（様式　乙 8）

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学 位 論 文 内 容 の 要 旨

Introduction

An ideal bone substitute should satisfy criteria such as being osteoinductive, osteoconductive, and bioresorbable. An outstanding bone substitute, α-calcium sulfate hemihydrate (α-CSH), can be used instead of autologous bone grafts thanks to its excellent biocompatibility, osteoconductivity, easy availability, and biodegradability. The α-CSH is regarded as a prime type of bone substitute that plays a crucial role in bone formation by facilitating the ingrowth of bone. An innovative α-CSH bioceramic have been synthesized from green processing technology (microwave-irradiation treatment) with superior blood wettability and biocompatibility. Histopathological evaluation from an *in vivo* test of chick chorioallantoic membrane (CAM) confirmed that the innovative α-CSH bioceramic not only induces angiogenesis but also enables osteogenesis potential. In the present study, the innovative α-CSH bioceramic as a bone graft substitute has been implanted in artificially-created defects of rabbit models to analyze new bone formation and material degradation.

Materials and methods

The rabbit model (New Zealand White rabbits) was used in this study. The rabbits were divided into three groups based on the type of filling materials namely α-CSH group, control group, and blank group. The bilateral implantation was performed among rabbits in 6 mm diameter and 7 mm depth artificially-created defects. The α-CSH (n = 4) and control material (n = 4) were randomly implanted in the animal's left or right femur lateral condyle cortical bone after defect drilling; in the blank group (n = 4), the created defects were left unfilled. The materials were implanted as a powder with approximately 0.2 g of material added in each defect. The healing process and bone formation was observed at each time point (2, 4, 8, and 12 weeks). Samples were scanned using μ-CT (Bruker Skyscan 1176, Kontich, Belgium) at 18 μm resolution. For histopathological analysis, the samples were fixed in 10% neutral-buffered formalin, dehydrated in ethanol, embedded in paraffin wax, trimmed, and stained with hematoxylin and eosin (H&E) for histopathological observation. The post-hoc Tukey HSD (Honestly Significant Difference) was used for multiplicity of contrasts with $p < 0.05$ considered as the level of significance. The value of each variable from the multiple readings was presented as mean ± standard deviation.

Results

The innovative α-CSH with uniform nanostructured crystals can be synthesized using microwave-irradiation treatment. The μ-CT analysis results from the rabbit model showed that the new bone volume was lower than 10% in all treatment groups at an early stage, in weeks 2 and 4. Further, after 8 weeks, the new bone volume in defects showed a trend of increase more in the α-CSH group than other groups. At the final observation at 12 weeks, the tissue remodeling by α-CSH group had decreased the new bone volume, yet in other groups this increased at the same time as regular mineral density. This unique phenomenon could be explained due to the tissue remodeling process that caused the dense callus to be withdrawn in α-CSH but not in blank and control material. Moreover, the degradation of α-CSH and control material was similar at 4 and 8 weeks (87% at 4 weeks and 47~50% at 8 weeks). However, the α-CSH group it reduced more at 12 weeks (21% remained), whereas in the control material, 38% residues remained in the defects.

In histopathological evaluation, the α-CSH group was repaired with lamellar bone and well-grown bone marrow infiltration similar to the control material. Therefore, analytical results demonstrate that the α-CSH possessed a faster degradation rate, more new bone volume formation, and better healing progress than the control material in rabbit model under the same conditions.

Discussion

Calcium sulfate has been recommended for use in the management of osseous defects after curettage of osteomyelitis, benign lesion, and trauma. A high bone formation cloud have also been discovered in previous studies which found that calcium sulfate produces significantly more new bone formation and is completely effective in preserving alveolar bone in post-extraction ridge dimensions. The new bone mineralization rate of calcium sulfate is similar to the rate of autograft material leading to its promising application in clinical practice. In the previous study, the innovative α-

CSH with uniform and specific crystal structure had more water absorption ability in a dehydrated state for setting. The rapid setting causes the proteins and growth factors in blood to be quickly absorbed into the bone defect area with implanted α-CSH, hence offering enough nutrition for the osteoblasts to promote the formation of angiogenesis. In addition, when calcium sulfate is resorbed, it serves as a concentrated source of calcium, which is needed during mineralization in active bone remodeling as calcium ions play an impartment role in offering osteoblasts for bone formation.

Therefore, the analysis results have proved the potential to enhance *in vivo* biocompatibility of the α-CSH group in the rabbit model. As investigated above, the microwave-synthesized innovative α-CSH bioceramic is believed to possess superior bone healing and regeneration ability for clinical applications. However, future tests will require larger sample sizes as well as larger animals such as pig or beagle models to get a statistical difference and to strengthen the present findings of the potential of the innovative α-CSH bioceramic as a bone substitute in clinical applications.

The present in vivo study was conducted at Lepto Biotech Co., Ltd. (Taipei, Taiwan) according to the concept of ISO 10993-6: 2007 "Biological evaluation of medical devices—Part 6: Tests for local effects after implantation".

（様式　乙 9）

論　文　審　査　結　果　の　要　旨

申請者は、これまでにマイクロ波照射処理を行った新規骨補填剤 α-硫酸カルシウム半水和物（以下 α-CSH）を開発し、鶏卵漿尿膜法を用いて血管新生のみならず高い骨形成能を持つことを明らかにしてきた。本研究では骨欠損モデルであるウサギ大腿骨外顆皮質骨欠損部に α-CSH を埋め込み、治癒過程での骨新生の速度、人工骨の吸収の程度をマイクロ CT を用いた X 線学的観察と組織学的観察により経時的に評価した。その結果、α-CSH は骨欠損部でのコントロールと同様の良好な骨新生を誘導し、人工骨そのものは移植後に吸収・置換され骨欠損部の治癒状態が自家骨に近づくことを明らかとした。

本研究から申請者は、マイクロ波照射処理した α-CSH 移植用人工骨は、従来の材料と同等の生体内安全性、骨形成能を持つ素材であり、さらに適切な経過で吸収されて置換されることにより、自家骨に近い骨を形成することができる素材となることを示した。

この研究成果は骨修復分野での新規人工骨補填材開発に直結する新規性の高いものであると考えられる。

以上により、本論文は本学学位規程第 3 条第 2 項に定めるところの博士（医学）の学位を授与するに値するものと認める。

（主論文公表誌）

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