BONE EXTRACELLULAR MATRIX ORIENTATION COMPOSED OF APATITE AND COLLAGEN, AND DEVELOPMENT OF NOVEL MEDICAL DEVICES FOR PROMOTING THE ORIENTATION BY METAL 3D PRINTING

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Abstract - Bone tissue exhibits specific oriented extracellular matrix (ECM) architecture derived from apatite orientation closely related to the collagen fiber alignment. Preferential orientation and the degree of collagen/apatite in bone ECM change depending on the anatomical position, *in vivo* stress distribution, regeneration, pathology, gene-defect, etc.

A novel design concept was proposed for medical devices, e.g., an intervertebral spacer that guides a sound bone characterized by preferentially oriented collagen/apatite along the cephalocaudal axis in the vertebral body. The spacer with a honeycomb tree structure (HTS) composed of through-pore and grooved substrate manufactured by metal 3D printing, concretely, laser powder bed fusion (PBF-LB/M), was developed and subsequently started to be applied as the clinical use since 2022. The novel spacer with HTS significantly shortened the period for guiding the healthy ECM preferential orientation compared to the conventional spacer with crushed autologous bone.

Keywords: spinal spacer, honeycomb tree structure (HTS), metal additive manufacturing, bone quality, orientation of apatite crystal

Introduction

It is known in the field of biomaterials science that crystallographic texture and orientation—long-range ordered arrangements of atoms, ions, and molecules—strongly dominate the mechanical and/or functional properties of materials like metals, ceramics, polymers, and their composites. The biological apatite crystal, for example, is a major inorganic component of bone ECM that belongs to a hexagonal crystal system, which normally has greater anisotropy than the cubic crystal system, providing the optimal strength in bone.

In this article, preferential apatite/collagen orientation is introduced as a bone quality parameter [1], and subsequently the medical device design, for example, an intervertebral spacer which is suitable for the preferential orientation of apatite and the related collagen fiber in bone tissues was proposed [2,3].

Experimental

The preferential alignment of the apatite *c*-axis and the related properties were analyzed using the bio-material scientific and biological techniques, µXRD, pQCT, nano-indentation, etc. Subsequently, devices design was developed on the basis of bone microstructural anisotropy.

The spacer possessing an anisotropic through-pore with a grooved substrate, that we termed “honeycomb tree structure (HTS)”, was designed for guiding bone ECM orientation; it was manufactured using a laser beam powder bed fusion method through 3D printing processes [2,3].

The newly designed spacers were implanted within and between sheep vertebral bodies and for 8 and 16 weeks. An autologous bone was not installed in the newly designed spacer. A push-out test was performed to evaluate the mechanical fixation of the spacer/bone interface. Additionally, the preferential orientation of bone matrix dominantly consisting of collagen fiber and apatite crystal was determined.

Results and Discussion

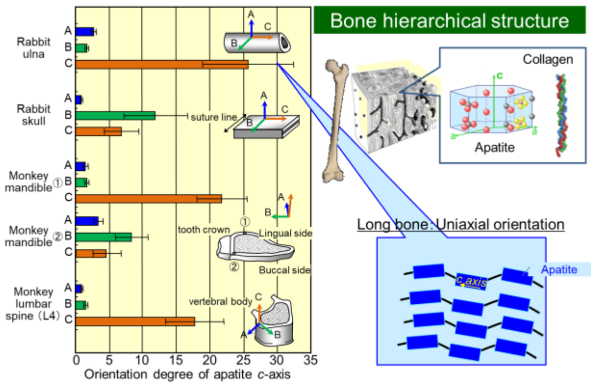

Figure 1 Preferential orientation degree of apatite *c*-axis depending on the anatomical portion in various cortical bones.

Figure 1 shows variation in preferential orientation degree of apatite crystallites in typical cortical bones of rabbit ulna, rabbit skull, monkey dentulous mandible and monkey vertebral body. Preferential alignment and degree of the *c*-axis of the apatite was evaluated by the relative intensity between (002) and (310) µXRD peaks. Preferential alignment of apatite in each bone tissue change strongly depending on the shape and *in vivo* stress distribution; that is, the *c*-axes of apatite in the rabbit ulna and monkey vertebral body are preferentially oriented as a one-dimensional orientation along the longitudinal axis and the cephalocaudal axis, respectively. In contrast, the rabbit skull bone has a two-dimensional orientation along the surface. The *c*-axis of apatite in a monkey dentulous mandible basically alignes along the mesiodistal direction, but this alignment changes along the biting direction near the tooth crown due to the mastication force. This suggests that the microscale measurement of apatite crystallographic orientation related to the collagen template is one of the bone quality indices for evaluating mechanical function and *in vivo* stress distribution in calcified tissues.

During the bone regeneration process, new bone formation is active, filling the bone defect with new bone tissue and increasing BMD. However, the recovery of bone ECM orientation is significantly delayed compared to the recovery of BMD. As a result, recovery of mechanical properties such as Young's modulus is also significantly retarded. In other words, it is essential that bone formation around the bone device be strategized in such a way as to restore the apatite/collagen orientation of the bone ECM [4].

Based on the preferential alignment of bone ECM composed of collagen/apatite, the potential of a novel intervertebral spacer with HTS in Fig. 2 developed using a design strategy for guiding an anisotropic bone ECM structure. The novel spacer enabled functional fusion to the surrounding bone with one dimensional orientation by inducing new one dimensionally oriented bone with a highly oriented collagen/apatite micro-organization along the cephalocaudal axis, in which maximum principal stress is applied.

The HTS shows potential for replacing autologous bone grafts, as bone formation occurred directly on the HTS surface, avoiding the bone resorption. Thus, accelerated guidance of the bone ECM orientation of collagen/apatite can be achieved by the HTS structure. The proposed intervertebral spacer with the HTS can overcome the limitations of innate regeneration of the oriented bone ECM microstructure,. The HTS allows for immediate texture formation of the regenerated bone matrix, which cannot occur during the innate recovery process. Mature regeneration of the anisotropic bone matrix enables stress transmission from the host bone, resulting in the formation of fully functionalized vertebrae. Osteoblast extension and alignment is one factor leading to the generation of the oriented collagen/apatite microstructure [5], which can be induced by an anisotropic topology with optimal dimensions in the substrate plane.

The spacer with HTS composed of through-pore and grooved substrate was designed and manufactured using metal 3D printing technology (PBF-LB/M) and subsequently implanted in the sheep vertebral body. The newly designed spacer with HTS induced hierarchically anisotropic trabecular-like bone with preferentially elongated trabeculae at micro-millimeter scale and bone ECM orientation at nanometer scale within a trabecula, both of which were parallel to the groove direction. This hierarchical anisotropic structure, mimetic to normal trabecular bone, was already formed 8 weeks spacer implantation. HTS was shown to have a strong ability to guide the ECM-oriented functional bone.

The novel spacer exhibited substantially greater strength at the spacer/host bone interface at 8 weeks post-implantation than a conventional and gold-standard spacer with autologous bone graft. The HTS equipped in the novel spinal spacer aids the rapid fixation between vertebral bodies as shown in Fig. 2. This is because the new bone ECM structure composed of apatite *c*-axis and collagen fiber aligns parallel to the grooved direction, which is similar to the sound vertebral body with one-dimensionally orientation of ECM along the cephalocaudal axis.

Furthermore, autologous bone tends to delay bone formation in the cage and maturation due to the need for the absorption. It was suggested that the introduction of autologous bone may have a negative effect on the functional recovery of the bone.

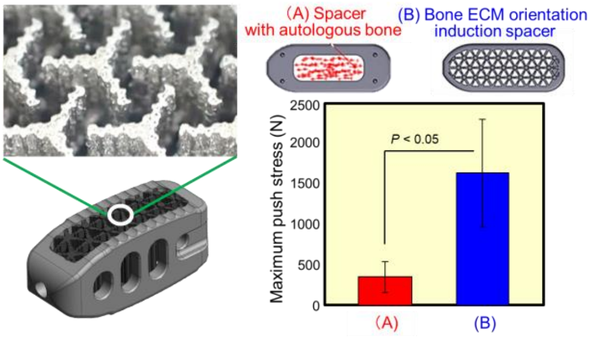

Figure 2 The novel intervertebral spacer with honeycomb tree structure guiding the preferential orientation of apatite and collagen in the early stage of new bone formation, resulting in strong fixation strength.

Conclusions

HTS provided a direct scaffold for bone formation with the preferential alignment of apatite/collagen orienation, resulting in functional fusion between the novel spacer and bone at an early stage. This new concept focusing on the artificial formation of the sound bone ECM orientation provides useful guidelines for the design of highly functionalized bone devices in near future.

Acknowledgements

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References

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