Rapid bone induction of rat compact bone using ultrasonic irradiation and acidic electrolyzed water

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Abstract

1. Introduction

Bone regeneration has always been a major research field that continues to explore the design of new graft material. It is well known that the surface area and the 3D-interconnected porous structures are the major factors for better cellular performances in bone induction and conduction. The human skeleton has a unique ability to regenerate itself. Healthy living bone has physiological microcracks because the daily activities and repetitive loading there accumulates nano-micro cracks. These physiologic cracks due to micro-damage may be a significant factor in the initiation of intracortical bone modeling. Bone induction occurs predominantly in crack area than the smooth surface. Additionally, the previous study showed a partially demineralized bone matrix had better performance in bone induction than calcified bone. Based on these facts, we tried to combine the mechanochemical cracks formation by ultrasonic treatment along with the partial demineralization by commercially available acidic electrolyzed water (AEW). Vibratory action and bubble cavitation effects created by the ultrasonic instrument can cause surface roughness. While AEW etches the surface on contact thus creating the partially demineralized state. We assume that the cracks created in the partially demineralized area of bone should be involved in the release of bone matrix-derived growth factors thus accelerating bone formation. The aim of this study is to evaluate the surface changes and estimate the osteoinductivity of the excised skull bone treated with or without ultrasonic irradiation and acidic electrolyzed water.

1. Materials and method

Saturated NaCl solution was continuously and effectively electrolyzed at 9.1 V and 9.0 A under a flow rate of 4,200 cm^3min^{-1} by the 3 chambers-double in-type electrolytic system. AEW (pH 2.4 to 2.7) was collected from the anode region in the system. For ectopic studies, adult Wistar rat parietal bone was exposed and continuously treated by ultrasonic bath

machine for 20 minutes (study 1) or piezoelectric ultrasonic scaler tip for 1 minute (study 2) using AEW or DW (pH 5.6) as irrigation solutions. Then the treated bone was cut into fragments (5X5X1mm^3). Each fragment was implanted into syngeneic rat back skin. While for Orthotopic study (study 3), exposed parietal skull bone was treated by ultrasonic scaler tip for 1 minute using AEW or DW then flap was repositioned and sutured. Fresh bone without any treatment was taken as a control in all three studies. The explants were processed accordingly to a standard protocol and stained with HE. The microstructures were observed by SEM for ectopic studies. The results were compared using the Mann-Whitney U test with a p-value <0.05 accepted as statistically significant.

2. Results

SEM

Study 1

AEW bone showed numerous microcracks, exposed collagen fibers, and the decalcified surface as compared to DW bone. In addition, numerous destroyed osteoblastic processes and dead osteoblasts were found in both groups. Untreated bone revealed homogenous compact structure. SEM-EDS revealed that the residual calcium content was lower in AEW treated group as compared to DW and normal saline treated group.

Study 2

AEW bone showed a non-homogeneous surface with an extension of deep microcracks and partially decalcified surface compared to DW bone, while the surface of fresh bone was smooth and dense.

Animal assay

Osteoblast differentiation and localized new bone induction were seen just at 2 weeks in AEW bone group in both study 1 and study 2 in comparison to DW bone group, while fresh bone did not induce bone and cartilage until 4 weeks.

In study 3, the histological result showed cavitation defect area on the treated outer cortical plate. Osteogenic cell differentiation in this defect area as well as along the surface area was observed in AEW bone group at 2 weeks, while in the fresh bone group, normal periosteal regeneration pattern was seen.

Histomorphometric analysis

Histomorphometric analysis revealed that the amount of new bone formation was significantly higher in the AEW group as compared to the DW bone group in study 1 and 3 ($p<0.05$).

3. Conclusion

Direct new bone induction was observed at 2 weeks in AEW bone. It was concluded that ultrasonic AEW demineralized bone has enhanced bone inductive capacity and accelerate bone formation and modeling than the fresh bone. Our mechanochemical surface alteration of compact bone with the combination of ultrasonic irradiation and AEW could contribute to improving the surface area and 3D architecture of dense cortical bone.