Three phase bone scintigraphy in evaluating revascularization and bone formation of natural biomaterial bone graft by using Tc^{99m} MDP in rabbits

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Abstract

Three phase bone scintigraphy (TPBS) examined the viability of bone graft, revascularization, and bone formation using single photon emission computed tomography (SPECT) and the intravenous injection of ^{99M} Technetium methylenediphosphonate (^{99M}Tc-MDP). Seven New Zealand White rabbits, aged 8 months had osteotomy of ulna performed, the left ulna substituted for natural biomaterial bone graft, containing gelatin sponge and hyaluronic acid, and the right ulna was replaced by vascularized autogenous bone graft. A post-operative scanning of bone using TPBS was done at 7 days and weekly, at week 1, 2, 4, 6, 8, 12 to evaluate revascularization and bone formation of natural biomaterial bone graft comparing with autogenous bone graft. The results demonstrated that the perfusion index and RCP of natural biomaterial bone graft and the autogenous bone graft in phases 1, 2, and 3 had no statistically significant difference when compared in between each week. The study also showed that, the ratio of the mean counts/pixel of the natural biomaterial bone graft in phase 3 had slightly lower than autogenous bone graft. This study provided model through which TPBS could evaluate revascularization and bone formation in this model. Natural biomaterial bone graft may be the same and as good a revascularization as autogenous bone graft although the bone formation of autogenous bone graft still has more efficiency than natural biomaterial bone.

Keywords: Three phase bone scintigraphy, revascularization, bone graft, SPECT, ^{99M}Tc-MDP

การตรวจสแกนกระดูก 3 เฟส เพื่อประเมินการเกิดหลอดเลือดและการสร้างกระดูกใหม่ของการปลูกถ่ายกระดูกด้วยวัสดุชีวภาพโดยการใช้สารเภสัชรังสีเทคนิเชียม^{99m} MDP ในกระต่าย

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บทคัดย่อ

เทคนิคการตรวจสแกนกระดูก 3 เฟสสามารถตรวจดูการมีชีวิตอยู่ของการปลูกถ่ายกระดูก การสร้างหลอดเลือดและการงอกของกระดูกใหม่ โดยการใช้เครื่อง single photon emission computed tomography (SPECT) ร่วมกับการฉีดสารเภสัชรังสีชนิด ^{99M}Technetium methylenediphosphonate (^{99M}Tc-MDP) กระต่ายทดลองสายพันธุ์ New Zealand White อายุ 8 เดือน จำนวน 7 ตัวถูกทำการผ่าตัดกระดูก ulna ทั้งสองข้างออก โดยขาหน้าข้างซ้ายใส่ natural biomaterial bone graft ที่ใช้ gelatin sponge ร่วมกับ hyaluronic acid และขาหน้าข้างขวาใส่ vascularized autogenous bone graft ในขนาดเท่าที่ตัดออก หลังการผ่าตัด 7 วันจะทำการตรวจวัดกระดูกโดยใช้เทคนิคการตรวจสแกนกระดูก 3 เฟส ทุกสัปดาห์ที่ 1, 2, 4, 6, 8 และ 12 เพื่อประเมินการสร้างหลอดเลือดและการงอกของกระดูกใหม่ จากการปลูกถ่ายกระดูกแบบ natural biomaterial bone graft เปรียบเทียบกับแบบ vascularized autogenous bone graft ผลการศึกษาแสดงให้เห็นว่า ค่านับวัดสารเภสัชรังสีในช่วงเฟสที่ 1, 2 และ 3 ของขาซ้ายและขาขวาไม่แตกต่างกันในทุกสัปดาห์ ขณะที่ในช่วงเฟสที่ 3 ค่านับวัดของ natural biomaterial bone graft น้อยกว่า autogenous bone graft การศึกษานี้อาจกล่าวได้ว่าในการทดลองนี้สามารถใช้เทคนิคการตรวจสแกนกระดูก 3 เฟส เพื่อประเมินการเกิดหลอดเลือดและการงอกของกระดูกใหม่ได้ ในการทดลองนี้ natural biomaterial bone graft อาจจะให้ผลในการเกิดหลอดเลือดใหม่ได้ดีเท่ากับ autogenous bone graft แม้ว่า ประสิทธิภาพการงอกของกระดูกใหม่ของ autogenous bone graft อาจจะดีกว่า natural biomaterial bone graft

คำสำคัญ : เทคนิคการตรวจสแกนกระดูก 3 เฟส การเกิดหลอดเลือดใหม่ การปลูกถ่ายกระดูก เครื่อง SPECT สารเภสัชรังสี ^{99M}Tc-MDP

Introduction

Bone scintigraphy has been frequently used for evaluating new bone growth after surgical implantation of bone grafts, ischemic injury, vascular infarction, acute inflammatory processes, and bone graft viability (Van Bruggen et al., 2010; Shehab et al., 2006; Minoves 2003; Harada et al., 2000). This is based on the evidence that ^{99m}Tc methylene diphosphonate (^{99m}Tc-MDP) is accumulated at the site of mineralization in proportion to the level of newly formed calcium phosphate crystals (Debruyn et al., 2013). It is more sensitive than radiographs, CT or MRI in detecting revascularization process, abnormalities at a very early stage within a few hours after transplantation, whereas radiological abnormalities are detectable after days (Nakayama et al., 2009). Therefore, bone scintigraphy provides a way of predicting graft failure before X-ray or clinical changes are apparent, and also before nonviable bone tissue becomes necrotic, infected, or the region fibrosis (Craemer et al., 1984). It can also be used when MRI cannot be used due to ferromagnetic effects in composite implants such as metallic implants or clips, with cardiac pacemakers, or with ferromagnetic foreign bodies (O'Mara et al., 1996).

Three phase bone scintigraphy (TPBS) is a noninvasive method for the study of changes in blood flow, blood distraction, blood circulation, angiogenesis from bone transplant that can evaluate new bone formation and measure metabolic activity of osteoblast and vascular function (Nutton et al., 1985). Studies of the vascular phase or perfusion of the bone scan have been made by recording a rapid sequence of radionuclide images for a short time immediately after injection of isotope (Berding et al., 1994).

Recently, TPBS for vascularized bone graft was shown to be a sensitive and reliable method for evaluating graft potency and viability in postoperative stages, a variety of osteoinductive agents or distraction osteogenesis (Zbigniew et al., 2007; Kawano et al., 2003). Clinically, bone scanning can be practically divided into three phases. In phase 1, an angiographic image was obtained while the isotope was still in the large vessels, immediately after injection. In phase 2, a blood-pool image recorded an isotope angiogram, which is thought to represent tracer in the capillary bed and indicates relative vascularity. In phase 3, a standard delayed image was used to demonstrate regions of abnormal uptake of tracer (Patka et al., 1985).

Autogenous bone graft remains the gold standard in clinical practice for the bone graft replacement and reconstruction of the bone defects (Samartzis et al., 2005, Deliberador et al., 2006). Since it present minimum immunological rejection, complete histocompatibility and gives the best osteoconductive, osteogenic and osteoinductive properties (Samartzis et al., 2005). Although, the effort of several studies is focused on synthetic materials to avoid the limited amount of potential donor material available, postoperative complication in harvesting can sometimes be deleterious to the patients, and may present a high incidence of donor site morbidity (Pollock et al., 2008).

Previous studies using gelatine scaffold sponges in bone remodeling found that this supported cell growth because of the presence of pore sizes, new cells can enter the sponge resulting in new bone formation (Rohanizadeh et al., 2008; Kang et al., 2010). Hyaluronic acid (HA) supports the increasing osteoblastic bone synthesis, this was the focus of an experiment bone grafting in the tibia of rabbits, and the results showed that it had higher bone score than spongiosal bone graft (Aslan et al., 2006). However, there are only few reports of using natural biomaterial bone graft containing gelatin sponge and HA model that have been published. Therefore, the purpose of this study were to assess the value of non-invasive technique, to evaluate the experiment and to compare between the natural biomaterial bone graft and the autogenous bone graft using Tc^{99m} MDP in rabbits.

Materials and methods

Animals and surgery procedure

Seven New Zealand White male rabbits at 7-8 months of age and body weight 2.5-3.5 kilogramswere used in the present study. These rabbits were acclimatized in this environment for 2 weeks prior to starting the experiment. All rabbits were caged in the same environment throughout the course of the experiment. Food was withheld for 12 hours and water was withheld for 6 hours before general anesthesia. Hair at the right and left front leg area was clipped and prepared for operation. Rabbits were induction and maintained general anesthetized by xylazine hydrochloride 5 mg/kg (Xylavet®, Thai meiji pharmaceutical, Thailand) and combination with ketamine 35 mg/kg (Ketava®, Atlantic lab, India) by intravascular injection. Antibiotic (enrofloxacin (Baytril®, Bayer Animal Health, USA)) 5 mg/kg and non-steroidal anti-inflammatory drugs (Carpofen (Rimadyl®, Pfizer, USA)) 4 mg/kg were administered subcutaneously just before skin incision.

The natural biomaterial bone graft was prepared aseptically from absorbable gelatin sponge (Spongostan, Ethicon™, Somerville, NJ, USA) in combination with hyaluronic acid (AdantDispo®, Meiji, Japan).

All rabbits were placed in the lateral recumbency and the aseptic technique was applied over the operating area of both front legs. Skin incision was performed at the lateral side of both forelimbs to expose mid part of the ulna. Osteotomy was performed on the middle part of the ulna and its segment of 12 mm length or the 1.5 time of the circumference length of the ulna was removed. A left ulna substituted for natural biomaterial bone graft and right ulna replaced by autogenous bone graft. This study received ethical approval from the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC).

Scanning Method

Three phase bone scintigraphy

Three phase bone scintigraphy was performed immediately after the bolus infection of 5-7 mCi of ^{99m}Tc-MDP into the marginal ear vein followed by flushing with 20 ml of saline, first-pass radionuclide angiographic data were obtained using a 128x128 matrix in the anterior view every 1 s for 60 s (phase 1). Next, blood-pool images were obtained at 10 min after injection using 256x256 matrix (phase 2). Finally, delayed images were obtained 3 h later in the same position with 256x256 matrix (phase 3). Postoperative was taken to each ulna at 1, 2, 4, 6, 8, 12 weeks. All data were obtained using a large-field-of-view gamma camera (Symbia E®, Siemens, USA) equipped with a low-energy, high-resolution, parallel-hole collimator. The uptake volume of ^{99m}Tc-MDP was reported as the ratio of mean counts/pixel (RCP). RCP equaled to mean counts/pixel of the individual ulna divided by mean counts/pixel of the normal that indicated at the middle part of the humerus of each front leg. The schematic of the lesion area of both front legs and normal as shown in Fig.1.

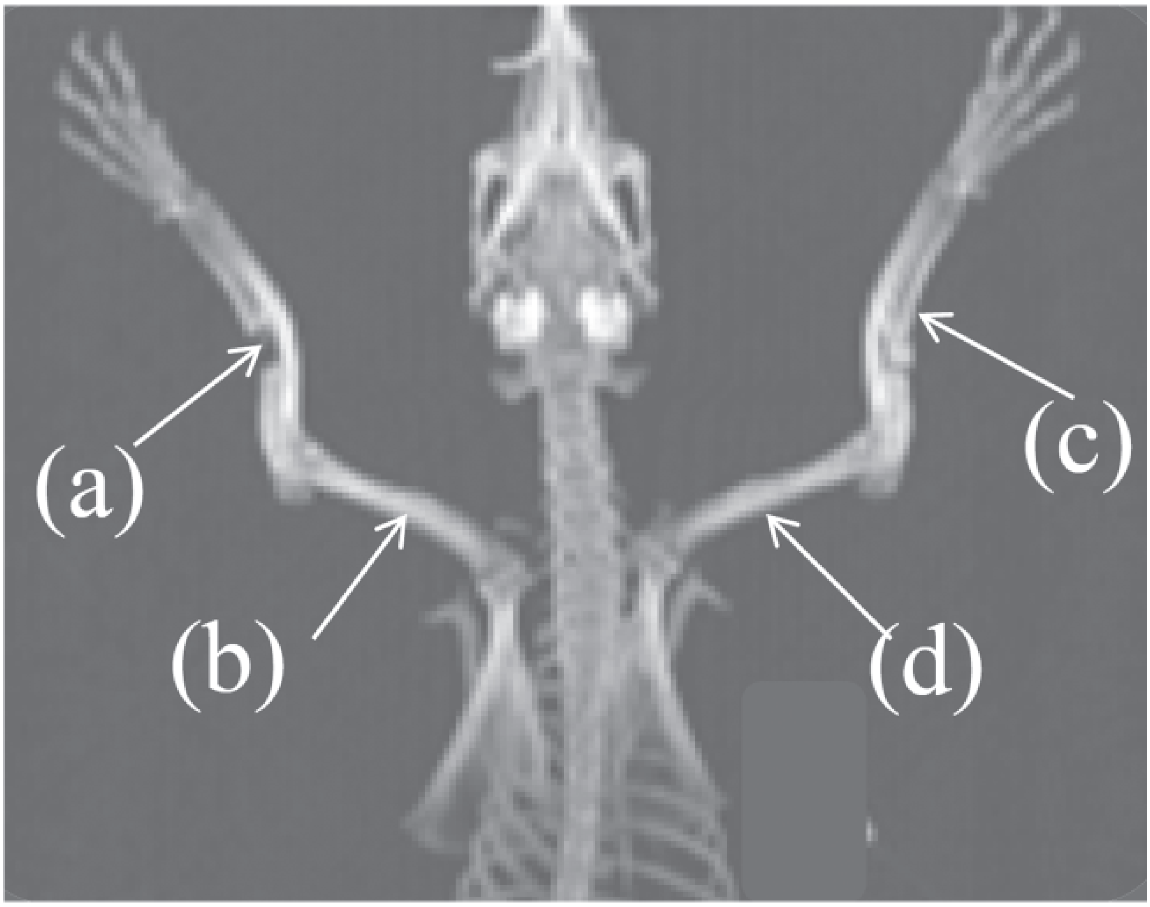

Figure 1: The schematic representation of lesion in the right ulna (a) and normal (b), the left ulna (c) and normal (d) of rabbit.

Statistical analyses

Computerized statistical software (SPSS 19.0 for Windows, Chicago, IL, USA) was used for analyses. Comparisons of the ratio of each phase at week 1, 2, 4, 6, 8 and 12. The differences of the left and the right ulna at each phase were examined by nonparametric using MannñWhitney *U* test. Values are expressed as mean ± standard deviation (SD). A *p-value* < 0.05 was considered statistically significant.

Results

Three phase bone scintigraphy technique

The ^{99m}Tc-MDP accumulation at the bone graft was detected postoperatively in bone scintigraphic images of week 1 to 12. In three hours after the injection of ^{99m}Tc-MDP, about half of the tracer was deposited in the bone, and delayed images were obtained. The perfusion index and the uptake ratio (lesion/normal) in each ulna, tended to increase with time but the rate at which it was increasing decreased from week 6 to 12 after surgery.

Phase 1: The perfusion index of the left ulna (natural biomaterial bone graft; gelatin sponge and HA) and the right ulna (vascularized autogenous bone graft) had no statistically significant difference when compared within the weeks (Table 1).

Phase 2: The ration of lesion/normal ulna in the blood-pool phase demonstrated that there no statistically significant difference the same was observed in the vascular phase when compared with each week and also when compared with the same ulna. Moreover, RCP of the left and right ulna peaked at 2 weeks in this phase (Fig. 2).

Phase 3: In the delayed phase, the RCP of the left and the right ulna peaked at week 4 and the picture of SPECT was as shown in Fig. 3. When comparing the same ulna, between week 1 and week 4 and between week 4 and week 12 (P = 0.003, 0.004; left ulna), (P = 0.004, 0.007; right ulna) respectively (Fig. 4) there was a statistically significant difference. However, there was no statistically significant difference between the left and the right ulna when compared at each week. Therefore, three phase bone scintigraphy model could evaluate revascularization and bone formation.

Table 1: The perfusion index at each week in the vascular phase (phase 1).

Week	Ratio	
	Right Lesion/Normal	Left Lesion/Normal
1	0.96 ± 0.13	1.03 ± 0.14
2	1.13 ± 0.12	1.09 ± 0.12
4	0.98 ± 0.15	0.96 ± 0.10
6	1.12 ± 0.11	1.14 ± 0.14
8	1.04 ± 0.09	1.06 ± 0.07
12	1.11 ± 0.08	1.09 ± 0.07

Values are mean ± SD for n= 5-7

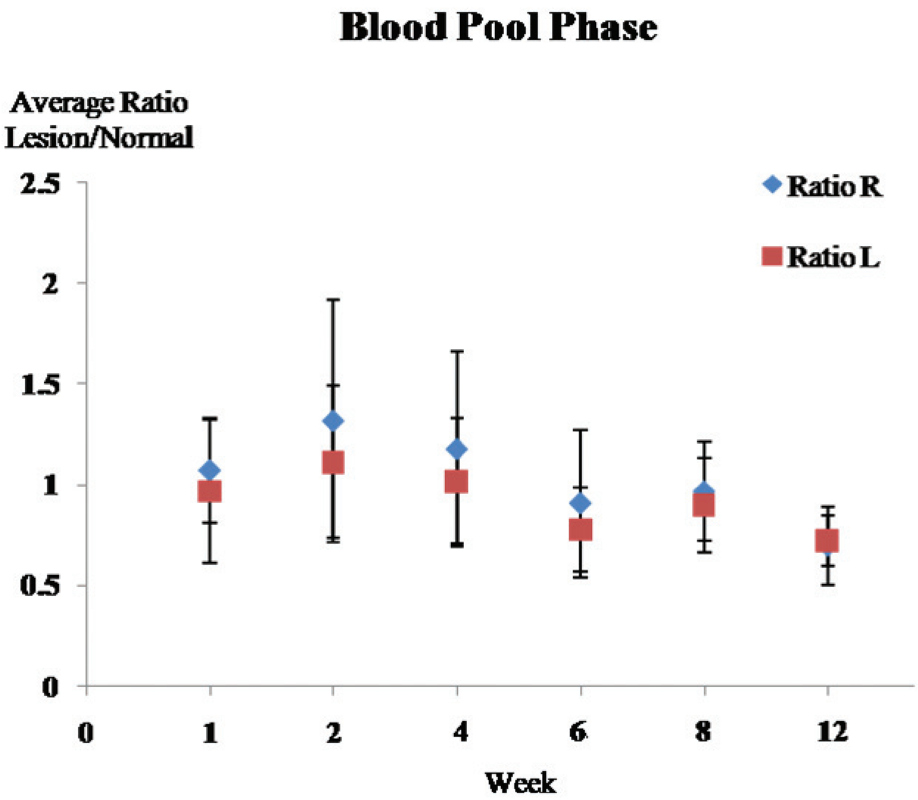

Figure 2: The ratio of lesion right/normal and lesion left/normal at each week in the blood pool phase (phase 2). Data represent mean ± SD for n = 5-7.

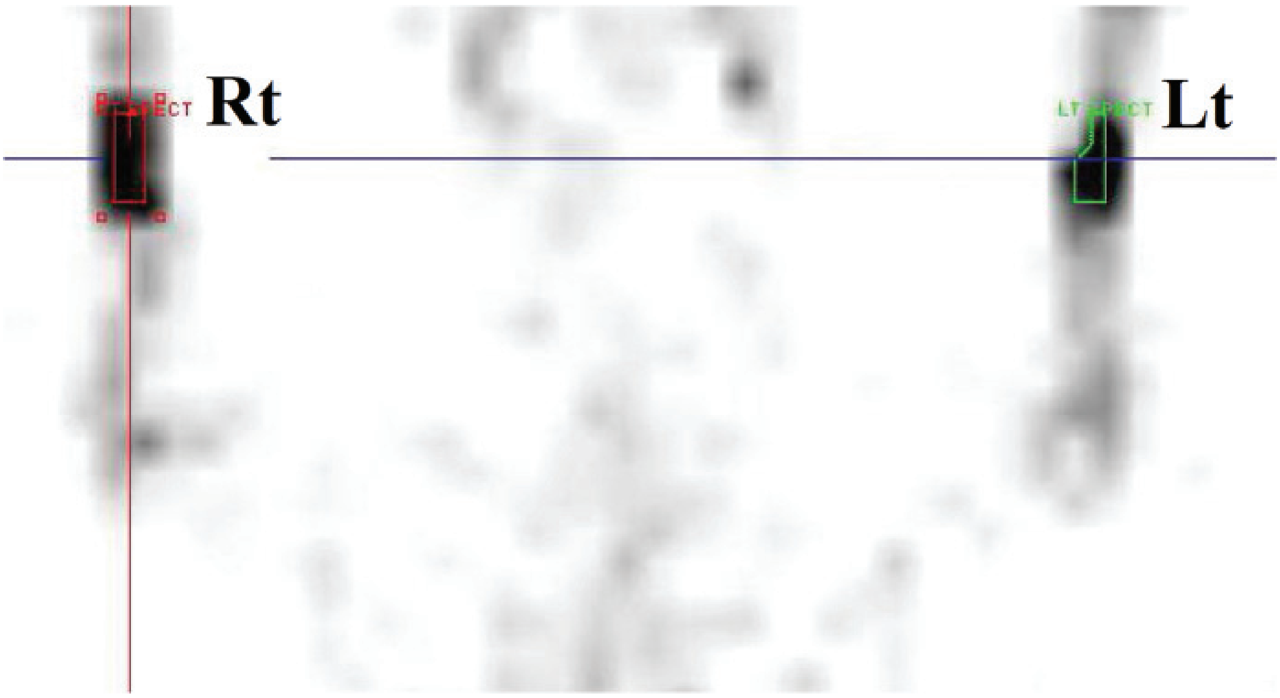

Figure 3: Representative photograph of the natural biomaterial in the left (Lt) and autogenous bone graft in the right (Rt) ulna of the rabbit in the bone phase (phase 3) at week 4 in SPECT images.

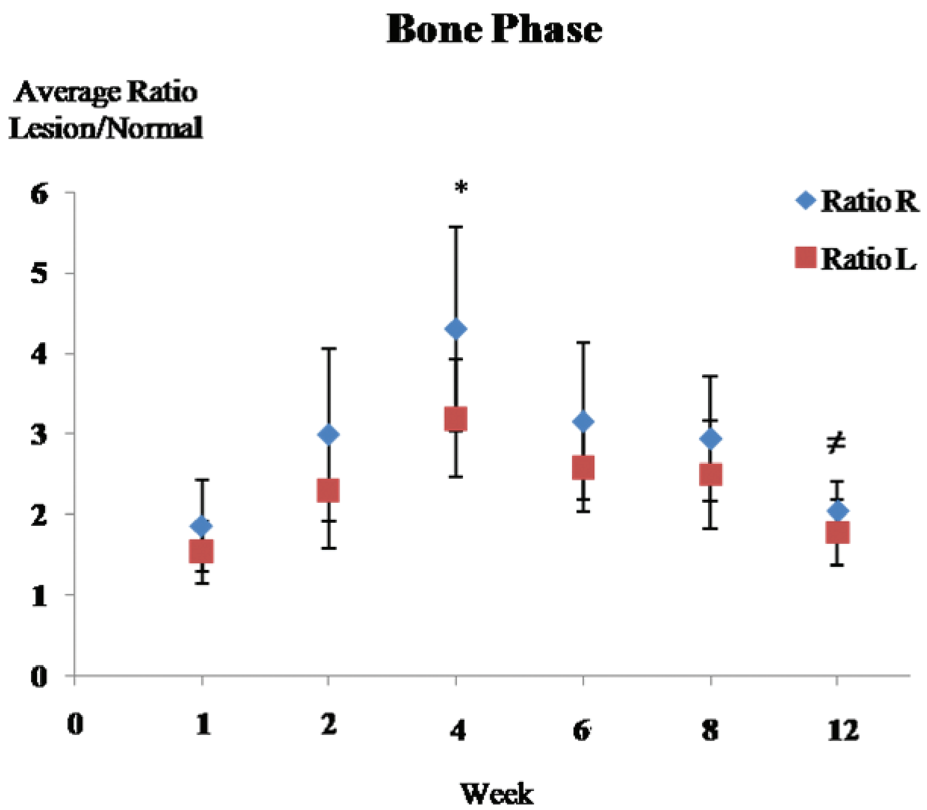

Figure 4: The ratio of the right lesion/normal and the left lesion/normal at each week in the bone phase (phase 3). Data represent mean ± SD for n = 5-7.
* compared with the same ulna at 1 week, $p<0.05$
≠ compared with the same ulna at 4 weeks, $p<0.05$

Comparison between the natural biomaterial bone graft and autologenous bone graft

The efficiency of natural biomaterial bone graft containing gelatin sponge and hyaluronic acid was not different from the autogenous bone graft. In the vascular and the blood pool phase (phase 1 and 2) revealed that natural biomaterial bone graft may be the same and as good vascularization as the autogenous bone graft. Nevertheless, bone formation of the autogenous bone graft was still more efficient than the natural biomaterial bone graft demonstrated by the bone phase (phase 3).

Discussion

Bone scintigraphy with radioactive tracers has found widespread use in the imaging of blood flow and metabolism in bone. The uptake of the radiopharmaceutical depends both on an adequate delivery system and on a living network of osteocytes (Schimming et al., 2000). ^{99m}Tc-MDP is a highly sensitive marker for blood flow and metabolic activity in bone tissue (Ryan and Fogelman, 1995), this is consistent with other reports that show TPBS has a high sensitivity for the beginning of bone formation (Malizos et al., 1995; Nakai et al., 2000; Droll et al., 2007). Therefore, it can evaluate vascularization and predict the success of the availability of bone formation. This technique may be considered to be the gold standard for demonstrating successful grafting (Smeele et al., 1996).

The results showed that the RCP peaked at 2 weeks in blood pool phase, this demonstrated that vascularization of bone graft were the highest in this week, meaning there was availability of natural biomaterial bone graft. In their study, Lauer et al found that as early as 6-11 days after mandibular reconstruction, increased tracer uptake proves that the surgery has been successful and indicates the presence of a normal healing process (Lauer et al., 2000). TPBS performed within 10 days after the mandibular reconstruction is a useful tool to monitor the viability and the early complications of vascularized mandibular bone grafts (Buyukdere et al., 2006).

In the delayed phase, RCP of the left and right ulna peaked at 4 weeks, revealed the occurring of bone formation while corresponding with radiograph images of the preliminary study of our group that had shown signs of bone formation at 4 weeks. The RCP tended to increase with time in each group and decreased from 6 to12 weeks after surgery because of the beginning of the mineralized processing, resulting in an increasing osteoconductive matrix, alternatively osteoblast number decreased. These findings showed provided that the vascularization of bone grafts were rapid during the first 6 weeks and entered a mature stage from week 6 to week 12 after surgery, which is consistent with the findings of other researchers (Chang et al., 2000).

The RCP of the natural biomaterial bone graft in the delay phase had slightly lower than autogenous bone graft of all the weeks. The results showed that the natural biomaterial bone graft had less bone formation and remodeling than the autogenous bone graft. This observation is expected to be so due to the drawback of the using gelatin scaffold sponge which was available during 6-8 weeks after which it was degraded by the process of the development of foreign body giant cells (Cegielski et al., 2008).

An ideal bone graft material should provide three elements; osteoconductive matrix, osteoinductive factors, and osteogenic cells which have space for infiltrating cells to attach, proliferate, and produce new extracellular matrix (Griffith et al., 2006). Previous studies showed that pore sizes > 300 μM and porosity ranging from 70-80% were optimal for enhanced vascularization and osteogenesis, due to appropriate exchange and supply of oxygen and nutrients (Karageorgiou et al., 2005; Kasten et al., 2008). However, natural biomaterial bone graft of this study had 100-300 μM the pore sizes and 95% porosity of sponge. That may be help to support the results of this present study.

In the present studied using natural biomaterial bone graft in combination; gelatin sponge and HA, which can support the osteogenic cell, the osteoinductive molecule and the osteoconductive filler. These were supported by several studies, for example, Cegielski et al. indicated that gelatin sponge fulfils its function as a cell scaffold and induces no inflammatory reaction (Cegielski et al., 2008), HA inhibits cell differentiation at the same time as it enhances cell proliferation and migration (Schlag and Redl, 1994). HA shares bone induction characteristics with osteogenic substrates, such as calcitonin and bone morphogenic protein (Pirnazar et al., 1997). Hyaluronan primarily acts locally and in combination at the fracture site to promote healing by stimulating callus formation (Radomsky et al., 1999).

There were however several limitations in this study; the short duration in evaluating the viability of the bone graft and the small number of animals used in this study. Therefore, further studies are necessary using a large number animals and also to investigate the correlation of revascularization between TPBS and histopathology study.

In conclusion, this study showed that three phase bone scintigraphy is an efficient noninvasive method to evaluate revascularization and bone formation in bone graft. The efficiency of the natural biomaterial bone graft containing gelatin sponge and hyaluronic acid was not differenct from the autogenous bone graft. The natural biomaterial bone graft may be the same and as good vascularization as the autogenous bone graft. However, the bone formation of the autogenous bone graft was still more efficienct than the natural biomaterial bone graft.

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