting materials.

2.2. Implant preparation and characterization

The porosity of the porous NiTi and Ti implants was in the range of 42–48 vol%. The implants were machined into bars 5 mm in diameter and 10 mm long and then mechanically polished with SiC paper. The bars were washed three times with acetone and deionized water ultrasonically and heated in an autoclave at 120 °C for 30 min for sterilization. The surface morphology and microstructure of the porous specimens were evaluated by scanning electron microscopy (SEM; JSM5200) and optical microscopy (OM; Olympus BH2-UMA). For comparison, commercial Ti and NiTi samples were used as-received without any surface treatment.

2.3. In vitro study

The osteoblasts isolated from calvarial bones of 2-day-old mice that ubiquitously expressed an enhanced green fluorescent rotein (EGFP) were cultured in a Dulbecco's modified eagle medium (DMEM) (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Biowest, France), antibiotics (100 U ml^{-1} of penicillin and 100 μg ml^{-1} of streptomycin), and 2 mm l-glutamine at 37 °C in an atmosphere of 5% CO_2 and 95% air. The specimens which were 2 mm thick and 5 mm in diameter were affixed to the bottom of a 24-well tissue culture plate (Falcon) using 1% (w/v) agarose. A cell suspension consisting of 15,000 cells in 100 μl of the medium was seeded onto the surface of the porous NiTi and porous Ti samples, and wells without any metal disks served as the control. The cells were allowed to settle and attach to the surface of the disks for 4 h. One ml of the medium was then added to the wells and changed every 3 days. Four identical samples were tested to improve the statistics and cell proliferation was examined after 8 days of culturing. The cells were allowed to attain confluence during the examination period. The morphology of the attached living EGFP-expressing osteoblasts was examined by SEM after the disks with attached cells were washed gently in PBS before fixation in 2.5% glutaraldehyde buffered at a pH of 7.42 with 0.1 M sodium cacodylate for 24 h at 4 °C. The excess fixatives were removed by washing in 0.1 M sucrose in a cacodylate buffer and dehydration was carried out in a series of baths containing different ratios of ethanol to water up to 100% ethanol. The cells were then critical point dried and sputter coated with gold before examination by SEM (JSM5200).

2.4. In vivo animal studies

2.4.1. Surgical procedures

To investigate the biocompatibility of the porous NiTi implants in animals, *in vivo* tests were carried out using the following procedures. All aspects of the animal care complied with the Animal Welfare Act and the recommendations by the NIH-PHS Guide for the Care and Use of Laboratory animals. Approval from the Hong Kong animal care committee was also obtained. The animals were adult female New Zealand white rabbits 5 months of age weighting between 3.5 and 4 kg. The operation sites were femur and tibia. All the femurs were subjected to push-out test and the tibias were used in the histological study.

Before the surgical experiments, the neighboring areas of the rabbit femurs were shaved and the skin was sterilized by brushing with chlorhexidin. A dose of 10 mg/kg of pentobarbital sodium was injected intravenously and the animals were anesthetized by intramuscular administration of ketamine hydrochloride (50 mg/kg) followed by diazepam (5 mg) and atropine sulfate (0.5 mg) without endotracheal intubation. A longitudinal incision was made medially at the distal part of femur/tibia, and sterile surgical techniques were used to fold back the dermal and subdermal layers to expose the underlying periosteal connective tissue layer. This layer was carefully sectioned in the direction of the tissue fibers to expose the femur/tibia, and a 5 mm diameter hole was drilled through the femoral/tibial condyles. After washing the hole with sterile saline (0.9%, w/v), the porous cylinders were implanted into the holes. The fascia was closed with absorbable sutures and the skin was closed with 4–0 nylon sutures. The rabbits were allowed to move freely in their cages after the operation with no external support and were observed daily for activity and weight bearing on the operated limbs.

2.4.2. Radiographic evaluation

X-ray radiography (Faxitron X-ray Corporation) was conducted at 4 weeks to monitor the implant conditions. The radiographs acquired after sacrificing the rabbits after 15 weeks were also employed to evaluate the osseointegration between the implants and surrounding bone tissues.

2.4.3. Histological evaluation

The animals were sacrificed 15 weeks after surgery using an overdose of intravenous pentobarbital sodium. The bones with the implants were fixed in 10% phosphate buffered formalin at a pH of 7.25 for 7 days, dehydrated in a series of