Perbandingan *Demineralized Bone Matrix* dan *Freeze Dried* dalam Pembentukan Tulang Baru di Otot Tikus Putih

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ABSTRAK

Pendahuluan. Salah satu alternatif pengobatan terhadap hilangnya sebagian tulang yang luas adalah penggunaan donor tulang. Donor tulang autogenous merupakan bahan standar donor tulang tetapi mempunyai keterbatasan sumber pengambilannya. *Demineralized bone matrix* (DBM) merupakan bahan pengganti yang ideal karena mempunyai kemampuan osteoinduksi dan osteokonduksi. Di sisi lain donor tulang beku kering atau *freeze dried* (FD) yang hanya mempunyai kemampuan osteokonduksi telah secara luas digunakan di klinik. Tujuan dari penelitian ini adalah membandingkan DBM dan FD dalam pembentukan tulang baru.

Bahan dan cara kerja. Delapan belas ekor tikus putih yang berumur 60 hari dan berat badan 200-300 gram dijadikan subyek. Tikus tersebut dibagi menjadi tiga kelompok pengamatan, masing-masing kelompok tikus akan mengalami pembedahan dan ditanami dengan donor tulang pada otot adduktornya. Masing-masing kelompok terdiri dari enam paha yang ditanami dengan DBM di satu sisi sedangkan sisi yang lain akan ditanami dengan donor FD dan akan dikorbankan pada minggu kedua, keempat, dan keenam untuk diamati (6 tikus per periode pangamatan). Pemeriksaan histologi dilakkan untuk menilai jumlah sel osteoblas dan pembentukan matriks tulang baru..Data akan dites kenormalannya dengan menggunakan tes Kolmogorov-Smirnov dan hasilnya akan dianalisa meggunakan tes T one sample test.

Hasil. Didapatkan sel-sel osteoblas dan pembentukan matriks tulang baru di dalam otot yang ditanami dengan DBM pada semua kelompok pengamatan dan tidak didapatkan sel-sel osteoblas dan pembentukan matriks tulang baru pada otot yang ditanami dengan donor tulang beku kering.

Simpulan. *Demineralized bone matrix* lebih efektif daripada *freeze-dried allograft* dalam pembentukan tulang baru.

Kata kunci: demineralized bone matrix, osteoinduksi, donor tulang beku kering

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Comparison between Demineralized Bone Matrix and Freeze Dried Graft for New Bone Formation in White Rats Muscle

ABSTRACT

Introduction. One alternative for the treatment of extensive bone defects is the use of bone graft. Autogenous bone remains the established standard of bone grafting material but bone available for harvest is limited. Demineralized bone matrix (DBM) was an ideal bone graft substitute because it has osteoinduction and osteoconduction mechanism. On the other hand, freeze dried allograft (FDA), which as osteoconduction mechanism only, has been widely used in clinic. Thus this study aims to compare the new bone formation between DBM and FDA.

Materials and methods. An experimental study was conducted to ascertain which both bone graft types was better by using post test only control group design, involving 18 male white rats of 60 days old and weighing about 200-300 grams. The rats were divided into three observation groups, each were surgically implanted with a graft in the adductor muscle. Each group consisted of six thighs which implanted with DBM and the other tighs were implanted with FDA. They were sacrificed in 2nd week, 4th week and 6th week after implantation (6 rats/period). Histological examination had been performed to assess the number of osteoblast cells and bone matrix formation. The data were tested for normality using the Kolmogorov-Smirnov test and the result was analyzed with T one-sample test.

Results. There were osteoblast cells and matrix formation in muscle transplanted with DBM in all groups and no seen osteoblast cell and matrix formation in muscle transplanted with FDA.

Conclusions. Demineralized bone matrix is more effective than freeze dried allograft in new bone formation.

Keywords: demineralized bone matrix (DBM), bone morphogenetic proteins (BMPs), freeze dried (FD)

Introduction

The majority of fractures can be healed normally but 5% -10% are leaving problems in the healing process. The key to success of bone healing are biomechanical stability and biological functions of bone.[1] In order to obtain mechanical stability, it is sufficient in most cases to restore bone length (alignment) and stable fixation on the bone. However, in most of the other cases it requires donor bone (bone graft) or bone-graft substitutes to fill the defect or void on bone loss.[2,3] Donor bone may reduce morbidity in patients due to non-union and delayed union and became one of alternative solutions to problems associated with trauma or bone defects after tumor resection. Thus, in the state of partial loss of bone but extremity tissue is still regarded as viable, the act of amputation can be avoided.[4] It can reduce the number of disability that will impact value of economic productivity.

The use of corticocancellous autograft from the iliac crest bone is still the gold standard. However, a meta-analysis study found that 40% of the patients still complained pain after surgery up to 5 years thereafter. For this reason, bone graft substitutes appear to increase and improve donor bone procedures.[5] The ideal bone replacement donors are expected to have osteoconduction and osteoinduction ability for bone regenerate and but does not cause side effects.[6,7]

One alternative is demineralized bone matrix (DBM) allograft. This technique is based on the initial discovery that the organic matrix of bone still contains a number of proteins capable of stimulating heterotopic osteogenesis with endochondral ossification mechanism. This matrix protein is called bone morphogenetic proteins (BMPs).[8]

The use of DBM bone donor will be more popular and growing. This is because of its ability to form new bone by two mechanisms, the osteoconduction and osteoinduction.[2] However, the use of DBM in the field is

still limited. Some histological studies showed that new bone formation is predominantly occured by osteoconductive process than osteoinductive.[9]

On the other hand, the use of freeze dried (FD) bone donor at the Dr. Soetomo Hospital is increasing, whereas the ability to form new bone is only through osteoconductive mechanism. It is recorded that the development of FD bone allograft production showed an increasing from year to year. In 2001 as many as 148 preparations had been produced from several tissue types and in 2006 it reached the number of 199.[10] Hence, the aim of this study is to compare DBM and FD bone donor effect to the new bone formation.

Materials and methods

This study was an experimental laboratory study using post-test only control group design. Subjects consisted of male white rats (*Rattus norvegicus*) Wistar strain of 50-60 days of age, body weight about 200-300 grams. As many as 18 rats were divided into three groups randomly based on the observation time. The observation time was on 2, 4 and 6 weeks. Two treatments were used in each rat. They were planted with DBM and FD allograft on the right and left femoral adductor muscle.

In every observation time, difference of formed bone matrix width and the number of osteoblasts between femoral adductor muscle groups receiving DBM and freeze dried cancellous allograft were assessed.

Results

Histological observation in week 2, 4 and 6 reveals clear profile of bone matrix trabeculars and osteoblasts in DBM group, while the FD group shows no bone matrix formation and osteoblast cells (Figure 1 and 2).

The data obtained in group treated with DBM were subjected to normality test and it was found that the number of osteoblasts and bone matrix area of each group has normal distribution ($p>0.05$). Because in FD group there were no osteoblasts found (osteoblast number = 0) and no formation of bone matrix (bone matrix area = 0 mm^2), we used one-tailed one-sample T test. The results are seen in Table 1.

The results of statistical tests using one-sample T test shows that there are significant differences in number of osteoblasts between two groups in week 2, 4 and 6 with a p value <0.05.

The results of statistical tests using a one-sample T test shows that there are significant differences in bone matrix area formed between two groups in week 2, 4 and 6 with p value <0.05.

Discussions

To isolate osteoinductive ability of DBM, the donor material was planted in rat muscle in hopes of being able to stimulate heterotopic osteogenesis. In this study observations were made in week 2, 4 and 6, and it was expected that new bone has been formed through the mechanism of endochondral ossification.[11] Based on the results of previous studies, it was found that in week 2 after donor bone planting, osteoblast profile and new bone formation process started to appear.[12] This has been histologically proven. The cartilage began to be absorbed and replaced by the bone. It appears that the chondrocyte cells began to enlarge and mature and osteoblasts appear to line at the edge of cartilage matrix.

In week 4, it was expected that new bone has been formed and bone marrow differentiation started to appear.[12] This has also been histologically proven. Much more cartilage has been absorbed. There were the presence of osteoblasts and osteoclasts, which were in the process of remodeling, along with the profile of bone marrow and erythrocytes in a row. However, the results were not consistent among histologically-observed preparations. The results obtained in week 6 showed that remodeling process has been completed with the marked images of disappearing cartilage and replaced by wide bone matrix with osteoblasts and osteocytes.

In observation week 4 and 6, the osteoblasts count was found decreasing. This can be caused by several factors. The formed osteoblasts act to produce bone matrix and subsequently, these osteoblasts will be submerged in the matrix to become osteocytes, and some may experience cell death (apoptosis). Another contributing factor is that the allograft material implanted in muscles, which are physiologically not the load area so that osteoblast cells may be faster to die and disappear because they are not required to the process of bone remodeling.

Based on result of this experiment, we have other alternative allograft which is more promising due to two mechanism bone formation. Other consideration is minimize the risk to disease transmission and in the future, DBM able to use as scaffold for stem cell implantation.

This experiment could be further investigated to looking for side effect and allogenic reaction potential. This issue due to protein component in the BMP which taken out from DBM.

Conclusions

Demineralized bone matrix allograft is more effective in new bone formation than the freeze-dried cancellous allograft.

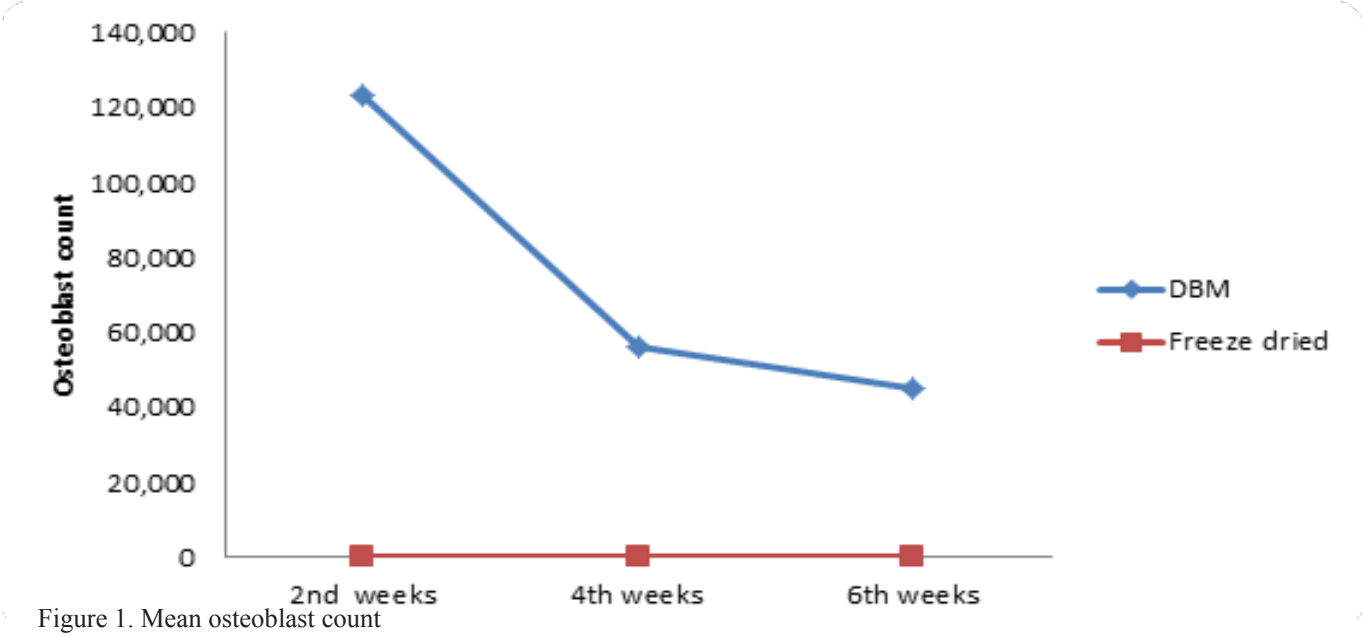

Figure 1. Mean osteoblast count

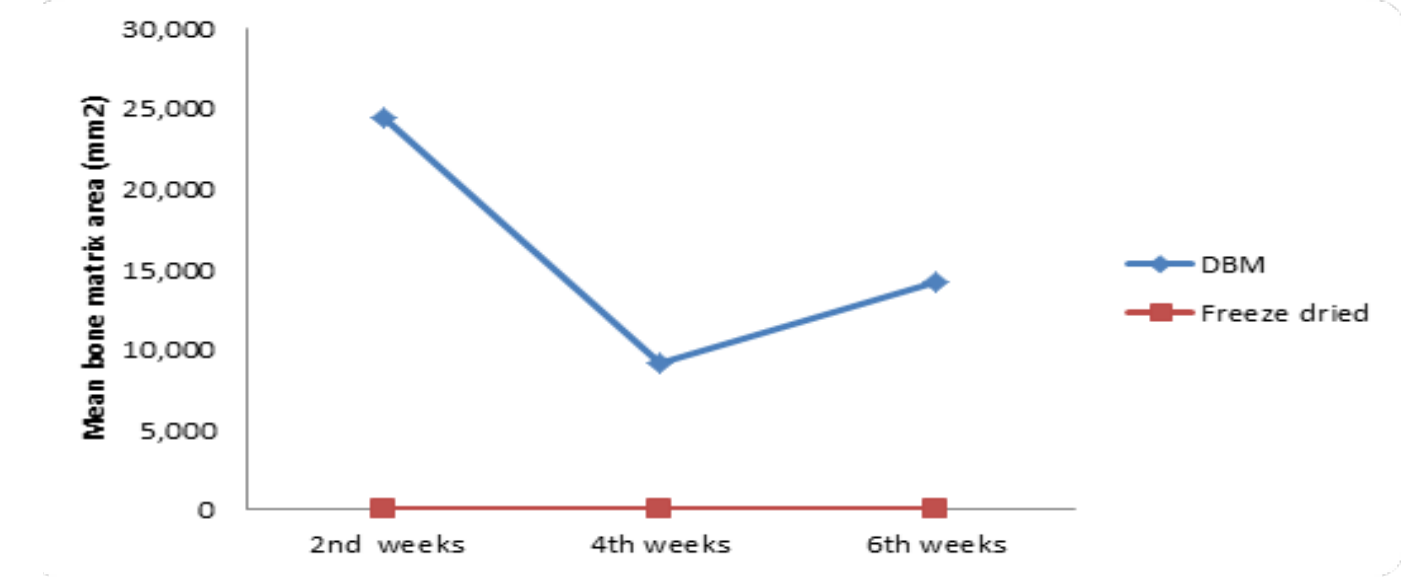

Figure 2. Mean bone matrix area

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