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Bone Plate Composed of a Ternary Nanohydroxyapatite/Polyamide 66/Glass Fiber Composite: Biocompatibility In Vivo and Internal Fixation for Canine Femur Fractures

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Bone plates have been applied to fix fractures for over a hundred years. Metal plates are the gold standard. However, an increasing number of clinical practices and animal experiments have shown that metal plates have had incidents of failure due to their rigid fixation and long-term complications. Degradable composites present the advantages of a lower elastic modulus and absorbable properties but are unsuitable for load-bearing applications. Nondegradable bone plates composed of a nanohydroxyapatite/polyamide 66/glass fiber (n-HA/PA66/GF) composite are prepared, which have enough strength and a low elastic modulus for an internal fixation device. To better assess its function as a bone plate, animal experiments are conducted using a canine load-bearing femur fracture model. The results show that the n-HA/PA66/GF plate can fix fractures effectively. Gross observation, radiographic films, and histological analysis all show that the n-HA/PA66/GF plate leads to a secondary (indirect) union with obvious callus formation, whereas the titanium plate leads to primary (direct) union due to rigid fixation. Furthermore, the histological results reveal that new bone grows at the interface and that the n-HA/PA66/GF plate can integrate with native bone tissue. Consequently, the n-HA/PA66/GF composite shows good potential as a bone plate to fix loading-bearing bone fractures.

1. Introduction

Internal fixation using bone plates has shown advantages for long bones fractures. Metal materials such as stainless steel and titanium and its alloys remain the gold standard, as they can fix fractures very well. However, they are not the ideal bone plate considering the negative effects on callus formation and the healing of fractures caused by the high elastic modulus and biomechanical mismatch with bone. Therefore, metal plates may cause regional osteoporosis and fatigue breakage with long-term implantation in the body. Thus, a second surgery may sometimes be needed to remove the metal plates. Many efforts have been made to obtain a better or more ideal plate material. Some candidates are degradable polylactic acid (PLA), poly(L-lactide) (PLLA) and its composites,[1] titanium alloys with a lower elastic modulus and magnesium based metal.[2,3] Additionally, degradable materials intrigue the interest of scientists and doctors because they can be absorbed and may be conducive to osteogenesis. However, PLA and PLLA and their composites are only used in maxillofacial and malleolar fractures since their mechanical properties and long-term reactions associated with their acidic degradation products often limit the application in load-bearing bones.[4] Numerous advancements have been achieved in magnesium-based metals allowing Mg and its alloys to be tailored to accommodate the desired mechanical properties and degradation behavior. However, hydrogen gas formation and degradation rate ultimately prevented their clinical success.[3c]

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Based on clinical experience, bone plates with sufficient strength to fix fractures, an elastic modulus matched with bone, nondegradable properties without complications due to degradation products, and no interference with radiographic film are promising alternatives compared to metal plates. These nondegradable plates would cease to function after a fracture heals because of their matched biomechanical properties. Although a small number of publications have reported their efforts in making a nondegradable bone plate, animal experiments, particularly using large animals, have rarely been reported. We thought the main reasons might be due to the biocompatibility and biomechanical properties. We have been focusing on the study of nondegradable biomaterials for decades. A composite of nanohydroxyapatite/polyamide 66 (n-HA/PA66) is one such biomaterial used in the clinic successfully as an interbody fusion cage in spinal surgery and bone defect filling material.[5] Clinical experience and previous results have suggested that the n-HA/PA66 composite can remain permanently in the host tissue as a bioactive nondegradable material that meets the requirements of a bone analog.[6] However, insufficient strength limits its internal fixation applications since the bending strength of the n-HA/PA66 composite is almost 30–40 Mpa.[5e] Adding fibers has been a method frequently used to reinforce the strength of materials. Therefore, we fabricated a bone plate made of a ternary nanohydroxyapatite/polyamide 66/glass fiber (n-HA/PA66/GF) composite. The bending strength of this composite was nearly 180 MPa when the load was located at the third hole of the plate. Further experiments showed that the n-HA/PA66/GF plate had good biocompatibility and biomechanical properties, which showed its potential as an internal fixation device.[7] In the present study, we employed a canine femur fracture model of to assess the effects of the n-HA/PA66/GF plate on fracture healing and callus formation. Meanwhile, biocompatibility in vivo was evaluated.

2. Results

2.1. Biocompatibility In Vivo

2.1.1. Biocompatibility with Muscle

After implantation for 1 week, erector spinae muscles embedded with the n-HA/PA66/GF rod had no swelling or infection by gross observation. Histological analysis showed that muscle fibers had slight disarray and edema after the first week, while myolysis, necrosis, or inflammatory response was discovered by hematoxylin and eosin staining. After 4 weeks, the n-HA/PA66/GF rod was still wrapped by muscles, with no change in appearance by gross observation compared to the first week. Histological results showed that the muscle fibers arrayed in parallel without morphologic abnormalities, edema, necrosis, or inflammation. Similar results were observed at 12 week by both gross and histological analysis (**Figure 1**A).

2.1.2. Biocompatibility with Bone

We applied a bone implantation experiment to evaluate the biocompatibility of n-HA/PA66/GF with bone. After implantation for 1 week, the gross specimen showed that the n-HA/PA66/GF rods were fixed in the tibia, and the surrounding bone

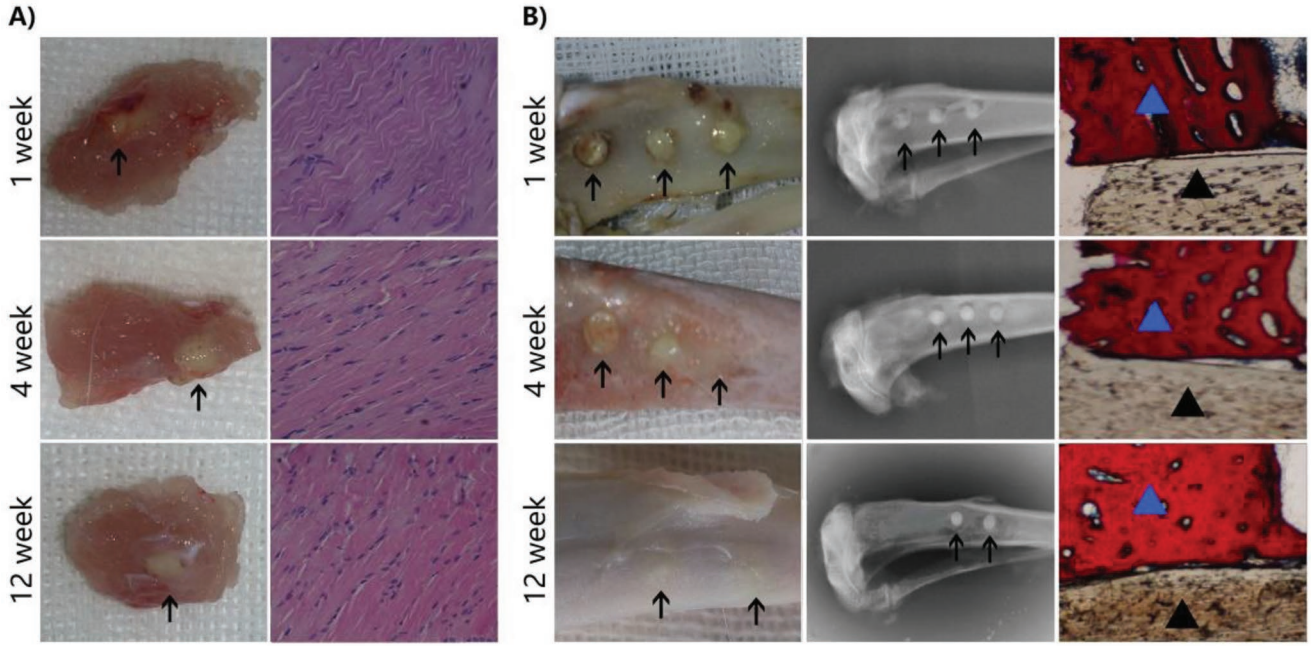

Figure 1. A) Biocompatibility of n-HA/PA66/GF with muscle at 1, 4, and 12 weeks. (Left: gross specimen of the n-HA/PA66/GF rod embedded in the erector spinae muscle; Right: hematoxylin and eosin staining of muscle adhered to the n-HA/PA66/GF rod; all at × 100 magnification). B) Biocompatibility of n-HA/PA66/GF with bone at 1, 4, and 12 weeks. (Left: gross specimen of tibia with the n-HA/PA66/GF rod; Middle: X-ray film of the n-HA/PA66/GF rod implanted in the tibia; Right: Van Gieson staining at the interface of the n-HA/PA66/GF rod and the tibia cortex; all at × 100 magnification). Note: Arrow represents the n-HA/PA66/GF rod; the blue triangle represents cortical bone; the black triangle represents the n-HA/PA66/GF plate.

tissue did not have an abnormal appearance. We identified radiolucent lines at the margin of the n-HA/PA66/GF rods on X-ray film. Histological results by Van Gieson staining revealed that there was no necrosis, osteolysis, or inflammatory response in the surrounding bone tissue. Additionally no fibrous tissue was identified at the interface between the bone and the n-HA/PA66/GF rod. After 4 weeks, bone tissue gradually grew on the bottom surface of the n-HA/PA66/GF rod on gross specimen. However, there were no obvious changes on X-ray images in comparison to the first week. We also could discover that bone tissue began to grow on the surface of n-HA/PA66/GF rod by Van Gieson staining. At 12 weeks, the radiolucent lines at the margin of the n-HA/PA66/GF rods disappeared on X-ray film. Van Gieson staining showed that bone tissue was able to attach tightly to the n-HA/PA66/GF rod. Additionally, the n-HA/PA66/GF rods implanted in the tibia did not degrade or become absorbed at each time point by gross and histological assessment. (Figure 1B).

2.2. Internal Fixation of Canine Femur Fractures

2.2.1. General Condition of Animals

The physical condition of all animals was good after surgery. There was no infection or exudate at the surgical wound site. After internal fixation, the animals could limp without a cast or immobilization. All animals fixed with the titanium or n-HA/PA66/GF plate could walk without claudication after three months.

2.2.2. Fracture Healing and Callus Formation by Radiographic Assessment

The n-HA/PA66/GF plate was conducive to observation and evaluation of the healing status and callus formation of the fractures because of the radiolucency of n-HA/PA66/GF. However, fracture healing was hard to assess after titanium plate fixation, specifically on anteroposterior X-ray film (**Figure 2**A). During the healing process, obvious callus formation surrounding the fracture gap was observed which was considered secondary healing in the n-HA/PA66/GF group on radiographic films. However, animals had primary fracture healing using the titanium plate, given that we did not discover callus formation at the fracture gap on either X-ray or computed tomography (CT) films (Figure 2B).

All femur fractures had a union in two groups by consecutive observation on radiographic film. There was no breakage of the n-HA/PA66/GF plates, titanium plates, or screws. Additionally, we did not observe any postoperative fixation reduction loss of the fractures in the two groups. According to the X-ray film, four animals had bone union at 12 weeks when the fractures were fixed by n-HA/PA66/GF plates, whereas the other five had union at 16 weeks. The calluses were considerable at 8 weeks and decreased and disappeared gradually 24 and 52 weeks after the fracture healed. In the titanium plate group, all animals obtained bone union at 12 weeks on X-ray film. However, we could identify the fracture gap more easily on CT film at 8 weeks, which could possibly be missed on X-ray. Furthermore, the X-ray and CT films showed no visible callus formation surrounding the fracture gap at each time point during the healing process. (**Figure 3**).

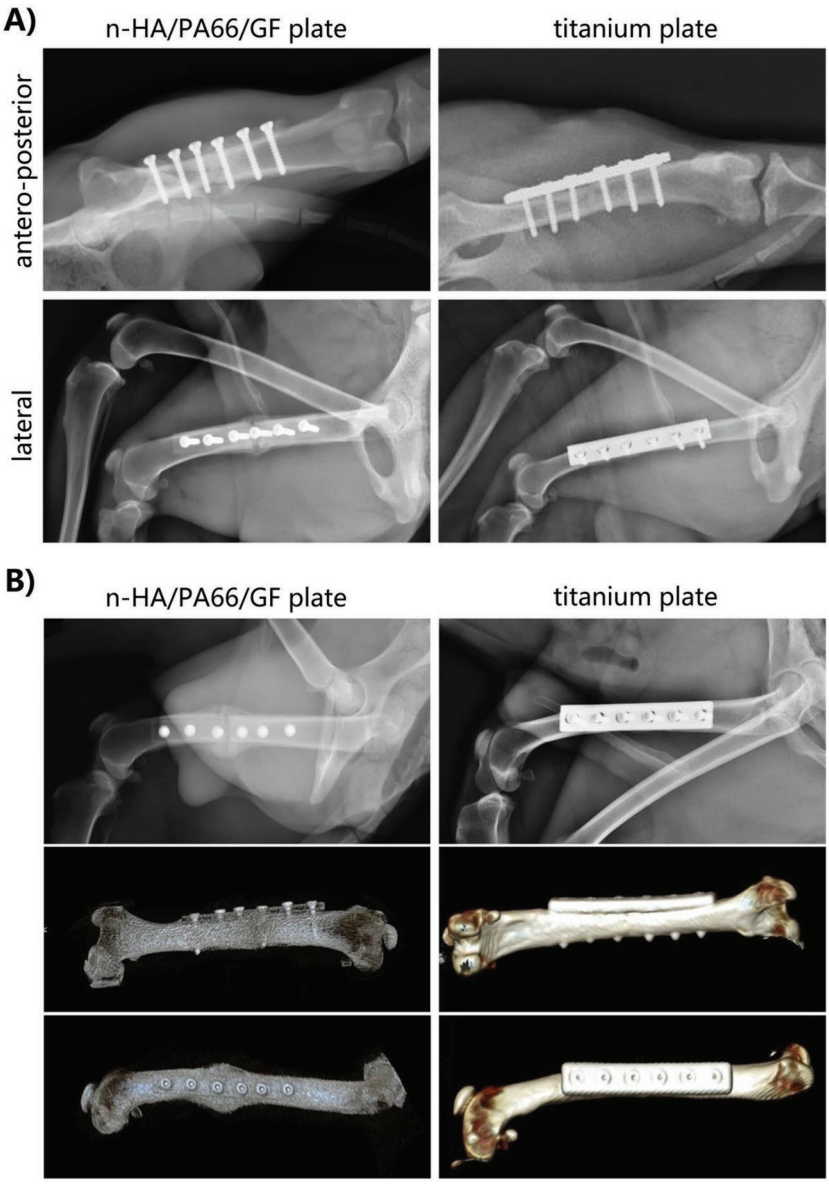

Figure 2. A) Antero-posterior and lateral views of X-ray film to assess the healing status of femur fractures (Left: fracture fixed by the n-HA/PA66/GF plate; Right: fracture fixed by a titanium plate). B) Callus formation of fractures at 8 weeks on X-ray and CT films in the n-HA/PA66/GF and titanium groups (Left: fracture fixed by the n-HA/PA66/GF plate; Right: fracture fixed by a titanium plate).

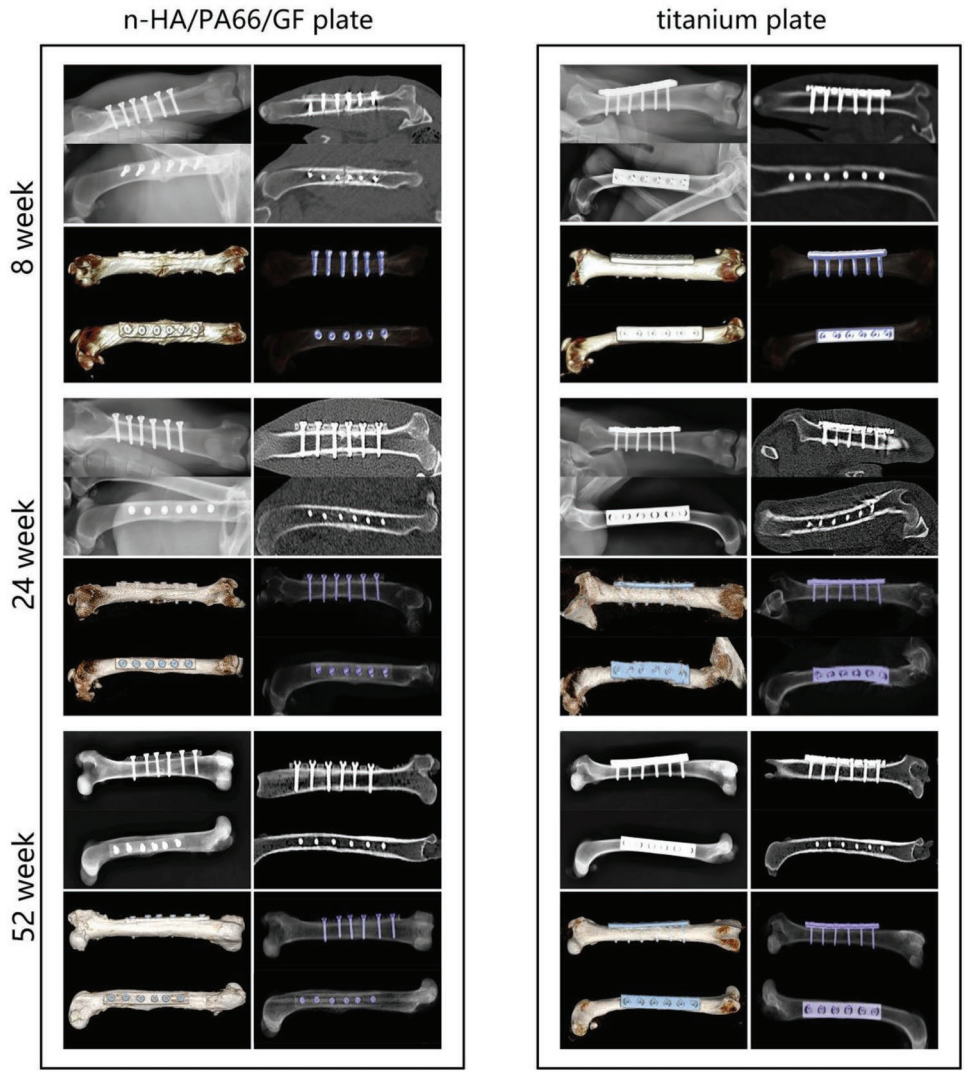

Figure 3. Healing process of the femur fracture on X-ray and CT films at 8, 24, and 52 weeks in the n-HA/PA66/GF and titanium groups (Left: fracture fixed by the n-HA/PA66/GF plate; Right: fracture fixed by a titanium plate). Note: High-definition pictures can be viewed in the supplemental data.

2.2.3. Fracture Healing and Callus Formation by Gross Assessment

Gross specimens of plate-bone sets showed similar patterns of facture healing and callus formation as the radiographic results between the two groups. Both the n-HA/PA66/GF and titanium plates could fix fractures without the loss of reduction of fracture and breakage of the plate. No osteonecrosis or visible osteoporosis beneath the plate was discovered. In the n-HA/PA66/GF group, we could identify considerable callus formation surrounding the fracture gap at 12 weeks, particularly on cross-section (**Figure 4**). The callus volume decreased gradually at 24 weeks and almost disappeared at 52 weeks, which is consistent with the healing process. The bone appearance and shape at the fracture gap had remolded well into comparatively normal bone after healing. Furthermore, we did not discover deformation or degradation of the n-HA/PA66/GF plates. In the titanium group, there was no callus formation at each time point. At 12 and 24 weeks when fractures had healed, we discovered an abnormal appearance at the fracture line, which had been remolded at 52 weeks (Figure 4). More specifically, we found that in some cases in the titanium group, the X-rays showed that animals had bone union in the early months, although there was a lower density area and distinctly different appearance compared to normal bone at the facture gap which could be identified on the CT images or gross specimen (**Figure 5**).

2.2.4. Callus Formation and the Interface of the Bone-Plate by Histological Assessment

After 12 weeks of fixation by the n-HA/PA66/GF plate, we discovered that considerable calluses were formed either longitudinally or transversely at the fracture gap, which was filled with immature bone tissue as evidenced by Van Gieson staining. Additionally, there was an obvious space between the plate and bone. At 24 weeks, bone tissue at the fracture gap matured and formed a compact lamellar bone gradually. The trabecular sheets

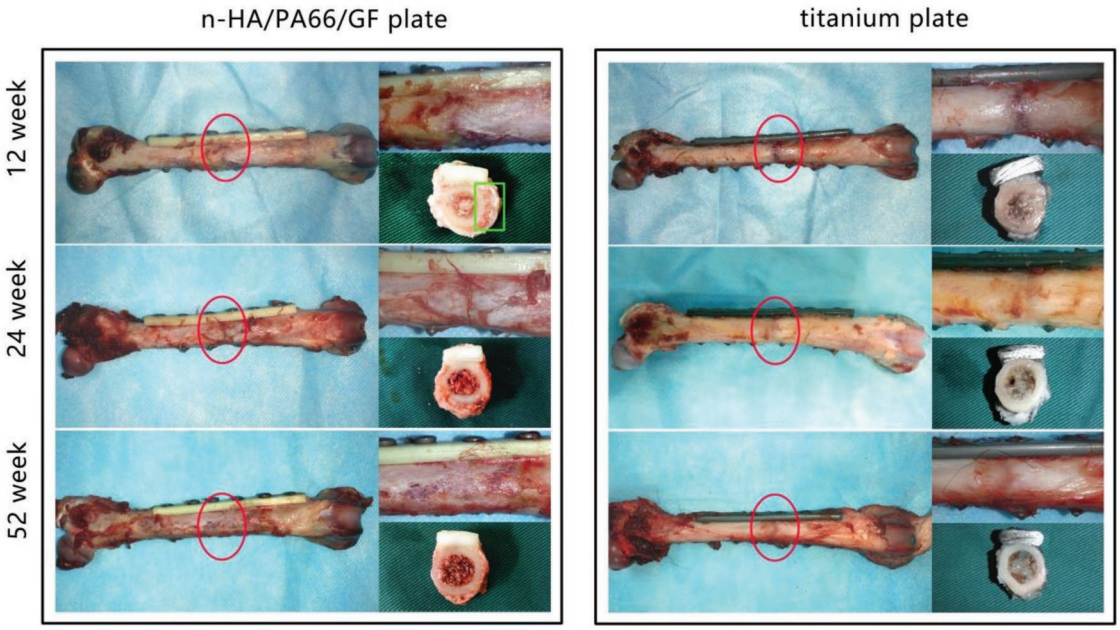

Figure 4. Gross specimen of bone-plate sets to assess the healing process and callus formation at 12, 24, and 52 weeks in the n-HA/PA66/GF and titanium groups (Left: femur fracture fixed by the n-HA/PA66/GF plate; Right: femur fracture fixed by a titanium plate). Note: Right pictures of each group represent the lateral and cross-section views of the fracture gap. The red circle represents the fracture gap; the green box represents a callus.

at the fracture gap became thick and compact and the intertrabecular spaces became narrow or gradually closed during this period. New bone began to grow into the interface between the n-HA/PA66/GF plate and bone. At 52 weeks, the bone tissue at the fracture had continued to change shape. The fracture gap had disappeared and was filled with thicker trabecula, which had almost turned into mature lamellar bone. At the interface between the plate and bone, we observed mature bone tissue formation which was attached tightly to the n-HA/PA66/GF plate. Overall, there were more new bone than at 24 weeks at the interface, and hence, more regions of apparent contact of bone with the n-HA/PA66/GF plate. Additionally, the n-HA/PA66/GF plate did not show any degradation during the healing process. At each time point, there was no resorption, osteonecrosis of bone, or inflammatory response beneath the plate by histological observation. (**Figure 6**) In the titanium plate group, there was no obvious or very few calluses formation around the fracture gap at each time point. New bone grew into the interface between the titanium plate and the bone as the same as that in the n-HA/PA66/GF group. However, we identified a space in almost all regions at the interface between the titanium plate and bone tissue at 52 weeks which suggested that new bone could hardly grow on the surface of the titanium. Furthermore, bone tissue resorption or necrosis was observed in some region beneath the titanium plate. (Figure 6).

3. Discussion

Metal plates have been the gold standard for internal fixation in load-bearing bone fractures in clinical practice because of their high strength advantage. In the 1950s, the Association for the

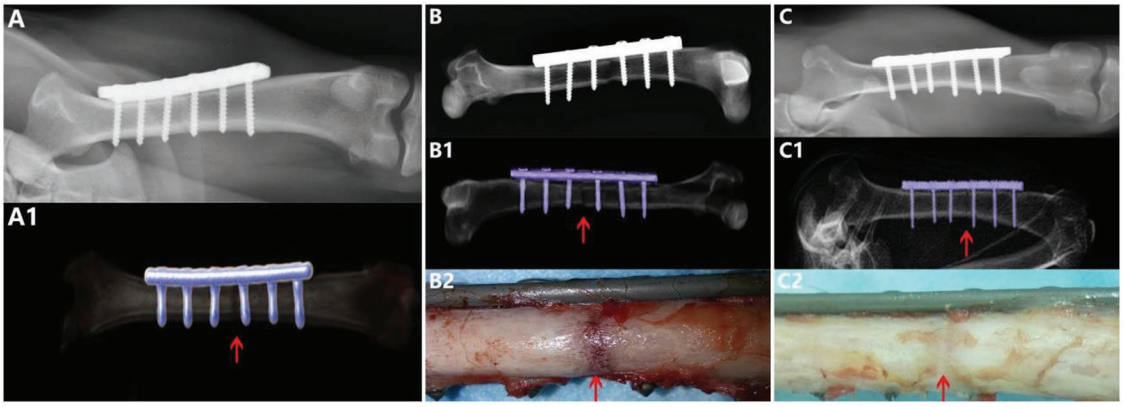

Figure 5. Appearance of the fracture gap in the titanium group. Left (8 weeks): A) X-ray; (A-1) CT film; Middle (12 weeks): B) X-ray; (B-1) CT film; (B-2) gross specimen; Right (12 weeks): C) X-ray; (C-1) CT film; (C-2) gross specimen. Note: Red arrow represents the fracture gap.

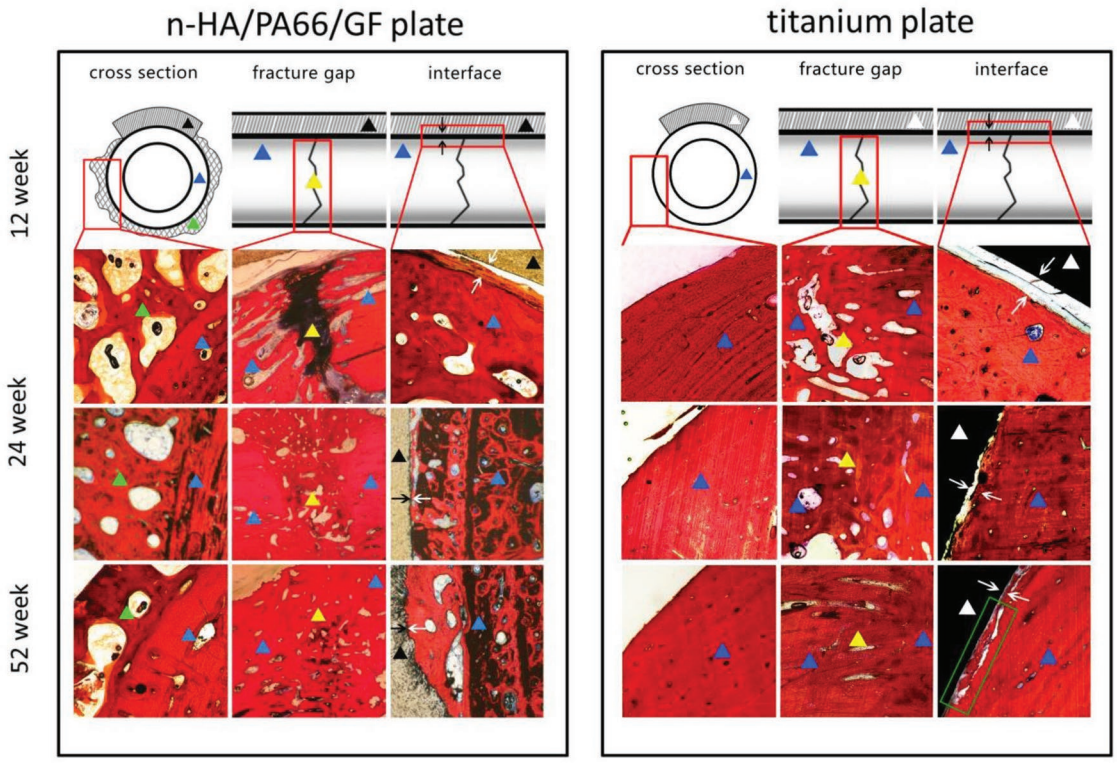

Figure 6. Van Gieson staining to assess callus formation and the interface of the bone and plate at 12, 24 and 52 weeks in the n-HA/PA66/GF and titanium groups. Left: callus formation at the cross section of the fracture gap (at ×100 magnification); Middle: healing status at the fracture gap (at ×40 magnification); Right: interface between the n-HA/PA66/GF or titanium plate and bone (at ×100 magnification). Note: The blue triangle represents cortical bone; the green triangle represents a callus; the yellow triangle represents the fracture line; the black triangle represents the n-HA/PA66/GF plate; the white triangle represents the titanium plate; the green box represents necrosis of bone tissues beneath the titanium plate; and arrows represent the bone-plate interface.

Study of Internal Fixation (AO/ASIF) was established whose principles and techniques emphasized the operative treatment of fractures, the mechanical aspects of internal fixation, and the absolute stability of fractures.[8] Rigid fixation became the goal and facilitated the primary bone healing of fractures. However, a rigid plate could lead to cortical osteoporosis, delayed bridging, and refractures after plate removal.[9] Practically, implants in contact with bone always participate in load transmission and may cause stress shielding. The amount of stress shielding depends on the rigidity of the implant and the coupling between the implant and bone.[8] The mismatch between the modulus of the plate and the cortical bone results in a situation where the majority of the load is transferred by the plate rather than by the underlying bone.[10] After rigid fixation, primary healing with insufficient callus formation decreases the intensity of bone,[11] and sometimes a second surgery to remove the plate is necessary. To overcome the shortcomings of the metal plate, degradable PLA, PLLA composites, and magnesium were assessed as internal fixation devices. These materials have a lower elastic modulus and can be absorbed. However, the strength and side effects of the degradation products have remained unsolved. Under this background, there was a time when nondegradable materials intrigued the interest of scientists and orthopedists since they have a lower Young's modulus, stable properties, and no interference with radiographic imaging.[12] Some of these have even been used in the clinic.[13] However, no attempt has been made in decades, probably because of the inferior mechanical strength and biocompatibility. As a matter of fact, there were no impressive achievements reported on nondegradable internal fixation devices.

Our goal was to fabricate a bone plate made up of a new material which can combine the advantages of both metal and biodegradable plates. The bone plate could fix fractures, facilitate fracture healing, and can exist in the body for eternity. Since n-HA/PA66 was first described as a biomimetic bone scaffold material by Wang et al.,[5e] it has been used in the clinic for over ten years. However, the mechanical strength limited its applications in internal fixation. Reinforcement with fibers is one of the most successful approaches to toughen brittle biomaterials. Glass fiber has good biocompatibility and is normally used to reinforce many composites.[14] For this reason, we made a new nondegradable composite of n-HA/PA66/GF for bone plates by adding glass fibers to reinforce the strength of n-HA/PA66. Biomechanical analysis indicated that the n-HA/PA66/GF plate had lower stiffness and satisfactory strength compared with titanium plates in previous experiments.[7b] Therefore, we further assessed the effects of the n-HA/PA66/GF plate as an internal fixation device in animal experiments.

In the present study, the n-HA/PA66/GF plate could fix canine femur shaft fractures without immobilizing the broken leg. This suggested that the n-HA/PA66/GF plate had good strength in vivo which was the first important factor for an

internal fixing plate. The elastic modulus of the n-HA/PA66/GF composites was 10–20 GPa,[7a] which was much lower than for metal plates. The bending and torsion stiffness for fixation was reduced by 34.1% and 56.8%, respectively, compared with titanium plate.[7b] During the fracture healing process, we found that there was visible callus formation around the fracture gap using the n-HA/PA66/GF plate, whereas no callus was formed using a titanium plate by gross observation, radiographic film, and histological assessment. This proved again that rigid fixation leads to the primary healing of fractures, but elastic fixation leads to secondary healing. Sufficient strength guaranteed the fragments without loss of reduction, but lower stiffness of fixation provided reduced stress shielding and facilitated interfragmentary movement, allowing the bone to carry more of a load and stimulating the formation of new bone to form a callus. Previous reviews and clinical experiences support that secondary healing of fractures with a callus may bring more strength to the new bone,[15] which may help decrease the risk of refractures. Based on these results, we easily obtained the conclusion that the n-HA/PA66/GF plate led to callus formation. However, previous in vitro experiments did not indicate that n-HA/PA66/GF could promote growth and osteogenic differentiation of mesenchymal stem cells (MSCs). One possible explanation is that the n-HA particles on the surface of n-HA/PA66/GF decreased when the glass fibers were added. Second, it was hard to imitate the biological circumstances at the fractures by performing cell experiments. Therefore, mechanical stimulation provided by the n-HA/PA66/GF plate may be the main factor for callus formation.

Femur shaft fractures took more time to heal after fixation with the n-HA/PA66/GF plate than titanium plate based on X-ray observations. Similar to the results in the present experiment, primary healing in clinical practice normally takes less time than secondary healing because fractures primarily heal directly through the haversian system. However, we found an interesting phenomenon in the titanium plate group, as the fracture line did not completely disappear by CT scan or gross specimen analysis in some cases, even when the X-rays supported that fractures had union in the early stage. First, the metal plate may interfere with the observation and evaluation of the healing status. Second, primary healing means a direct connection of the broken ends of the fracture through the haversian system. Thus, we may identify the healed scar at the original fracture site after a few months. This may enhance the reasonability that we should not remove the metal plate from a load-bearing bone before one year or even longer after fracture in clinical practice. In fact, there have been controversies as to which pattern of healing is better. However, even after union of the fracture, the stiffness or modulus mismatch between the bone and the plate can lead to osteopenia or even refractures.[16] Therefore, the ideal internal fixation should obtain a balance between biology and mechanical stability.[15c] Long-term implantation of the n-HA/PA66/GF plate in the body would have a less negative influence on bone loss given that the elastic modulus of the n-HA/PA66/GF composite is much lower than that of metal materials. Accordingly, we at least could conclude that the n-HA/PA66/GF plate facilitated callus formation by elastic fixation and can stay in the body after union.

An ideal bone plate should be matched and supported by a screw of the same material. Therefore, a screw made of n-HA/PA66/GF was prepared previously and was shown to fix intercondylar fractures.[7a] However, we experienced frequent screw breakage in fixing femur shaft fractures in our preliminary experiments. The strength of the n-HA/PA66/GF screw was not enough in a loading-bearing bone. In addition, unlike man, it was impractical to make the limb operated on unloaded and casted in dogs, so there was much more force on the plates and screws compared with clinical use. However, the stress shielding was mainly derived from plates. We considered that fixing factures using an n-HA/PA66/GF plate and metal screw would not create obvious stress shielding on the bone and could exist in the body eternally. The callus formation and secondary healing of fractures fixed by an n-HA/PA66/GF plate and metal screws proved this point.

Like metal, the n-HA/PA66/GF plate is nondegradable. The interface between bone and the implant influences the long-term effects of the internal fixation device. A good interface is beneficial to the stability of the implant and a decrease of the shear force at the interface of bone-plate.[17] In vitro tests showed that the n-HA/PA66/GF composite had good cytocompatibility with MSC and MC3T3-E1 cells which could adhere tightly to the surface of the material.[7] Adherence of cells, especially MSCs and osteoblasts on the surface of the material is the first step in good interface formation. The rough surface of the composite with n-HA particles may facilitate cell adherence and calcium deposition. From our results, we discovered there was a space between the bone and plate in the early weeks after implantation. Then, the space filled with new bone gradually, which suggested that the n-HA/PA66/GF surface had good biocompatibility with bone. Furthermore, the n-HA/PA66/GF plate could integrate and be connected with native bone in some regions where new bone grew into the n-HA/PA66/GF plate. This would also decrease the shield stress derived from internal devices. We also assessed the in vivo biocompatibility for long-term implantation. The results showed the n-HA/PA66/GF composite had no negative effects on muscle and bone tissues. Therefore, it is safe for the n-HA/PA66/GF plate to stay in the body for eternity.

4. Conclusions

In this study, we demonstrated that the n-HA/PA66/GF plate could fix fractures without breakage and had no interference with radiographic imaging. Compared with titanium plates, the n-HA/PA66/GF plate facilitated callus formation which led to secondary fractures healing. The n-HA/PA66/GF plate could integrate with native bone after long-term implantation. No negative effects on muscle or bone were found. Therefore, all these data support that the n-HA/PA66/GF plate could be used as an internal fixation device, and nondegradable materials may now be considered.

5. Experimental Section

Preparation of the n-HA/PA66/GF Bone Plate: The flow diagram provides information on the n-HA/PA66/GF bone plate preparation and

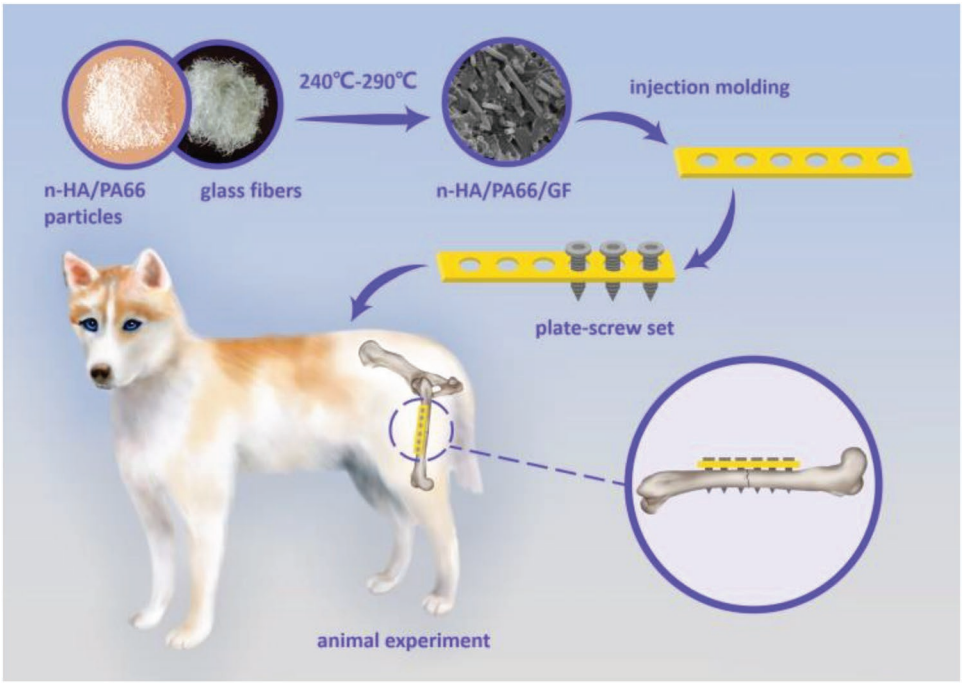

Figure 7. Schematic diagram: preparation of the n-HA/PA66/GF plate and experiment procedure.

animal experiments (**Figure 7**). In brief, PA66 (A3K, BASF, Germany) was completely dissolved in ethanol solution at a temperature of 70 °C. Then n-HA slurry (Sichuan Guona Technology Co., LTD) was prepared and gradually added into the PA66 and ethanol solution with vigorously stirring for 2 h. The mixture then was precipitated for 24 h at room temperature. After totally washing by deionized water and ethanol and being dried at 100 °C, the n-HA/PA66 composite particles were obtained for preparing n-HA/PA66/GF composite.[5d] The n-HA/PA66 particles were mixed with glass fibers with a 2:5:3 weight ratio of hydroxyapatite, PA66, and glass fibers. An extrusion method was used to prepare the n-HA/PA66/GF composite at 240–290 °C.[7a] Subsequently, the n-HA/PA66/GF bone plate (10 × 75 × 3 mm, with six holes) was obtained using an injection molding method. The titanium plate (10 × 75 × 3 mm, with six holes) and screw (Φ3.5 mm, 18 mm, or 20 mm length) used in this study were purchased from BaiDe Medical Instrument Co, Ltd. (Jiangsu, People's Republic of China). In addition, an n-HA/PA66/GF rod (Φ3.5 mm, 5 mm, or 10 mm) was used to study the biocompatibility in vivo. All devices, including the n-HA/PA66/GF bone plates and rods, and titanium plates and screws were sterilized by steam sterilization before implantation in animals.

Biocompatibility Assessment In Vivo: All animal experiments were performed in accordance with institutional guidelines under the approved protocols by the Chongqing Administration Rule of Laboratory Animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twelve skeletally mature New Zealand white rabbits with weight between 2.0 and 2.5 kg and at 4–6 months of age were used for this study, and bone and muscle implantation were performed. Before surgery, animals were anesthetized with a 3% pentobarbital sodium (1 mL kg^{-1}). For bone implantation, the hindlimb was shaved and disinfected, and the tibia shaft was exposed through sharp dissection. Two or three holes were drilled using slow drill speeds and n-HA/PA66/GF rods (Φ3.5 mm, 10 mm) were implanted. For muscle implantation, the lumbosacral region was shaved and disinfected. The unilateral erector spinae muscle was exposed with one n-HA/PA66/GF rod (Φ3.5 mm, 5 mm) embedded. After surgery, the animals were allowed to resume activity. X-ray films were taken at 1, 4, and 12 weeks after the operation. The X-ray tube was set at 60 kV and 40 mA. Then, animals were euthanized by an overdose of sodium pentobarbital via intravenous injection. Tibia with the n-HA/PA66/GF rod were harvested and fixed in 4% phosphate-buffered paraformaldehyde for Van Gieson staining. Muscle tissues directly connected to the n-HA/PA66/GF rod were detached carefully and fixed for hematoxylin and eosin staining.

Internal Fixation for Canine Femur Fractures: Eighteen male mongrel dogs were divided into two groups randomly according to implant materials. Under intraperitoneal anesthesia with 3% pentobarbital sodium (1 mL kg^{-1}) and standard aseptic surgical techniques, the femur was exposed through a lateral approach. A transverse fracture model was made with a 1–1.5 mm width defect by using Gigli saw at the middle part of the femur. The broken ends of the fracture were reduced by a clamp and fixed with either a titanium or n-HA/PA66/GF plate. Each plate-and-screw set consisted of 1 plate and 6 titanium screws. The fracture gap was located between the third and fourth holes. After fixation, the incision was closed in layers and the femur was left uncasted. The animals were kept in individual cages under standard conditions and allowed to resume activity after anesthesia recovery.

X-ray and CT scanning films were taken at 8, 12, 24, and 52 weeks after internal fixation to investigate the process of fracture healing. If the animals had no radiographic evidence of a bone union after 12 weeks, radiographic films were taken every four weeks until the fracture healed. The X-ray tube was set at 70 kV and 100 mA. CT images were obtained using dual-energy scanning (Somatom Definition Flash, Siemens Medical Solutions, Forchheim, Germany) at 80 and 140 kV. Then, the femurs with the plates and screws were extracted to observe the callus formation surrounding the fracture gap after euthanasia by intraperitoneal pentobarbital sodium overdose at 12, 24, or 52 weeks.

Histological Observation: All bone and muscle specimens were fixed in 4% phosphate-buffered paraformaldehyde immediately after harvesting. For hematoxylin and eosin staining, muscle tissues were embedded in paraffin and serially cut for histological analysis. For Van Gieson staining, the n-HA/PA66/GF or titanium plate-bone sets were dehydrated using graded ethanol solutions (60%, 70%, 80%, 90%, 100%, and 100%) and then embedded in epoxy resin. After that, 50 µm slides were made to assess the callus formation and the interface between bone and the titanium or n-HA/PA66/GF plate. The plate-bone sets were sectioned either longitudinally or transversely at the fracture gap.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biocompatibility, bone plate, fractures, internal fixation, nanohydroxyapatite/polyamide 66/glass fiber

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[1] V. Guarino, F. Causa, P. Taddei, M. D. Foggia, G. Ciapetti, D. Martini, C. Fagnano, N. Baldini, L. Ambrosio, *Biomaterials* **2008**, *29*, 3662.

[2] a) G. J. Thompson, D. A. Puleo, *Biomaterials* **1996**, *17*, 1949; b) Y. Guo, D. Chen, W. Lu, Y. Jia, L. Wang, X. Zhang, *Biomed. Mater.* **2013**, *8*, 055004.

[3] a) P. Han, P. Cheng, S. Zhang, C. Zhao, J. Ni, Y. Zhang, W. Zhong, P. Hou, X. Zhang, Y. Zheng, *Biomaterials* **2015**, *64*, 57; b) B. Schaller, N. Saulacic, T. Imwinkelried, S. Beck, E. W. Liu, J. Gralla, K. Nakahara, W. Hofstetter, T. Iizuka, *J. Cranio-Maxillofacial Surg.* **2016**, *44*, 309; c) M. P. Staiger, A. M. Pietak, J. Huadmai, G. Dias, *Biomaterials* **2006**, *27*, 1728; d) F. Witte, *Acta Biomater.* **2010**, *6*, 1680.

[4] a) A. Kukk, J. T. Nurmi, *Foot Ankle Surg.* **2009**, *15*, 192; b) K. C. Yerit, G. Enislidis, C. Schopper, D. Turhani, F. Wanschitz, A. Wagner, F. Watzinger, R. Ewers, *Oral Surg., Oral Med., Oral Pathol., Oral Radiol., Endod.* **2002**, *94*, 294; c) T. Nieminen, I. Rantala, I. Hiidenheimo, J. Keränen, H. Kainulainen, E. Wuolijoki, I. Kallela, *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 1155; d) T.-Y. Yang, C.-H. Huang, Y.-C. Lu, H.-C. Chen, C.-Y. Lin, C.-H. Huang, *Formosan J. Musculoskelet. Disorders* **2012**, *3*, 27.

[5] a) M. Huang, J. Feng, J. Wang, X. Zhang, Y. Li, Y. Yan, *J. Mater. Sci.: Mater. Med.* **2003**, *14*, 655; b) L. Zhang, Y. Li, X. Wang, J. Wei, X. Peng, *J. Mater. Sci.* **2005**, *40*, 107; c) Y. Xiong, C. Ren, B. Zhang, H. Yang, Y. Lang, L. Min, W. Zhang, F. Pei, Y. Yan, H. Li, *Int. J. Nanomed.* **2014**, *2014*, 485; d) H. Wang, Y. Li, Y. Zuo, J. Li, S. Ma, L. Cheng, *Biomaterials* **2007**, *28*, 3338; e) X. Wang, Y. Li, J. Wei, G. K. De, *Biomaterials* **2002**, *23*, 4787.

[6] a) Y. Zhang, Z. Quan, Z. Zhao, X. Luo, K. Tang, J. Li, X. Zhou, D. Jiang, *PLoS One* **2014**, *9*, e96265; b) X. Yang, Y. Song, L. Liu, H. Liu, J. Zeng, F. Pei, *Orthopedics* **2012**, *35*, 66; c) Y. Zhang, X. Deng, D. Jiang, X. Luo, K. Tang, Z. Zhao, W. Zhong, T. Lei, Z. Quan, *Sci. Rep.* **2016**, *6*, 26751.

[7] a) B. Su, X. Peng, D. Jiang, J. Wu, B. Qiao, W. Li, X. Qi, *PLoS One* **2013**, *8*, e68342; b) B. Qiao, J. Li, Q. Zhu, S. Guo, X. Qi, W. Li, J. Wu, Y. Liu, D. Jiang, *Int. J. Nanomed.* **2014**, *9*, 1423.

[8] D. L. Helfet, N. P. Haas, J. Schatzker, P. Matter, R. Moser, B. Hanson, *J. Bone Joint Surg., Am. Vol.* **2003**, *85-A*, 1156.

[9] H. K. Uhthoff, P. Poitras, D. S. Backman, *J. Orthop. Sci.* **2006**, *11*, 118.

[10] a) R. Huiskes, H. Weinans, R. B. Van, *Clin. Orthop. Relat. Res.* **1992**, *274*, 124; b) S. Benli, S. Aksoy, H. Havitcioğlu, M. Kucuk, *J. Biomech.* **2008**, *41*, 3229.

[11] A. Completo, F. Fonseca, J. A. Simões, *J. Biomech.* **2008**, *41*, 560.

[12] K. Fujihara, Z.-M. Huang, S. Ramakrishna, K. Satknanantham, H. Hamada, *Biomaterials* **2003**, *24*, 2661.

[13] M. S. Ali, T. A. French, G. W. Hastings, T. Rae, N. Rushton, E. R. Ross, C. H. Wynn-Jones, *J. Bone Jt. Surg., Br. Vol.* **1990**, *72*, 586.

[14] a) M. F. Arif, N. Saintier, F. Meraghni, J. Fitoussi, Y. Chemisky, G. Robert, *Composites, Part B* **2014**, *61*, 55; b) R. Krüger, J. Groll, *Biomaterials* **2012**, *33*, 5887.

[15] a) L. E. Claes, C. A. Heigele, C. Neidlinger-Wilke, D. Kaspar, W. Seidl, K. J. Margevicius, P. Augat, *Clin. Orthop. Relat. Res.* **1998**, *355*, S132; b) R. Marsell, T. A. Einhorn, *Injury* **2011**, *42*, 551; c) S. M. Perren, *J. Bone Jt. Surg., Br. Vol.* **2002**, *84*, 1093.

[16] H. K. Uhthoff, F. L. Dubuc, *Clin. Orthop. Relat. Res.* **1971**, *81*, 165.

[17] J. D. Bobyn, E. S. Mortimer, A. H. Glassman, C. A. Engh, J. E. Miller, C. E. Brooks, *Clin. Orthop. Relat. Res.* **1992**, *274*, 79.