Role of Sterile Lyophilized Amniotic Membrane in Treatment of Fracture with Bone Defect: an Experimental Study on *Sprague-Dawley*

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ABSTRACT

Introduction. In reconstruction of fracture with critical size bone defect, the restoration of the alignment and stable fixation for succesfull result is necessary. Bone graft or bone transport is usually needed for bone defect reconstruction because there are not any osteoinductive and osteoconductive components on fracture with bone defect. This fracture continues to be deficient in bone repair or become non-union.

Materials and methods. The research design is post test control group using twenty eight skeletally matured *Sprague-Dawley* rats, divided into four groups, 4 mm sized femoral defects were surgically created in the right femur of 28 rats. Seven rats were randomly assigned to each treatment group, in which the femoral defect was filled with sterile lyophilized amnion, morcalized bovine xenograft and combination. In the empty defect group (control group) defects were left empty. Animals were sacrificed at 8 weeks postoperatively. Then the radiologic and histopathologic examination were completed. Radiologic evaluation was conducted using Lane and Sandhu score, histologic evaluation using Salkeld score.

Results. Non-parametric Kruskal-Wallis statistic analysis for the radiologic score 8 weeks postoperatively revealed p-value of 0,25 which meant there was no significance difference between four groups. However, the histopathologic score statistic analysis examination revealed the p-value of 0,001 which meant there were significance differences between four groups.

Conclusions. Regarding the radiologic score, amniotic membrane group has similar radiological score to control group, however the histopathologic score is better. Xenograft have similar radiological and histopathological score to the control. Combination of amniotic membrane with xenograft has better histopathological score to control. Although the radiologic score is similar.

Keywords : sterile lyophilized amnion, bone defect fracture

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Peran Membran Amnion Liofilisasi Steril dalam Tatalaksana Fraktur dengan Defek Tulang: Sebuah Studi Eksperimental di Tikus Putih *Sprague-Dawley*

ABSTRAK

Pendahuluan. Pada rekonstruksi fraktur dengan defek tulang yang luas, diperlukan penggunaan graft tulang ataupun tindakan transport tulang. Hasil akhir dari penyembuhan tulang paska pembedahan pada beberapa kasus fraktur akan mengalami penyembuhan tulang yang kurang baik yang akhirnya akan menyebabkan defek ataupun *non-union* dari fraktur tersebut.

Bahan dan cara kerja. Desain penelitian adalah studi *post test control group design*. Sampel yang digunakan adalah dua puluh delapan tikus putih *Sprague-Dawley* yang telah mengalami maturasi skeletal (8-12 minggu), dibagi menjadi empat kelompok, tiap tikus akan dilakukan tindakan fraktur dengan defek tulang pada tulang femur selebar 4 mm, kemudian tikus dibagi berdasarkan implantasi yang diberikan, yaitu kelompok kontrol, kelompok implantasi amnion liofilisasi steril, kelompok implantasi xenograft morcalized bovine, dan kelompok implantasi kombinasi amnion dengan xenograft. Hewan coba dikorbankan setelah 8 minggu, kemudian dilakukan pemeriksaan radiologis dan histopatologis dari fraktur. Evaluasi radiologis menggunakan skor menurut Lane dan Sandhu, evaluasi histopatologis menggunakan skor menurut Salkeld.

Hasil. Berdasarkan uji statistik non parametrik Kruskal-Wallis terhadap skor radiologis tulang pada minggu ke-8 paska pembedahan didapat nilai p 0,25. Secara statistik dapat diambil kesimpulan bahwa tidak terdapat perbedaan bermakna perbandingan skor radiologis antara empat kelompok tersebut. uji statistik non parametrik Kruskal-Wallis pada skor histopatologis menurut Salkeld minggu ke-8 paska pembedahan didapat nilai p 0,001 secara statistik, dapat diambil kesimpulan bahwa terdapat perbedaan bermakna perbandingan skor histopatologis antara empat kelompok tersebut.

Simpulan. Berdasarkan skor radiologis, membran amnion liofilisasi steril memiliki skor radiologis yang tidak berbeda dengan kontrol, walaupun skor histopatologisnya lebih baik.
Xenograft memiliki skor radiologis dan histopatologis yang tidak berbeda dengan kontrol.
Kombinasi membran amnion liofilisasi steril dengan xenograft memiliki skor histologis yang lebih baik dibanding kontrol, walaupun skor radiologisnya tidak berbeda.

Kata kunci: amnion liofilisasi steril, fraktur dengan defek tulang

Introduction

One of the current chalenge on fracture treatment is reconstruction of fracture with critical size bone defect, where the restoration of the alignment dan stable fixation for succesfull result is necessary. Bone graft or bone transport is usually needed for bone defect reconstruction because there are not any osteoinductive and osteoconductive components on fracture with bone defect. Although new technologies and advances in orthopaedic surgery have enhanced fracture healing and surgical outcomes, there are fracture that continues to be deficient in bone repair or become non-union. Large osseous defect without aplication of osteogenic and osteoinductive material will result to bad bone regeneration.[1,2]

Four elements are integral to bone repair: an osteoconductive matrix, osteoinductive signals, osteogenic cells capable of responding to these signals, and a sufficient blood supply. Autologous bone-graft provides an osteoconductive matrix, osteoinductive factors, and osteogenic cells, resulting in a reliable approach for managing most nonunions and bone defects. However, there are associated morbidities with the harvesting procedure, a limited supply, and varying osteoinductive potential of the graft depending on the patient. As a result, new strategies to optimize nonunion repair are being developed.[2-4]

Successful cell seeding of the scaffold depends on the type and source of the living cells as well as the extracellular matrix (ECM) components of the scaffold. One of the oldest biomaterials used for scaffolds is the fetal membrane. The ECM of the amniotic membrane (AM) and its components, such as growth factors, suggest that the AM is an excellent candidate to use as a native scaffold for TE. In addition, the AM is a biomaterial that can be easily obtained, processed and transported.[5]

This study aims to investigate the role of amniotic membrane as a scaffold for bone defect treatment need further research.

Materials and Methods

The research design is post test control group using twenty eight skeletally matured *Sprague-Dawley* rats, divided into four groups. Four mm sized femoral defects were surgically created in the right femur of 28 rats. Seven rats were randomly assigned to each treatment group, in which the femoral defect was filled with sterile lyophilized amnion, morcalized bovine xenograft and combination. In the empty defect group (control group) defects were left empty. Animals were sacrificed at 8 weeks postoperatively. Then the radiologic and histopathologic examination for all groups were completed. Radiologic evaluation was conducted using Lane and Sandhu score (table 1). Femur bones were preserved in paraffin block for histopathology examination with Hematoxyillin Eosin staining and later evaluated using Salkeld score (table 2). The scoring were analyzed by non parametric Kruskal Wallis analysis.[6-9]

Results

All films were evaluated in a blind fashion by the same radiologist. Dorso-ventral and medio-lateral projection of the right femur after 8 weeks between 4 groups, first group/control group (Figure 1.A), second group with sterile liophylized amnion implantation (Figure 1.B), third group with xenograft *morcalized bovine* implantation (Figure 1.C), and the fourth group implanted with combination of sterile liophylized amnion and xenograft *morcalized bovine* (Figure 1.D) showed fracture healing with positive callus formation although the bone defect still remained.

Table 1. Lane and Sandhu modified for radiologic score[7-9]

Radiologic features	Score
Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of defect	1
Bone formation occupying 50% of defect	2
Bone formation occupying 75% of defect	3
Bone formation occupying 100% of defect	4
Union	
Non-union	0
Possible union	2
Radiographic union	4
Remodeling	
No evidence of remodeling	0
Remodeling of intramedullary canal	1
Full remodeling of cortex	2

Table 2. Histological grading scale for the degree of healing modified Salkeld score[10]

Quality of union	Score
No sign of fibrous or other union	0
Fibrous union	1
Fibrocartilaginous or cartilage union < 25%	2
Fibrocartilaginous or cartilage union 26-50%	3
Fibrocartilaginous or cartilage union 51-75%	4
Fibrocartilaginous or cartilage union > 75%	5
Mineralizing cartilage and bone union	6
Woven bone	7
Mature bone	8

Non parametric Kruskal-Wallis statistic analysis for the radiologic score at 8 weeks postoperative revealed p-value of 0,25 which means there is no significance difference between four groups.

All samples were evaluated in blind fashion by the same histopathologist. The results are that most of the samples from control group showed fibrous tissue healing (Salkeld score 1), two samples with Salkeld score 2 fibrocartilage healing less than 25%. For the AM implantation (second group), most of the samples showed healing with woven bone (Salkeld score 7), two samples healed with mineralized cartilage (Salkeld score 6) and one sample healed with mature bone (Salkeld score 8). Third group (xenograft morcalized bovine implantation) showed that histopathologic scores were dominated by fibrocartilage healing less than 25% of the defect. And for the fourth group (combination), healing with woven bone was found in five samples, and two samples healed with mineralized cartilage.

Non parametric Kruskal-Wallis statistic analysis for the histopathologic score revealed the p value 0.001 which mean there were significance differences between four groups. Post hoc analysis revealed that the differences where due to the difference between control and amnion group (p=0.001), control and amnion with graft (p=0.001), graft and amnion with graft (p=0.001) and not between control and graft (p=0.05) or amnion and amion with graft (p=0.54).

Discussions

Nonunion fracture was found in almost all subjects although there were positive callus formations. It is probably due to unstable implant (Kirschner-wire 1 mm)

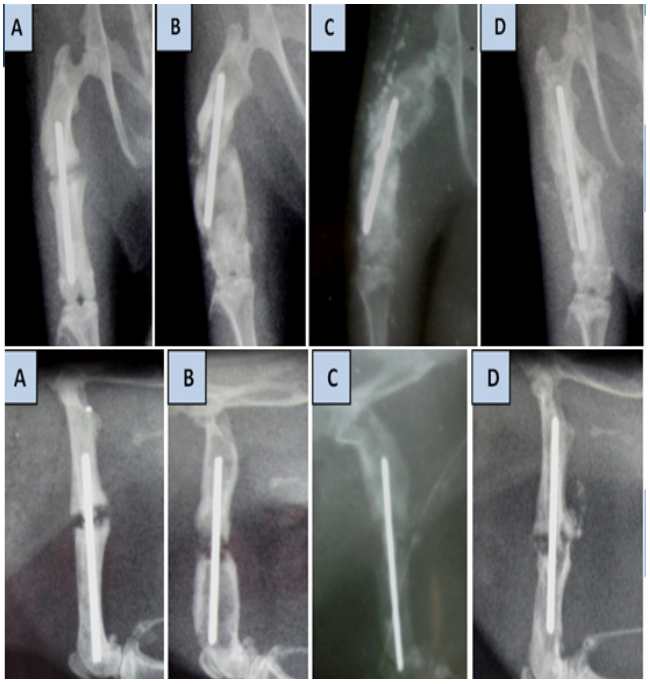

Figure 1. Radiographics appearance of right ffemur after 8 weeks. A. Control group; B. Lyophilized amnion; C.Xenograft; D. Combination

and uncontrolled early weight bearing of the rats.

Control group have lower score distribution compared to other groups because no implantation to the bone defect gives radioluscen appearance on the X-ray. Blokuish[12] said that radiology was not correlated with histopatology and biomechanic in process of bone healing. Radiography was not ideal to evaluate progression of fracture healing.[11,12]

As mentioned by Samandari[13], amniotic membrane has a positive effect on cell differentiation, migration, and invasion of various cell types. It provides therefore a mesenchymal signal for healing in bone, cartilage, nerve, and tendon. Samandari also said that amniotic membrane has different growth factors, assists in improving physiologic wounds, accelerates wound healing, and stimulates bone induction.[1,13,14]

Numerous growth factors have important role for bone remodeling and have positive effects on fracture healing. It was reported that basic FGF play an important role in the initial phase of the fracture-healing process because of its angiogenic properties and mitogenic activity on the osteoblast lineage. Basic FGF regulates the expression of local regulatory factors in osteoblasts and also promotes fracture healing by the stimulation of bone remodeling.

The application of recombinant human basic FGF increased the volume and mineral content of the calluses in rats. Jacobs reported that the composition of the amniotic membrane contains higher levels of EGF, KGF, HGF and basic FGF. Koizumi[15] also reported that human AM expresses mRNA for EGF, TGF-alpha, KGF, HGF, basic FGF, TGF-beta1, -beta2, -beta3, KGFR and HGFR. All of this research gives assume that amniotic membrane implantation would have a positive stimulating effect on the fracture healing.

Conclusions

Amniotic membrane has similar radiological score compared to control group, however its histopathologic score is better than control group. Xenograft have similar radiological and histopathological score compared to the control group. Combination of amniotic membrane with xenograft has better histopathological score than control group, with similar radiologic score.

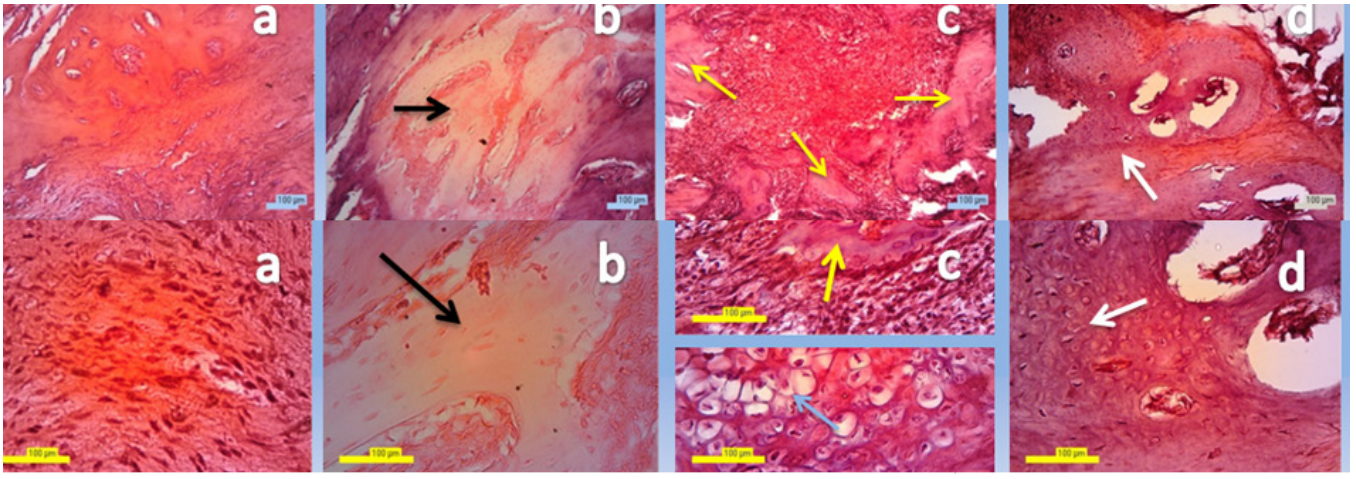

Figure 2. Histopathologic examination under hmatoxyllin-eosin staining (Top: 10x, Below: 40x). A. Control group; B. Lyophilized amnion; C.Xenograft; D. Combination

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