Research Note

Rapid Bone Induction of Cortical Bone Treated with Ultrasonic Demineralization in Acidic Electrolyzed Water

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Abstract: Healthy bone has many physiological microcracks, which may be involved in the release of growth factors and act to accelerate bone remodeling process. In this study, the cortical bone fragments (5 x 5 x 1 mm³) from rat skull were ultrasonically demineralized with acidic electrolyzed water (AEW: pH 2.7) in ultrasonic bath (120 W, 38 kHz, 20 min) designated as ultrasonic bone. Fresh bone and ultrasonic bone were grafted into subcutaneous tissue of 4 week-old male rats, and explanted at 2 and 4 weeks. Fresh bone had physiological micro-cracks, while ultrasonic bone showed clear enlargement and union of cracks on SEM. Ultrasonic bone revealed bone induction locally at 2 weeks, while fresh bone didn't induce bone and cartilage until 4 weeks. The new combination technique of ultrasonic irradiation and AEW will contribute to improve the surface area and the 3D-structure of the dense bone and should promote bone induction in the initial stage.

Key words: Bone induction, Cortical bone, Demineralization, Electrolyzed water, Ultrasound

Introduction

The ultrasonic technique has been widely applied in the medical and dental field, such as cleaning of instruments, echo examination, and so on¹⁾. The ultrasonic scalar tip has been most familiar in the dental field. We have previously applied ultrasonic technique for the improvement of commercially available biomaterials²⁻⁵⁾. Recently, newly developed ultrasonic instrumentation delivers high-energy ultrasound to wound surface via fluid medium, causing bubble cavitation, to facilitate wound debridement⁶⁻⁸⁾. We focused on bubble cavitation power via acidic electrolyzed water (AEW) by ultrasonic wave. We assume that the physiological and artificial cracks should be involved in the release of bone morphogenetic protein (BMP) molecules as trigger sites of bone induction⁹⁾. The ultrasonic and acid combination technique will contribute to modify cortical bone surface for rapid bone induction.

The aim of this study is to investigate the bone-inductive potency of rat cortical skull bone treated with or without ultrasonic irradiation via AEW in adult rat subcutaneous tissues.

Materials and Methods

Production of acidic electrolyzed water (AEW)

AEW (pH 2.5 to 6.5) can be generated from the anode region of a 3 chambered electrolytic apparatus (Redox technology Co., Japan) through effective electrolysis of saturated sodium chloride solution (26.5% NaCl). AEW of pH 2.7 was used in this study. The apparatus has a 3 chamber double in electrolytic system.

Preparation of graft bone fragments

Eight adult Wistar rats (female, 11 month-old) were sacrificed as donors, and parietal bone were harvested, and cut into fragments (5 x 5 x 1 mm³). The bone fragments were demineralized in AEW (pH

2.7, 1.0 liter) by ultrasonic irradiation (120 W, 38 kHz, 20 min) using ultrasonic bath machine (Powersonic 603, Hwashin Technology Co., Seoul, Korea) and designated as ultrasonic bone. Fresh bone fragments were taken as control. Ultrasonic bone and fresh bone were observed by scanning electron microscope (SEM) before the graft. The animal experiments were approved by Animal Research Center of Health Sciences University of Hokkaido, Japan (Authorized No 109). Wistar rats were purchased from Hokudo Co., Sapporo, Japan. All surgical procedures were performed in sterile conditions.

Subcutaneous grafts

Both ultrasonic bone and fresh bone were grafted into subcutaneous tissues of 14 Wistar rats (4 week-old, male), and explanted at 2 and 4 weeks. The tissues were fixed, demineralized, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). The specimens were observed by optical microscope.

Results

SEM

Fresh bone exhibited dense surface with physiological cracks (about 5 µm in length) (Fig. 1A). Ultrasonic bone showed clear enlargement and union of cracks (about 50-100 µm in length), and damaged fibers on the surface (Fig. 1D).

Histological findings

Fresh bone didn't induce bone and cartilage at 2 and 4 weeks (Fig. 1B, C), while ultrasonic bone revealed bone induction locally at 2 weeks (Fig. 1E). The original bone area of both fragments showed empty lacunae spaces of osteocytes, suggesting necrotic bone (Fig. 1B, C, E, F). Induced bone with cuboidal osteoblasts was found on acellular original bone at 4 weeks in ultrasonic bone group (Fig. 1F).

Discussion

Ultrasonically demineralized bone induced bone at 2 weeks in our

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Figure 1. SEM (A and D) and histological (B, C, E and F) pictures

A, B and C: Fresh bone

D, E and F: Ultrasonic bone

A. SEM: Smooth clear surface with scanty destroyed fibers and physiological cracks around 5 μ m in length (indicated by white arrow head).

D. SEM: Roughened surface with destroyed fibers along with enlarged, irregular cracks around 50-100 μ m in length (indicated by white arrow head).

B. and C. Fresh bone: Smooth outer cortical plate with empty lacunae space of osteocytes, encapsulated by fibrous connective tissue without distinct morphological changes at 2 and 4 weeks. Note: empty lacunae in grafted bone (HE).

E. Ultrasonic bone at 2 weeks: Local bone induction along outer cortical plate (HE).

F. Ultrasonic bone at 4 weeks: Distinct new bone with cuboidal osteoblasts along outer cortical plate (HE).

NB: New bone, EL: Empty lacunae (indicated by black arrow)

scale bar = 50 μ m

study. Generally, fresh autogenous bone remains the gold standard of treatment for nonunion and large bone defects¹⁰. However, cortical bone block inhibits the invasion of cells and blood vessels, because the calcified structure is highly dense like a castle wall. Our combination technique was based on the important reports that bone induction occurred predominantly in the cracks, rather than on the flat surfaces⁹, and that demineralized bone matrix (DBM) showed better performance in bone induction than calcified bone¹¹. SEM showed micro-cracks could be created on the skull bone treated with ultrasonic demineralization in AEW. Partial dissolution of cortical bone in AEW might be an effective procedure for the release of BMPs and bone matrix-derived growth factors^{12,13}. Living bone has physiological micro-fractures¹⁴. We believe that the physiological micro-cracks should be involved in the trigger sites of bone induction or remodeling.

It was concluded that the AEW-ultrasonic demineralized bone had better performance in bone induction than the fresh bone. Our micro-damage technique with the combination of the ultrasonic irradiation and AEW could contribute to improve the surface area and the 3D structure of the dense cortical bone, and accelerate bone induction.

Conflict of Interest

The authors have declared that no COI exists in this research.

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