Okayama University Medical Research Updates （OU-MRU） 2022.02 Vol.98

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Okayama University research: Okayama University launches clinical trials of a jawbone regeneration therapy using human BMP-2 transgenic protein derived from *Escherichia coli*.

(Okayama, 21 February) **Okayama University researchers in collaboration with Osteopharma Inc., have developed a new protocol for producing artificial bone for regenerating bone in defective human mandibular to the same level as conventional autologous bone drafts but without surgical complications associated with autologous methods.**

Chewing food is essential for healthy living. However, the onset of super-aging societies worldwide has led to increases in masticatory disorders due to tooth defects that in turn cause nutritional disorders in the elderly, resulting in demand for dental implant treatment becoming more widespread. Notably, patients with many missing teeth show absorption of the alveolar bone—the region of bone that contains the tooth sockets and which forms the base for implants— and bone augmentation as exemplified by autologous bone grafting is necessitated. However, autologous bone graft procedures can lead to complications including neurovascular damage and infection during bone collection.

The major issue to overcome is that currently available bone filling materials do not exhibit biological bone-forming activity and do not have the same bone-generation effect as autologous bone grafts. So there is strong demand for the development of alternative methods that offer the same bone growth properties as conventional autologous bone grafting but without procedural complications.

Professor KUBOKI Takuo at the School of Medicine, Dentistry and Pharmaceutical Sciences at Okayama University and colleagues at Osteopharma Inc., addressed these problems and succeeded in developing an industrially scalable manufacturing protocol (high-efficiency refolding technology) for producing rhBMP-2, which exhibits precise three-dimensional structure and biological activity, which to-date has been difficult for *E. coli* expression systems, and is compatible with Good Laboratory Practice (GLP).

Clinical trials were initiated in July 2021 at Okayama University Hospital for patients who wished to receive dental implant treatment to replace lost teeth but with insufficient bone mass to undergo such surgical procedures.

“Following a non-clinical study of *E. coli*, we succeeded in the development of a high quality manufacturing process that is compatible with Good Manufacturing Practice (GMP),” says Professor KUBOKI. “Our approach enables the efficient and inexpensive manufacture of high-quality rhBMP-2 preparation for bone regeneration in the human mandibular.”

**OKAYAMA University**
1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 Japan

Professor KUBOKI and his colleagues combined β-tricalcium phosphate (β-TCP)—a bioabsorbable ceramic artificial bone—and rhBMP-2 to yield sustained release at regeneration sites of rhBMP-2 as well as securing regeneration sites.

"We have clarified the effectiveness of our procedure using large animals such as pigs and dogs and confirmed that it induces bone formation in the maxillofacial region that is equivalent to or better than autologous bone grafts," explains Professor KUBOKI. "After we have completed our initial clinical trials, we will move forward to commercialization of this artificial bone."

This research is directly related to the promotion of Okayama University's Sustainable Development Goals (SDGs) because the clinical results address the medical needs for hard tissue-related diseases of the elderly in super-aging societies globally.

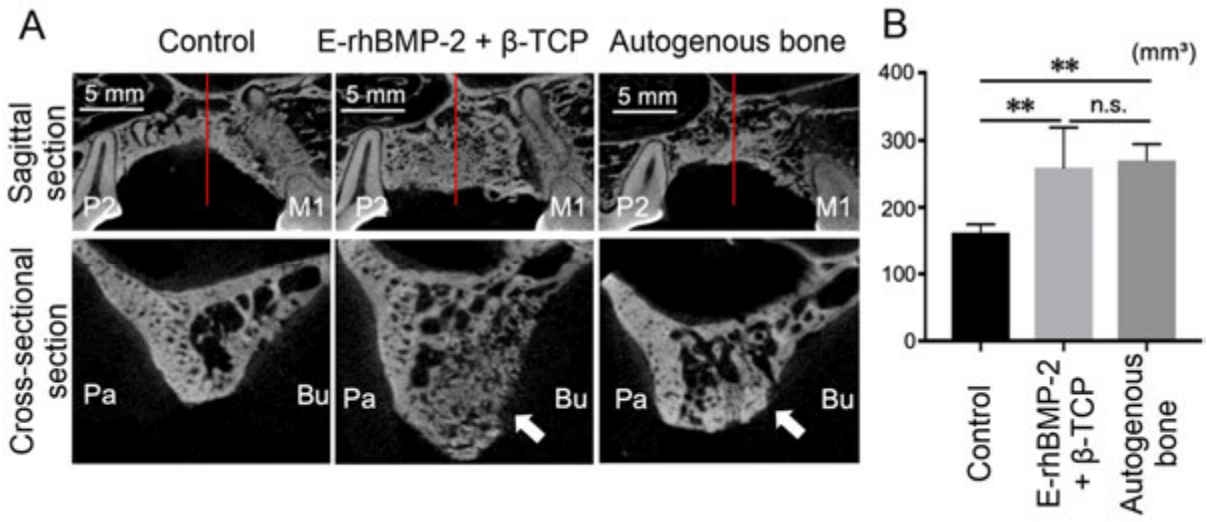


**Figure: Radiological comparison of the osteoconductive potential of E-rhBMP-2/β-TCP and autogenous bone transplantation.**
E-rhBMP-2/β-TCP granules and autogenous bone grafts collected from the chin were transplanted in bone defects and analyzed after 8 weeks. (A). Sagittal and cross-sectional micro-CT images of the bone augmentation site in the E-rhBMP-2/β-TCP, autogenous bone graft and control groups. (P2: second premolar, M1: first molar, Pa: palatal, Bu: buccal, white arrow: transplanted site.) (B). Graph shows the mean and standard deviation (+/−SD) of bone volume. Note that, at 8 weeks after transplantation, the bone volume in the groups transplanted with E-rhBMP-2/β-TCP and autogenous bone was significantly increased compared with that in the control group. (n.s.: not significant, **p<0.01, One-way ANOVA/Tukey). Summarized from "Nosho S, Ono M, Kuboki T, *et al*. *Journal of Prosthodontic Research*. 2021".

**Background**
Bone morphogenetic protein (BMP-2) -based bone regeneration therapy shows potential as being the most effective form of regenerative treatment. Human bones contain BMP-2, and

importantly, when this protein is transplanted together with a carrier to other parts of the body that do not have bones, then bones can be ectopically formed.

In Europe and the United States, human BMP-2 recombinant protein (rhBMP-2) products have been approved for clinical applications in alveolar bone augmentation surgery for dental implants and orthopedic surgery. However, rhBMP-2 products have not been approved in Japan, and the products in Europe and the United States are produced using a mammalian cell expression system, which has low production efficiency, and the resulting products are expensive. To be effective, the BMP-2 binds to receptors as a dimer in which two of the same subunits are bound, and it is necessary for this dimer to form the physically correct three-dimensional structure.

Research to-date has shown that although it is possible to mass-produce the rhBMP-2 subunit in the *E. coli* expression system, it is difficult to obtain the exact three-dimensional structure of this dimer and consequently it is difficult to mass-produce it on an industrial scale.

**Reference**

**1.** Mitsuaki Ono, Wataru Sonoyama, Katushi Yamamoto, Yasutaka Oida, Kentaro Akiyama, Shigehiko Shinkawa, Ryu Nakajima, Hai T Pham, Emilio S Hara, Takuo Kuboki. Efficient bone formation in a swine socket lift model using *Escherichia coli*-derived recombinant human bone morphogenetic protein-2 adsorbed in β-tricalcium phosphate. *Cell Tissues Organs*. 2014, 199(4), 249-255.
DOI：10.1159/000369061
https://www.karger.com/Article/Abstract/369061

**2.** Shuji Nosho, Mitsuaki Ono, Taishi Komori, Akihiro Mikai, Ikue Tosa, Kei Ishibashi, Yukie Tanaka, Aya Kimura-Ono, Emilio S Hara, Toshitaka Oohashi, Takuo Kuboki. Preclinical bioequivalence study of *E.coli*-derived rhBMP-2/β-TCP and autogenous bone in a canine guided-bone regeneration model. *Journal of Prosthodontic Research*. Advance Online Publication: June 25, 2021.
DOI：10.2186/jpr.JPR_D_20_00226
https://doi.org/10.2186/jpr.JPR_D_20_00226.

**Reference (Okayama University e-Bulletin & OU-MRU) : Professor KUBOKI's team**

e-Bulletin Vol.9：Bio-hybrid implants: Restoring organ functions
OU-MRU Vol.11：Compound-protein combination shows promise for arthritis treatment
OU-MRU Vol.19：Study links signalling protein to osteoarthritis
OU-MRU Vol.38：Bioengineered tooth restoration in a large mammal

**OKAYAMA University**
1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 Japan

**Correspondence to**
Professor KUBOKI Takuo, D.D.S., Ph.D.
Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama-shi, Okayama-ken, 700-8525, Japan.
E-mail: kuboki@md.okayama-u.ac.jp
http://www.okayama-u.ac.jp/user/implant/eng/index.html

Professor KUBOKI Takuo

**Further information**

Okayama University
1-1-1 Tsushima-naka , Kita-ku , Okayama 700-8530, Japan
Public Relations Division
E-mail: www-adm@adm.okayama-u.ac.jp
Website: http://www.okayama-u.ac.jp/index_e.html
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Okayama University Image Movie (2020):
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Okayama University supports the Sustainable Development Goals: https://sdgs.okayama-u.ac.jp/en/

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Vol.11 : Compound-protein combination shows promise for arthritis treatment
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Vol.13 : Peptide directs artificial tissue growth

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## ◆About Okayama University

Okayama University is one of the largest comprehensive universities in Japan with roots going back to the Medical Training Place sponsored by the Lord of Okayama and established in 1870. Now with 1,300 faculty and 13,000 students, the University offers courses in specialties ranging from medicine and pharmacy to humanities and physical sciences.

Okayama University is located in the heart of Japan approximately 3 hours west of Tokyo by Shinkansen.

Website: http://www.okayama-u.ac.jp/index_e.html


**Japan（日本）**


Hirofumi Makino, M.D., Ph.D.
President , Okayama University

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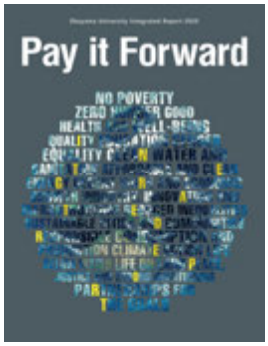


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An integrated report is intended to explain how an organization creates value over time through an organic integration of the vision and the combination of financial information and other information. Through this report we hope to promote greater interest in Okayama University among readers everywhere. In order to help us make improvements in future editions, we encourage you to contact us with any comments and suggestions you may have.

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1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 Japan