Role of Scaffold's Biocompatibility in Influencing Comminuted Fracture Healing in Sprague-Dawley Rats

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ABSTRACT

Introduction. The use of bone graft is still debatable for treating comminuted fracture. Autograft is the gold standard of bone graft. However, it has a limitation in supply. Therefore, the use of other source of graft (allograft, xenograft, or synthetic) is increasing. Graft must have good biocompatibility in order to enhance fracture healing.

Materials and methods. Randomized post test only control group was conducted in 30 Sprague-Dawley rats in order to evaluate biocompatibility of the scaffold. We used hidroxyapatite (HA)-Bongros®, nanocrystalline (HA)-$CaSO_4$ (Perossal®), nanocrystalline HA (Ostim®), morselized bovine xenograft (BATAN), dan local HA from dr. Sutomo Hospital as the scaffold. Tissue reaction (foreign body giant cell (FBGC) and lymphocyte), radiological and histological score was evaluated at 8^{th} weeks.

Results. The amount of FBGC and histological score showed significant difference (p=0,003 and p=0,013). Local HA scaffold showed the most FBGC accumulation. There was no significant difference in the amount of lymphocyte (p=0,397) and radiological score (p=0,204 for antero-posterior projection and p=0,506 for medio-lateral projection). There was significant correlation between the amount of foreign body giant cell and histological score (p=0,034).

Conclusions. Both physical and chemical factor influenced biocompatibility of scaffold. Scaffolds that have pores showed better histological score compared to that has none. Chemical compound of the scaffold play important role in tissue reaction. The amount of FBGC showed the cytotoxic level of the scaffold.

Key words: hydroxyapatite, scaffold, biocompatibility, tissue reaction

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Biokompatibilitas *Scaffold* secara *in Vivo* dan Pengaruhnya terhadap Penyembuhan Fraktur Kominutif Tikus *Sprague-Dawley*

ABSTRAK

Pendahuluan. Penggunaan tandur tulang pada fraktur kominutif masih diperdebatkan. Tandur tulang autologus merupakan baku emas dalam penggunaan tandur tulang, namun keterbatasannya adalah persediaan yang terbatas. Untuk itu banyak beredar pengganti seperti *allograft, xenograft,* dan tandur tulang sintetik (biomaterial *scaffold*). Tandur tulang harus mempunyai biokompatibilitas yang baik guna mendukung penyembuhan fraktur.

Bahan dan cara kerja. Dilakukan *randomized post test only control group* terhadap 30 tikus *Sprague Dawley* guna menilai biokompatibilitas *scaffold* secara *in vivo. Scaffold* yang digunakan adalah hidroksiapatit (HA)-Bongros®, nanokristalin HA-$CaSO_4$ (Perossal®), nanokristalin HA (Ostim®), *morselized bovine xenograft* (BATAN), dan HA-lokal bank jaringan dr. Sutomo. Dilakukan penilaian reaksi jaringan (jumlah sel datia benda asing dan limfosit), skor radiologis dan histologis pada minggu ke-8.

Hasil. Perbedaan bermakna ditunjukkan pada jumlah sel datia benda asing memberikan perbedaan bermakna (p=0,003), namun tidak dengan limfosit (p=0,397). Scaffold HA-lokal menunjukkan jumlah sela datia benda asing paling banyak. Skor histologis memberikan perbedaan bermakna (p=0,013) , namun skor radiologis tidak menunjukkan perbedaan bermakna (p = 0,204 untuk proyeksi antero-posterior dan p = 0,506 untuk proyeksi mediolateral). Terdapat korelasi yang bermakna antara jumlah sel datia benda asing dan skor histologis (p=0,034).

Kesimpulan. Biokompatibilitas *scaffold* secara *in vivo* ditentukan oleh komponen fisik dan kimia pembentuknya. Secara fisik, *scaffold* yang memiliki pori-pori menunjukkan skor histologis yang lebih baik. Komponen kimia pembentuk *scaffold* dapat memengaruhi reaksi jaringan. Jumlah sel datia benda asing berhubungan dengan sitotoksisitas *scaffold*.

Kata kunci : hidroksiapatit, *scaffold*, biokompatibilitas, reaksi jaringan

Introduction

Comminuted fracture is a result from high energy trauma, that may ended with nonunion. Treating this complication is a challenge for orthopaedic surgeon.[1] Therefore, it's better to prevent nonunion rather than to treat. The role of bone graft in comminuted type of fracture is still debatable.[2,3]

Autograft has been long considered as the gold standard of bone graft in fracture. However, it has a limited supply.[4] Nowadays, there are many options in using bone graft such as allograft, xenograft, or biomaterial scaffold (synthetic graft).[5,6]

Scaffold, as a biomaterial, must have a good biocompatibility for human. Evaluation of biocompatibility could be done by many means, include in vitro (cytotoxicity) and in vivo (implantation test).[7]

This study was performed to evaluate which scaffold, that were available in Indonesia, has the best biocompatibility and how does it influence comminuted fracture healing in rat in vivo.

Materials and methods

Randomized post test only control group was done to 30 skeletally mature (12 weeks) Sprague-Dawley rats weighted $\pm$273 g. Each rat was anaesthetized using intraperitoneal injection of ketamin and xylazine (60-80 mg/kgBW and 5-10 mg/kgBW). A lateral longitudinal approach was used at the left femur. A 5 mm comminuted fracture was made in the middle of the femoral diaphysis. Fixation was performed using retrograde intramedullary K-wire. At this point, the rats were randomly allocated into 6 groups (control and 5 intervention groups) according to the graft that will be implanted to the fracture site as follows; group I (local-HA; dr. Sutomo tissue bank), group II (morselized bovine xenograft; BATAN), group III (nano-crystalline HA-$CaSO_4$; Perossal®), group

IV (HA; Bongros®), group V (nano-crystalline HA, Ostim®). All of the surgery was done by the first author. Post operative treatments include oral amoxicillin 100 mg/BW and paracetamol 100 mg/BW for the first 3 days. Evaluation of signs of infection was performed daily.

Fracture healing was evaluated both radiologically and histologically at 8 weeks after intervention. Both dorso-ventral and medio-lateral x-ray view of the left femur was performed. Lane and Sandhu radiological score was used to evaluate the fracture healing radiologically.[8] Radiological score was evaluated by one radiology specialist. Histological sample was made from the femoral bone and the surrounding soft tissue. Allen score was used to assess the healing score histologically.[9] Using a 400x magnification microscope, the amount of lymphocyte and foreign body giant cell (FBGC) surrounding the scaffold's crystal were counted in 5 microscopic view. The histological score was evaluated by one pathology anatomy specialist. Both the surgeon and the evaluators were double blinded during the entire test. Ethical clearance was approved by Health Research Ethics Committee, Faculty of Medicine-Universitas Indonesia, Cipto Mangunkusumo Hospital.

Results

Two subjects were drop-out due to implant failure and osteomyelitis. FBGC count in control, group I, II, II, IV, and V were 0, 14.8, 3, 2.8, 6.4, and 2.8 respectively and were statistically different (p=0.003). All scaffolds present with a very low median of lymphocyte count (<1), with no significant differences between groups.

Group I showed the lowest median histological healing score while the other groups showed median score of 3. There wass significant difference in histological healing score (p=0.013). All of the intervention group showed no significant difference if compared to control group. However, group I showed significant difference if compared to the other intervention group.

Both radiological score (dorso-ventral view and medio-lateral view) showed no significant difference with an average median score 2 in all groups.

There was significant correlation between FBGC and histological score (p=0.034). However, other variables didn't give any correlation.

Discussions

The amount of FBGC is influenced by several factors, include physical and chemical. Scaffold that has a smooth surface will give less amount of FBGC. The toxicity effect of the scaffold will stimulate phagocytosis by the macrophage and release of cytokine (TNF-α, IL-β, IL-6 and prostaglandin E2) that result in inflammation that will impair fracture healing.[10,11] The chemical component in group I scaffold may be toxic for the macrophage. In order to avoid apoptosis of the macrophage, they fuse each other to perform a FBGC.

The amount of lymphocyte is minimal, indicating that there was no chronic inflammation process. The tissue reaction that was seen microscopically may due to the result of acute inflammation previously. However, it was still unclear because we didn't perform time evaluation in our study.

In this study, we used x-ray that was calibrated for human use, not for animal. The gold standard for radiographic evaluation for animal bone is using micro CT, however it is not available in Indonesia. Therefore, the radiological healing score were similar between groups.

Scaffold in group I has a granule form physically. Other scaffolds have a ratio between pore size and porosity. This difference in physical component may influence histological healing score. Those with pore achieved healing by vascular growth into the pore, while the granule form dependently counting on adhesion of osteoinductive and osteoprogenitor cells onto the surface.[12,13]

This study showed that scaffold in group I has the lowest biocompatibility. Meanwhile, Kamal et al.[14] in his study comparing the same scaffolds in vitro, showed that scaffold in group I has the best biocompatibility. In vitro study can't evaluate the responds of living tissue toward scaffold, while in vivo study can show tissue reaction by implantation of the scaffold.[15,16] This may be the factor that gave different result in vitro and in vivo.

Using bone graft in comminuted fracture didn't enhance healing compared to control group. But we must take into consideration that the model of comminuted fracture in this study wasn't a result in high energy trauma like that was encountered in real life situation.

Conclusions

Biocompatibility of scaffold does influence fracture healing. Scaffolds with low biocompatibility will give low healing score. This study showed that local HA from dr. Soetomo tissue bank has the lowest biocompatibility in vivo.

References

1. Salminen S. Femoral shaft fractures in adults: epidemiology, fracture patterns, nonunions, and fatigue fractures [dissertation]. Helsinksi: University of Helsinski; 2005.
2. Wright RR, Schmeling GJ, Schwab JP. The necessity of acute bone graft ing in diaphyseal forearm fractures: a retrospective review. J Orthop Trauma.1997;11(4):288-94.
3. Wei SY, Born CT, Abene A, Ong A, Hayda R, DeLong WG Jr. Diaphyseal forearm fractures treated with and without bone graft . J Trauma. 1999 Jun;46(6):1045-8.
4. Ring D, Rhim R, Carpenter C, Jupiter JB. Comminuted diaphyseal fractures of the radius and ulna: does bone graft ing affect nonunion rate? J Trauma. 2005;59(2):438-41.
5. Marra KG. Biodegradables polymers and microspheres in tissue engineering. In: Hollinger JO, Einhorn TA, Doll BA, Sfeir C. Bone tissue engineering. Florida:CRC Press LLC, 2005.p.149-66.
6. Easter D. Allograft vs xenograft: practical considerations for biological scaffolds. San Diego: CME; 2008.
7. Silva GA, Marques AP, Gomes ME, Coutinho OP, Reis RL. Cytotoxicity screening of biodegradable polymeric systems. Florida:CRC Press LLC, 2005.p.339-53.
8. Kalender AM, Goktas U, Ozbag D, Ertork C, Okumus M, Guner S, et al. The effect of GSM mobile phone electromagnetic field on femur fracture healing in a rat model. J Clin Anal Med. 2012;3(4):388-92.
9. Allen H, Wase A, Bear WT. Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. Acta Orthop Scand. 1980;51(3):595-600.
10. MacLauchlan S, Skokos EA, Meznarich N, Zhu DH, Raoof S, Shipley JM, et al. Macrophage fusion, giant cell formation, and the foreign body response require matrix metalloproteinase 9. J Leukoc Biol. 2009;85(4):617-26.
11. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008;20(2):86-100.
12. Finkemeier CG. Current concepts review: bone grafting and bone graft substitutes. J Bone Joint Surg Am. 2002;84-A(3):454-63.
13. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26(27):5474–91.
14. Kamal AF. [Transplantasi sel punca mesenkimal, *recombinant human* BMP-2, dan kombinasinya dalam percepatan *union* osteotomi dan peningkatan kekuatan mekanik autograft femur yang mendapat pajanan *extracorporeal irradiation* pada model tikus] [disertation]. Jakarta: University of Indonesia; 2012.
15. Lorian V. Differences between in vitro and in vivo studies. Antimicrob Agents Chemother. 1988;32(10):1600–1.
16. Emami J. In vitro-in vivo correlation: from theory to applications. J Pharm Pharmaceut Sci. 2006;9(2):31-51.