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Biomechanical stability of novel mechanically adapted open-porous titanium scaffolds in metatarsal bone defects of sheep

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ABSTRACT

Open-porous titanium scaffolds for large segmental bone defects offer advantages like early weight-bearing and limited risk of implant failure. The objective of this experimental study was to determine the biomechanical behavior of novel open-porous titanium scaffolds with mechanical-adapted properties *in vivo*.

Two types of the custom-made, open-porous scaffolds made of Ti6Al4V (Young's modulus: 6–8 GPa and different pore sizes) were implanted into a 20 mm segmental defect in the mid-diaphysis of the metatarsus of sheep, and were stabilized with an osteosynthesis plate. After 12 and 24 weeks post-operatively, torsional testing was performed on the implanted bone and compared to the contralateral non-treated side. Maximum torque, maximum angle, torsional stiffness, fracture energy, shear modulus and shear stress were investigated. Furthermore, bone mineral density (BMD) of the newly formed bone was determined.

Mechanical loading capabilities for both scaffolds were similar and about 50% after 12 weeks (e.g., max. torque of approximately 20 Nm). A further increase after 24 weeks was found for most of the investigated parameters. Results for torsional stiffness and shear modulus as well as bone formation depended on the type of scaffold. Increased BMD after 24 weeks was found for one scaffold type but remained constant for the other one.

The present data showed the capability of mechanically adapted open-porous titanium scaffolds to function as bone scaffolds for large segmental defects and the influence of the scaffold's stiffness. A further increase in the biomechanical stability can be assumed for longer observation periods of greater than six months.

1. Introduction

Large bone defects caused by severe fracture, tumor or infection are a challenging issue for the regeneration ability. If the defect exceeds a critical size, the bone regeneration cannot be handled by the body's healing process itself and requires supportive therapeutic intervention like autografts and allografts [1,2]. Autografts offer high biocompatibility, but they have limited availability; furthermore, the grafting may cause additional complications like donor site morbidity, higher risk of infections and higher effort due to the second surgical procedure [3–5]. Therefore, synthetic materials have to be utilized to initially replace the bone defect and to support bone regeneration. They have to be fabricated in sufficient quantity in order to be suitable as a bone substitute material. Furthermore, biological (biocompatibility, adequate pore size and interconnectivity) as well as mechanical requirements (stiffness similar to surrounding tissue) have to be satisfied.

Calcium phosphate ceramic scaffolds offer the possibility of biodegradation and reveal good results. Bone defects up to 50 mm were filled with synthetic scaffolds, stabilized with by nail or plate with promising results [6–11]. Nevertheless, there are still risks associated with resorbable scaffolds [6,9,11–16]. In general, implant failure or failure of the applied osteosynthesis system may occur due to insufficient mechanical properties of the synthetic scaffold [8,17].

For large segmental defects within load-bearing areas, initially mechanical stable implants are indispensable. Metallic scaffolds (e.g., made of titanium) can cope with this requirement [18–21].

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These scaffolds offer advantages for two primary reasons: At least immediate partial weight-bearing with support of the osteosynthesis and long-term stability accompanied by its biocompatibility and bone ingrowth ability into the open-porous surface. In addition, the risk of late fractures due to scaffold instability is nearly negligible as long as the bone-graft-interface is well-incorporated with new bone. Scaffolds made of titanium are not biodegradable and therefore, cannot be replaced by newly formed bone. Therefore, the open-porous structure has to guarantee a sufficient pore size for bone ingrowth and nutrient supply [22,23].

Open-porous titanium implants revealed good biocompatibility and showed sufficient bone ingrowth *in vivo* [24–26]. However, the main disadvantage of the current types of scaffold is the poor adaptation to the mechanical properties of the surrounding tissue (i.e., cortical bone) and the risk of bone loss due to stress shielding [27–29]. In order to minimize the mismatch between scaffold and application area, additive manufacturing (AM) offers the possibility to gain control about the mechanical properties of the scaffold, which can be fabricated in a wide range of structural designs [22,30–32]. Furthermore, an open-porous structure with a defined pore size can be implemented. Pores with a size between 400 and 700 μm [33,34] and even up to 1.200 μm [26] showed sufficient results for bone ingrowth.

However, there are only a few studies about the capability of open-porous titanium scaffolds for healing and stabilization of large segmental defects in a biomechanical environment [35–37]. Although these studies reveal good results under mechanical and histological examination, none of the investigated porous titanium implants were either adapted to the mechanical requirements of the application region or to a surface structure with a defined pore design.

Therefore, the objective of this experimental study was to determine the biomechanical and bone ingrowth behavior of novel open-porous titanium scaffolds with mechanical-adapted (i.e., reduced stiffness) properties under biomechanical *in vivo* conditions in large segmental metatarsal bone defects of sheep that fulfilled the biological requirements for sufficient tissue ingrowth as ascertained *in vitro* [22,23].

2. Materials and methods

Two different types of custom-made cylindrical open-porous titanium (Ti6Al4V) implants were developed for this study. Both types of bone scaffolds were numerically investigated prior to manufacturing [38,39] in order to gain control about the mechanical properties and to match the elastic properties of human cortical bone.

2.1. Implant design and manufacturing

The first scaffold type was fabricated by selective laser melting (SLM Solutions GmbH, Lübeck, Germany) based on the CAD data of a biomechanically optimized scaffold design [38]. This scaffold exhibited a height and diameter of 20 and 17 mm, respectively (Fig. 1, left). Porosity was implemented as an open-porous structure with a rectangular pore size of 700 × 700 μm orientated perpendicularly in all three spatial directions. This pore design showed good results for cell spreading and vitality *in vitro* [22,23]. Rectangular strut width and height were 400 and 800 μm, respectively. Furthermore, the inner core was left empty in order to reduce the amount of artificial material as well as the structural stiffness.

The second scaffold type was made of a perforated titanium sheet with a thickness of 0.7 mm (provided by DOT GmbH, Rostock, Germany) (Fig. 1, right) and also based on the numerical findings of a previous study [39]. Rhombic holes were cut into the sheet by laser cutting in order to reduce the axial stiffness and to increase the radial flexibility ("Flex-Cage"). Struts exhibited a thickness of 0.7 mm and an angle of 30° to the vertical axis. The ends of the sheet were spot-welded to close the circle for the cylindrical scaffold. Afterwards, the star-shaped cross section was formed in a self-developed device by pressing six rods against the cylindrical shell. The scaffold's height and diameter were 22 and 17 mm, respectively.

After the fabrication process, all scaffolds were cleaned in an ultrasonic bath in order to remove all loose titanium particles and impurities. SLM fabricated scaffolds were additionally surface-coated with calcium phosphate (Bonit®, DOT GmbH, Rostock, Germany) in order to support bone regeneration [40,41]. The inner core of the Flex-Cages was filled with BONITmatrix® (CaP granules with a size of approximately 0.6 × 4 mm) embedded in a collagen matrix (three parts of 1% diluted collagen suspension and one part BONITmatrix®, provided by DOT GmbH, Rostock, Germany). The granules consisted of a mixture of the two calcium phosphates HA (particle size of approximately 90 nm) and β-TCP (particle size of approximately 400 nm) in the ratio of 60:40, embedded in a biological active silicon dioxide matrix (SiO_2, overall percentage: 13%). The granules offer a high interconnecting porosity between 60 and 80%.

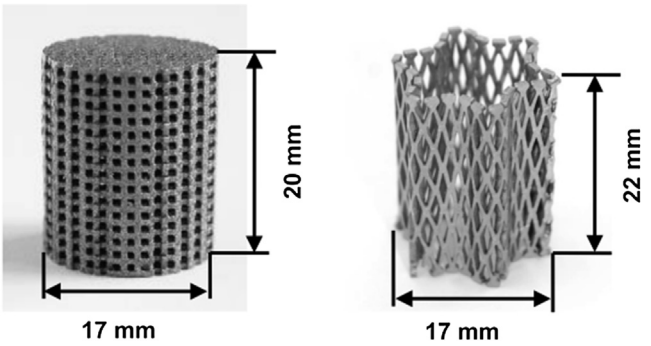

Fig. 1. Left: SLM fabricated scaffold with cylindrical cross-section and regularly orientated pores in all spational directions prior to surface coating. Right: Scaffold made of perforated titanium sheet with star-shaped cross-section ("Flex-Cage") without augmentation of collagen and CaP.

Finally, the scaffolds were prepared for surgical procedure by sterile packing and underwent sterilization with γ-irradiation (Synergy Health Radeberg GmbH, Radeberg, Germany) in accordance with ISO-standards (ISO 11137).

2.2. Mechanical testing of the implants

Scaffolds (n = 3 for each design) were tested under uniaxial compression using a universal testing machine (Z50, Zwick Roell, Ulm, Germany) with a velocity of 1 mm/min to determine the mechanical properties. Elastic deformation of the testing machine and the test setup was determined and taken into account for all subsequent testings in order to guarantee pure deformation of the specimens. Load-displacement relation was continually recorded during testing.

Elastic modulus E_S and compressive strength σ_{max} were calculated as follows:

$$E_s = \frac{\Delta F \cdot l_0}{\Delta l \cdot A} \quad (1)$$

where $\Delta F/\Delta l$ is the linear slope of the load-displacement relationship, l_0 is the initial length (i.e., 20 and 22 mm) and A is the cross-sectional area of the scaffolds (both scaffolds refer to a cross section of 227 mm^2 for a maximum diameter of 17 mm).

$$\sigma_{max} = \frac{F_{max}}{A} \quad (2)$$

2.3. Animal study

Three-year-old female sheep (breed: "Deutsches Schwarzkopf Fleischschaf") with a mean weight of 68.1 ± 8.4 kg were used for the study. All animals were treated according to current guidelines on animal well-being as previously approved by the Local Committee for Animal Experimentation (Reference number: LALLF M-V/TSD/7221.3-1.1-093/10). Animal examinations, housing, feeding and veterinary care were conducted using established procedures. The sheep were housed in an outside haddock with free access to food and water. Furthermore, animals could freely move in the outside territory during the study period.

2.3.1. Experimental design and implantation scheme

Manufactured titanium scaffolds were used for the investigation of the biomechanical stability of segmental metatarsal bone defects in comparison to the contralateral non-treated (intact) bone for two different time periods (12 and 24 weeks postoperatively). Animals were randomly divided into four groups. Animals from Group I and II were treated with the SLM fabricated scaffold for 12 (n = 10) and 24 weeks (n = 4), respectively. Animals from Group III and IV were treated with the Flex-Cage scaffold, respectively, for 12 (n = 7) and 24 weeks (n = 4).

2.3.2. Surgical procedure

One day prior to surgery the animals were kept without access to food in order to reduce the digestive process during surgery, which was performed under general anesthesia. In order to minimize the stress for the animals, an intramuscular administration of Xylazine (0.05 mg/kg BW) was given by injection to calm the animals before transporting them to the facility. A vein catheter was placed in the