ORIGINAL ARTICLE

Tissue-Engineered Hydroxyapatite Bone Scaffold Impregnated with Osteoprogenitor Cells Promotes Bone Regeneration in Sheep Model

Mohd Yazid Bajuri[1] · **Nanchappan Selvanathan**[1] · **Fatin Nadira Dzeidee Schaff**[1] ·
Muhammad Haziq Abdul Suki[1] · **Angela Min Hwei Ng**[2]

Abstract

BACKGROUND: Managing massive bone defects, a great challenge to orthopaedics reconstructive surgery. The problem arise is the supply of suitable bone is limited with many complications. Tissue-engineered hydroxyapatite bone (TEHB) scaffold impregnated with osteoprogenitor cells developed as an alternative to promote bone regeneration.

METHODS: This animal protocol has been approved by Universiti Kebangsaan Malaysia Animal Ethical Committee. The TEHB scaffold prepared from hydroxyapatite using gel casting method. A total of six adolescent female sheep were chosen for this study. Later, all the sheep were euthanized in a proper manner and the bone harvested for biomechanical study. Bone marrow was collected from iliac crest of the sheep and bone marrow stem cells (BMSCs) isolated and cultured. BMSCs then cultured in osteogenic medium for osteoprogenitor cells development and the plasma collected was seeded with osteoprogenitor cells mixed with calcium chloride. Bone defect of 3 cm length of tibia bone created from each sheep leg and implanted with autologous and TEHB scaffold in 2 different groups of sheep. Wound site was monitored weekly until the wound completely healed and conventional X-ray performed at week 1 and 24. Shear test was conducted to determine the shear force on the autologous bone and TEHB scaffold after implantation for 24 weeks.

RESULTS: All of the sheep survived without any complications during the study period and radiograph showed new bone formation. Later, the bone harvested was for biomechanical study. The highest shear force for the autologous group was 13 MPa and the lowest was 5 MPa while for the scaffold group, the highest was 10 MPa and the lowest was 3 MPa. Although, proximal and distal interface of autologous bone graft shows higher shear strength compared to the TEHB scaffold but there is no significant difference in both groups, p value > 0.05. Histologically in both proximal and distal interface in both arms shows bone healing and woven bone formation.

CONCLUSION: TEHB scaffold impregnated with osteoprogenitor cells has the potential to be developed as a bone substitute in view of its strength and capability to promote bone regeneration.

Keywords Bone scaffold · Hydroxyapatite · Tissue-engineered · Osteoprogenitor cells · Bone regeneration

✉ Mohd Yazid Bajuri
ezeds007@yahoo.com.my

1 Department of Orthopedics and Traumatology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Ya'acob Latif, Bandar Tun. Razak, 56000 Cheras, Kuala Lumpur, Malaysia

2 Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Ya'acob Latif, Bandar Tun. Razak, 56000 Cheras, Kuala Lumpur, Malaysia

1 Introduction

Bone graft can be autologous, allogeneic or synthetic [1]. Autograft which considered the "gold standard" of bone grafting as it has the ability to induce bone formation and regeneration through osteogenesis, osteoinduction and osteoconduction whereas allograft and xenograft despite readily available, risk of graft rejection and disease transmission made it less suitable for bone defect treatment [2, 3]. Alloplasts are synthetic material that only has osteoconductive property that limit the ability repairing the challenging bone defects [4]. The limitations of these bone grafts materials have revolutionized tissue engineering involving scaffolds and living cells which have been the leaders in biomedical research [2].

A viable synthetic graft can be enhanced by adding stem cells and growth factors to its three-dimensional porous scaffolds, either separately or in combination [5]. According to previous studies, the most suitable combination of scaffold and cells for bone regeneration are hydroxyapatite and osteoprogenitor cells [6, 7]. Few most intriguing properties in bone marrow stem cells (BMSC) makes it suitable for the development of osteoprogenitor cells impregnated on tissue-engineered hydroxyapatite bone (TEHB) scaffold.

First, plastic adherence is one of the well-known properties of BMSCs which proved that cells can isolate and culture *in vitro*. Moreover, surface antigen expression allows for rapid identification of a cell population by flow cytometry or other similar techniques which makes it easier to identify the BMSCs *in vitro* culture. Third, BMSCs is a multipotent cell of which it can differentiate into osteoblast *in vitro* which aid in osteogenesis. All these three properties proved BMSCs can be isolated and characterised from human bone marrow easily compared to other stem cells type [8].

Besides that, previous studies have proved that hydroxyapatite has good biocompatibility and biomimetic properties [9–11]. A scaffold based on hydroxyapatite able to support attachments and proliferation of osteoprogenitor cells at the implant site. Moreover, the previous findings also indicate that hydroxyapatite construct has strong potentials for bone tissue regeneration because it possesses the functional properties that facilitate cell growth and bone formation [12].

Hydroxyapatite based scaffold provides the necessary support for the cells to adhere, proliferate and in combination with host tissues, produce mineralised matrix. A combination of Hydroxyapatite based scaffold and BMSCs has also shown to promote osteogenesis by osteoblasts [13].

In this study, we aim to prove that BMSC seeded hydroxyapatite scaffold can promote osteogenesis via osteoinduction and osteoconduction.

2 Materials and methods

In this study, the autologous bone graft is compared with the dual-density TEHB scaffold impregnated with osteoprogenitor cells. The dual-density means the outer solid area and the inner porous layer. The solid area of TEHB scaffold provides stability and inner porous layer serves to facilitate vasculature to stimulate osteogenesis. TEHB scaffold is made from hydroxyapatite because of its osteoconductive property and it also promotes osteointegration.

2.1 Animals

The animal protocol was reviewed and been approved by the Universiti Kebangsaan Malaysia Animal Ethical Committee, approval number FF-2015-369. Six adolescent female sheep (female, 7–12 months and 20–25 kg) were chosen for this study. It was then were allowed to acclimatize for 2 weeks. The sheep are of the merino breed. There are two groups i.e. Group 1: Autologous bone graft; Group 2: cell-seeded TEHB grafts, n = 3 for each group. The sample size is small as this is a pilot research and a proof of concept study. For prophylactic antibiotics, IV Amoxycillin 15 mg/kg BW was administrated intramuscularly along with analgesia i.e. Meloxicam 2 mg/kg BW daily and IM tramal 2 mg/kg. The animal's vital signs were monitored daily.

2.2 TEHB scaffold preparation

Gel casting method was used to prepare the scaffold and results shown in Fig. 1. The components of the gel casting method were organic monomer and cross-linker: monofunctional acrylamide, $C_2H_3CONH_2$ (monomer) and difunctional methylenebisacrylamide $(C_2H_3CONH)_2CH_2$ (cross-linker). Ammonium persulphate $(NH_4)_2S_2O_8$ and N, N, N', N'-tetramethylethylenediamine (TEMED) was used as initiator and catalyst, respectively. β-TCP [β-$Ca_3(PO_4)_2$] and hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) powders were used to prepare slurry (both of them were nano-powder with a particle size of < 200 nm). Darvan C-N, a 25% aqueous solution of ammonium polymethacrylate, was used as a dispersant. The scaffolds were prepared using polymeric sponges which soaked in a ceramic slurry containing monomers, cross-linker.

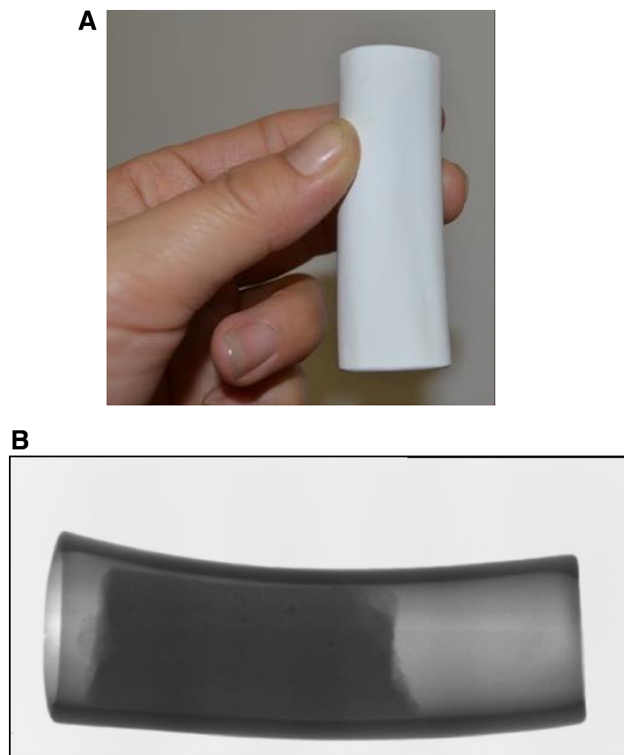Catalyst and initiator have been added for rapid gelation via in situ polymerization—a newly defined processing

Fig. 1 Tissue-engineered hydroxyapatite bone scaffold preparation. **A** Dual-density TEHB scaffold prepared through the Gel casting method. **B** The X-ray picture of dual-density TEHB scaffold (Solid outer layer and porous inner layer)

(gel-sponge) method that combined the gel-casting and polymer sponge methods were used. In preparation of the porous scaffolds, polyurethane foam cut into desired shapes and sizes (40 mm long tubular shaped with diameter of about 20 mm) were soaked in the slurries. The polymerised samples were dried in air for 24 h and then underwent sintering process. The scaffolds heated at a rate of 1 °C/min to 600 °C and kept at this temperature for 1 h to burn out the polyurethane foams, and then were sintered with temperature increased at a rate of 3 °C/min to 1150 °C and kept in this temperature for 2 h. The scaffolds were individually packed and sterilised by gamma irradiation.

2.3 BMSCs isolation and culture

Aspirated bone marrow from the thoraco-lumbar spine of patients undergoing pedicle screw fixation was first diluted with standard culture medium supplemented with 10% fetal bovine serum. Then, mononuclear cells were isolated from the diluted bone marrow via gradient centrifugation (Universal 32R, Hettich, Germany) over a Ficoll-Paque layer at 5000 rpm for 20 min and subsequently washed twice with phosphate-buffered saline. The cells were resuspended in culture medium (F12: DMEM 50:50 supplemented with 10% fetal bovine serum) and plated onto a 25 cm^2 culture flask. All cultures were then incubated at 37 °C in a controlled environment of a humidified atmosphere of 5% CO_2. On day three, fresh medium was added upon it. Medium was changed upon substantial cell attachment and later, twice a week. Upon cell confluence, cells were detached by the addition of 0.05% of trypsin–EDTA solution and counted using trypan blue dye-exclusion-method and a haemacytometer. Subsequently, these cells were subcultured at a standard density of 5000 cells/cm^2. Subculturing was performed for 3 to 5 times (Passage 3–5) in order to expand the cells to approximately 30 million. One week before the implantation, cells were maintained in Osteogenic Medium (culture media supplemented with 10–7 M dexamethasone, 0.05 mg/ml ascorbate-2-phosphate, 10 mM b-glycerophosphate) for one week to induce osteogenic differentiation.

2.4 *In vitro* TEHB scaffold preparation

TCP/HA cylinders were firstly pre-wetted with medium. Approximately 30–50 million osteoprogenitor cells were seeded on each cylinder. Cells were premixed with plasma at the ratio of 1 × 106 cells to 100 ul plasma and dropped using a pipette onto the pre-wetted granules. Polymerisation of the fibrinogen in the plasma was initiated by the addition of 100 ul of 0.5 M $CaCl_2$. The conversion of fibrinogen into fibrin will trap the cells within the scaffold. The cell-seeded TEB scaffold (bone constructs) will then be immersed in osteogenic medium for one week in a CO_2 incubator to induce osteogenic differentiation. After 1 week, the cell-seeded scaffold will then be transported immersed in the osteogenic medium at 4–18 °C in a cold box to the operating theatre for implantation.

2.5 TEHB scaffold implantation

An autologous, tricortical bone graft harvested from the left iliac crest. A 5 cm incision was made along the iliac crest. The lid was removed slowly with a raspatory and tricortical bone harvest utilising a bone curette. The lid will be reinserted, the musculatures reattach and the wound closed in layers. An external fixator applied over the tibia. A longitudinal incision of approximately 5 cm length was made along the medial aspect of the limb to expose the right tibia. In order to avoid damage to proximate nerve and blood vessels during osteotomy, a wet compress was placed in between the bone and posterolateral soft tissue. A parallel osteotomy perpendicular to the bone's longitudinal axis were performed while the bone segment was excised about 3 cm in length. The periosteum within the defect area should be removed completely. The iliac bone fragments were placed realign and wound closed in layers.

Biplanar external fixators were applied in both groups to stabilize the fracture and create a rigid fixation. Postoperatively, the animals were allowed unrestricted weight-bearing.

The implantation method of the experimental group is of the same manner as the TEHB scaffold group. The only difference is the bone graft is replaced with the TEHB scaffold.

2.6 Radiological analysis

The bone formation at the implantation site of sheep was monitored radiologically at week 1 and week 24. Conventional X-ray analysis (3.2 mAs; 65 kV) in two standard planes (anterior–posterior and lateral) were performed to assess bone formation. For the definition of radiological bone union in this study, the radiographic union scale in tibial fractures (RUST) score was used [14].

2.7 Euthanasia

The sheep were euthanized according to Muslim ritual at week 24. It is done in lawful halal animals including sheep. Then, both hind limbs were disarticulated at the knee and tibia was dissected, external fixators were removed. Only the needed part was harvested and others were buried in a proper way.

2.8 Biomechanical testing

After 24 weeks, the harvested bone samples were tested to determine its biomechanical strength of dual-density TEHB scaffold and autologous bone. One end of the tibia was mounted in a custom-made jig that was designed for this test. Instron 8874 biaxial testing machine was used to provide the shear force towards the interface (Fig. 2). A shear test was conducted at a compressive load of 0.05 kN until the fracture point was reached. The contralateral tibia used as a paired reference. The shear strength values were calculated based on the following formula. Where,

Force = 0.05 kN used in the shear test

Surface area = average of 3 diameter from the axial dissection of the bone divided by 2 and times with πr^2

2.9 Histological analysis

For histological analysis, the mid-defect regions were sectioned in the transversal and sagittal plane. Callus tissue composition will be evaluated on 6 μm-thick methylmethacrylate embedded sections stained with Safranin

Fig. 2 Instron machine for shear stress test. Shows the shear stress test on the TEB scaffold using the Instron machine

Orange/von Kossa (mineralized tissue, black), and Movat's pentachrome to demonstrate bone (yellow), cartilage (deep green) and fibrous tissue (light green blue).

2.10 Statistical analysis

The data were analysed using statistical package for social science (SPSS) version 16. Data were expressed as mean ± SEM (Standard error of mean). P value less than or equal to 0.05 is considered statistically significant. Student's t test was performed to compare the mean of interface shear strength of TEHB and autologous group.

3 Results

All the sheep survived the experimental procedures without any serious complications that require the sheep to be dropped out from the study. One of the sheep from the TEHB scaffold group sustained foot drop following surgery. Due to weight-bearing on the limb, the sheep had an infected ulcer at the dorsum part of the hooves. The ulcer required debridement and placed a 3.00 mm wire to stabilize the ankle. Every other day dressing was done and wound healed after a month.

3.1 Evaluation of radiological analysis

The radiological analysis shows that the signs of union have occurred based on there is no breakage in the cancellous part of the bone. There is also no demarcation line in between the osteotomy site and the scaffold site based on the X-ray. It was tested clinically by moving the osteotomy site which is non-mobile on movement. Based on this, it is concluded that the bone has unite radiologically and clinically. Bone healing has been monitored by a serial radiological X-ray. Figure 3 shows the bone formation at the implantation site at week 1 and week 24.

3.2 Mechanical test

Overall, the shear strength of autologous group was higher for the autologous group compared with the scaffold group. The highest shear force for the scaffold group was 10 MPa and the lowest was 3 MPa. While for the autologous group, the highest shear force achieved was 13 MPa and lowest
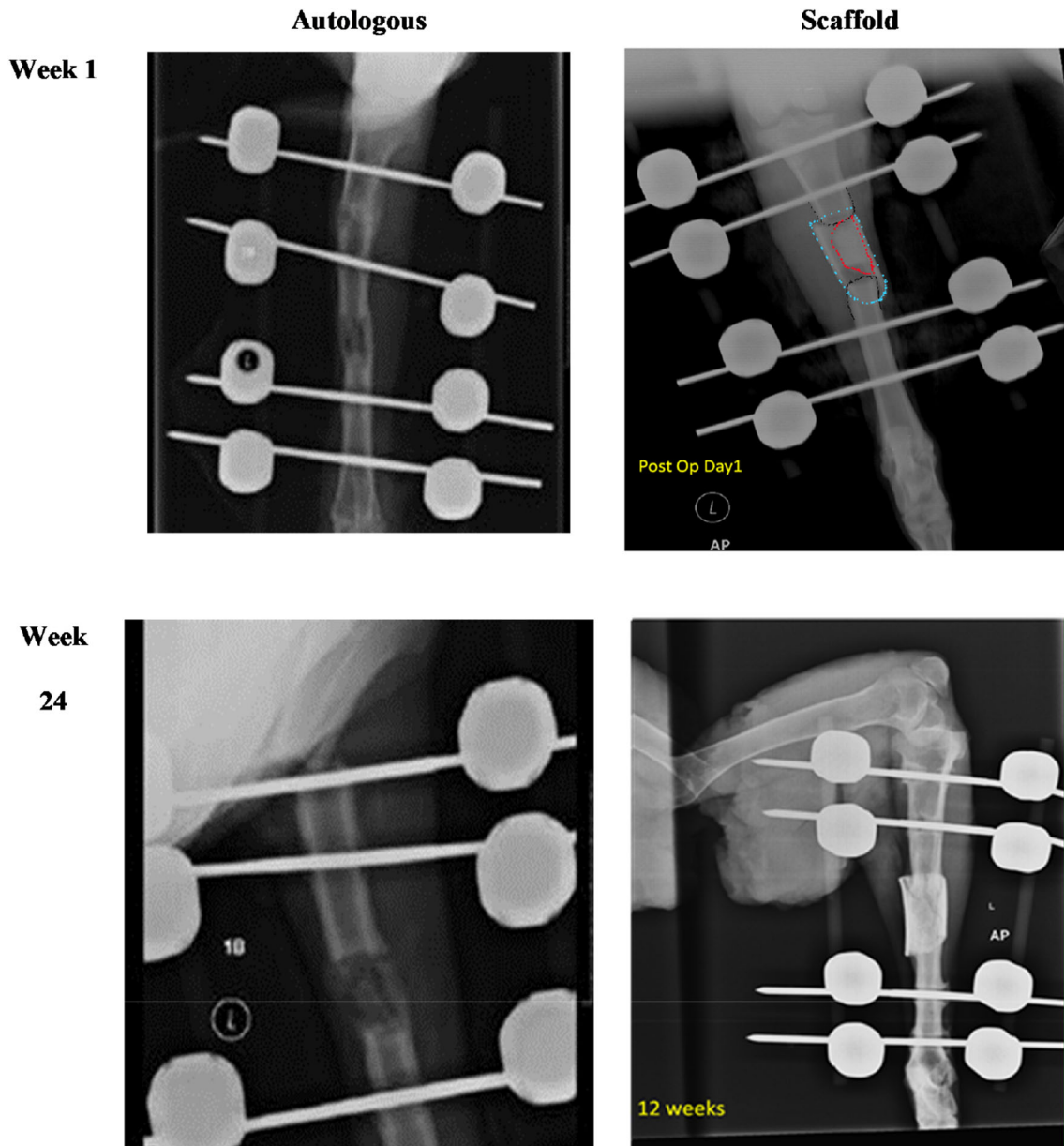

Fig. 3 Radiograph of bone regeneration at implantation site. The bone regeneration at the implantation site can be observed from week 1 until week 24. The callus formation can be clearly observed at week 24. The differences in the bone formation can be observed at the pictures above. Day one post-op X-ray radiograph for the scaffold group. The red dot outlines the porous scaffold, the black dot outlines the host bone and the blue dot outlines the solid dense scaffold. Three months (12 weeks) post-op X-ray radiograph for the scaffold group. The margin between host bone and porous scaffold cannot be clearly discerned. The dense scaffold was largely intact with slight cracks

was 5 MPa. At best, the shear strength for the scaffold group achieved 50% of the shear strength of its contralateral limb. While the autologous group achieved 65% of the shear strength of its contralateral limb in the best case.

For the mean and standard deviation, mean shear strength of distal interface between TEHB and host bone is 3.33 ± 1.25 MPa, mean shear strength of proximal interface between TEHB and host bone is 5.33 ± 3.5 MPa and mean shear strength of contralateral bone for TEHB group is 26.67 ± 11.96 MPa. While the mean shear strength of distal interface between autograft and host bone is 9.33 ± 4.57 MPa, mean shear strength of proximal interface between autograft and host bone is 8.33 ± 4.32 MPa and mean shear strength of contralateral bone for autograft group is 16 ± 7.13 MPa. Student's t test was performed to compare the mean of interface shear strength of TEHB and autologous group. No statistical difference was established ($p > 0.05$) (Fig. 4).

3.3 Histology analysis

3.3.1 Specimen 1 autologous graft

Autologous graft proximal interface A1P—Section shoes normal configuration of hyaline cartilage and bony tissue towards one end, along with presence of woven bone, intermixed with fragmented pieces of bony tissue and fibrocollagenous element towards the ceter.

Presence of osteocytes is conspicuous noted within the bony tissue.

Normal healing process.

Autologous graft distal interface A1—Section shows similar features as above, with the distal portion exhibiting normal smooth muscle componenet, fibrous tissue towards the periphery, rimming hyaline cartilage and juxtaposed normal woven bone.
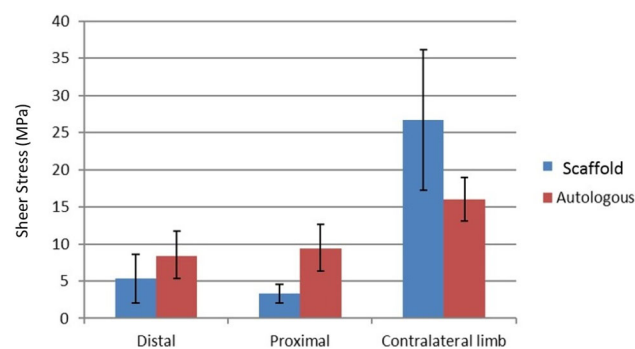
Normal healing process.

Fig. 4 Comparison between TEHB and autologous group. Comparison on the shear stress (MPa) force at distal and proximal interface and at contralateral limb between scaffold and autologous group

3.3.2 Specimen 2 autologous graft

Autologous graft proximal interface A2P—Section shows well-delineated hyaline cartilage and normal lamellar bone with fibrocillagenous tissue muscle at one end, along with areas of well delineated woven bone exhibiting osteocytes, and normal marrow content within.

New bone formation, normal in configuration.

Autologous graft distal interface A2—Section shows similar features as above, with focal area exhibiting of fibrocollagenous tissue towards the end.

Thickened blood vessels are seen towards the periphery.

New bone formation, normal in configuration.

3.3.3 Specimen 3 Autologous graft

Autologous graft proximal interface A3P Section shows transverse section of a well circumscribed woven bone, presence of numerous osteocytes rimmed by thickened and hyalinized fibrocollagenous tissue and skeletal muscle component towards the periphery.

Autologous graft distal interface A3—Section also shows similar feature as above.

Feature of normal healing process with well-delineated woven bone formation is noted.

3.3.4 Specimen 1 scaffold

Proximal scaffold interface S1 A—Section shows cental area of fatty marrow, at places showing purplish hydroxyapatite-like material and well delineated woven bone showing numerous osteocytes within.

Surrounding fibrocalllagenous tissue and interface of collagenous element with bone appear normal.

Distal scaffold interface S1—Similar features noted, but there is also evidence of presence of purplish granular material within the marrow at places and also within the woven bone in many areas.

Surrounding fibrocartillagenous tissue shows strip of similar material at the periphery.

3.3.5 Specimen 2 scaffold

Proximal scaffold interface S2 A—Section shows woven bone with osteocytes and the marrow exhibiting predominantly fatty element. Thin peripheral rim of fibrocollagenous tissue is noticed.

Distal scaffold interface S2—Similar feature as above noted with woven bony configuration, along with presence of osteocytes.

3.3.6 Specimen 3 scaffold

Proximal scaffold interface S3A—Section shows woven bony lamella on either sids, with central slip of fibrocollagenous and eosinophilic tissue embedded within which granular violaceous material is noticed focally.

Foci of metaplastic transformation of the fibrous tissue into woven bone is also distinctively noted.

Distal scaffold interface S3—Section shows similar configuration of woven bone on either side with surrounding intervening areas of fibrocollagenous and eosinophilic tissue, containing at places violaceous granular content.

Numerous osteocytes are seen within the woven bone.

Metaplastic transformation of the fibrous tissue into bony element is also noticed.

Figure 5A shows well defined bone formation, taken from the proximal end. There are scattered osteocytes within. Figure 5B shows an interphase zone showing partial formation of bone along with pale areas of acellular material.

4 Discussion

Our bone is one of the extraordinary systems in which its ability to heal without any scarring. However, perturbations of the fracture site may result in disruption of the repair process if the bone defects reach a critical size that leads to non-union. A clinical example of this situation is the principal bone defects created during the resection of neoplasms [8]. In view of the multiple limitations that associated with the use of autografts or bank bones for bone reconstruction, researchers have found the alternative, of implanted material which can promote bone healing also known as a bone graft, whether in combination with other material or by itself [6]. This augmentation of bone healing occurs accordingly by osteoconduction, osteoinduction and osteogenesis. Osteoconductive material allows new bone formation whereas osteoinductive graft allows recruitment and differentiation factors for bone-forming cells at the recipient site. An osteogenic graft helps to induce bone-forming cells to the recipient site. The feature of an ideal bone graft is the ability to have all of the three functions [4]. A study showed an intramedullary cement osteosynthesis using calcium phosphate cement (CPC) which consist the properties of osteopromotive effect helps provide bone stabilization which acts as bone substitutes used in an impending fracture or compromised bone such as osteoporosis or cyst [15].

The design of the scaffold is based on biological principles that have evolved from understanding how autografts function and how they are remodelled after transplantation. Scaffold for osteoprogenitor cells, regardless of the materials from which they are formed, should encourage osteoprogenitor cells adhesion, proliferation and differentiation to elicit bone formation [1]. The implanted scaffold has to be vascularised because osteogenesis needs a well-developed vascular supply. The scaffold porosity, pore size distribution and continuity also critical for vascular invasion. All these parameters help the interaction between the scaffolds and transplanted cells with the host environment. Pioneering studies found that pore sizes less than 15–50 mm helps in fibrovascular ingrowth whereas pore sizes of 50–150 mm promotes osteoid formation, and pore sizes more than 150 mm encourage the ingrowth of mineralised bone [12]. A TEHB scaffold, with an average pore size of 250 mm with an interconnected structure with
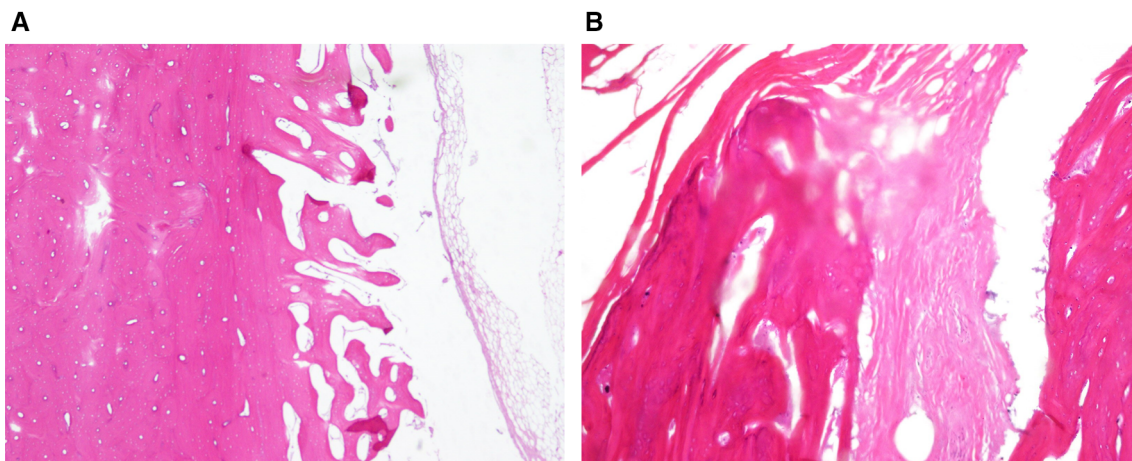

Fig. 5 Histological pattern of TEHB. **A** This area shows well defined bone formation, taken from the proximal end. There are scattered osteocytes within. **B** This is an interphase zone showing partial formation of bone along with pale areas of acellular material. Scale is: magnification—10x

no dead-end pockets, should facilitate vascular invasion and bone development. Ideally, the TEHB scaffold should be resorbed at a rate proportionate with new bone formation. In this respect, TEHB scaffold impregnated with osteoprogenitor cells was developed to induce osteogenesis at the defected site by osteoconduction and osteoinduction.

The *in vivo* performance of TEHB scaffold was examined using a sheep tibial bone defect. A tibial bone defect was done on sheep model by removing 3 cm bone and the bone regeneration was assessed by serial radiological X-ray and the bone tissue formation was assessed after 6 months of implantation. Tibial bone formation was observed in the defected area of sheep with TEHB scaffold and autologous graft. The bone union can be observed in both autologous and scaffold group when comparing the X-ray taken at week 1 and week 24. Moreover, comparison of shear test was done between the scaffold and the autologous bone graft. In all 3 samples, the proximal and distal interface of autologous bone graft shows higher shear strength compared to the scaffold. In the second sample of the scaffold group, it shows 10 MPa of shear force at the proximal interface. Relatively comparable with the autologous bone graft in view of the highest shear strength in the autologous group proximal interface is 13 MPa. Highest shear strength in the scaffold at the distal interface is 5 MPa in relation to the autologous group which is 2 times more, 10 MPa. The shear force for the contralateral leg is a benchmark for a normal shear force in a non-defect bone. The lowest is 13–15 MPa. The highest for the scaffold group is 10 MPa and the autologous group is 15 MPa bearing the fact that the interface is only bony union and not remodelled.

The bone formation in autologous sheep group is higher compared to scaffold sheep group. This is because the autologous bone has all the three functions which allow it to be better incorporated based on their porous architecture,

which allows easy cellular and vascular invasion [1]. The cancellous part of bone graft has a large surface area that is covered by osteoblasts, which make it osteogenic as well as osteoconductive for three-dimensional bone growth. In addition to that due to the extensive vascular invasion, the bone matrix can readily be demineralised, and its proteins exposed through the actions of osteoclasts. This later will lead to the release of osteoinductive matrix proteins [12]. By contrast, autogenous cortical bone grafts are more solid which provides mechanical stability for graft at the defect site. Therefore, the new bone formation at the interface of the autologous bone graft is faster compared to bone scaffold which supports the facts that shear strength of the autograft higher that TEHB scaffold [16]. These findings were similar with a fracture fixation in which the higher the screw tension will reduce the displacement of the bone segment and allow the fracture to heal accordingly [17].

The lower shear strength and the slow bone formation in the scaffold group sheep because of slow rate of scaffold degradation. This is because the rate of bone formation should be similar to the rate of scaffold degradation [8]. The bone formation at the defect area implanted with TEHB scaffold is slower because of slow reabsorption of TEHB scaffold. Similar to autograft, osteoclast is responsible for reabsorption of TEHB scaffold.

Histologically in both proximal and distal interface in both arms shows bone healing and woven bone formation (Fig. 6). Generally, clinical union is assessed by demonstrating maneuver of forces at the site of bone defect, and radiological union is by assessing 3 out of 4 cortices at the site of bone defect. Having all 3 parameters favours the evidence of union at the interface.

Therefore, from this study we conclude that bone reunion is autograft group sheep is faster compared scaffold group based on the serial radiological X-ray and shear
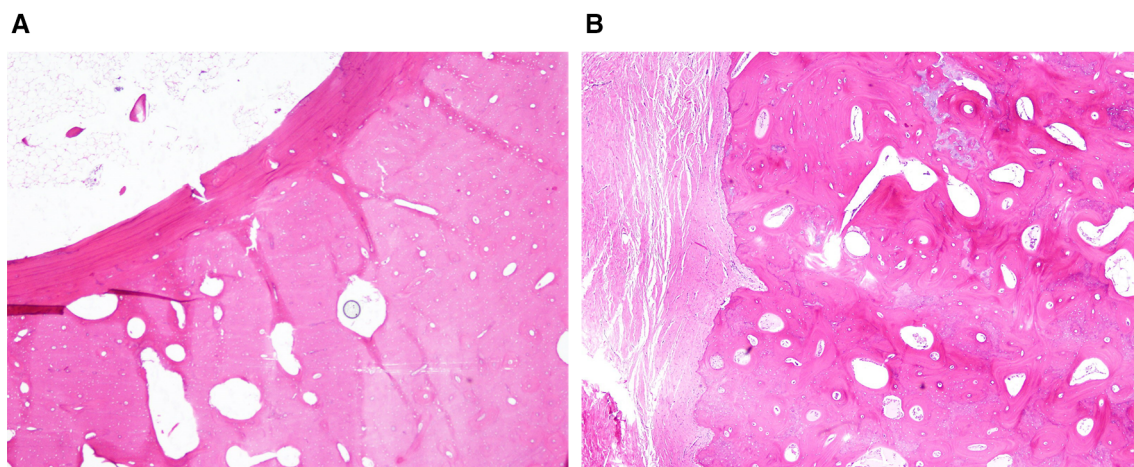
A

B

Fig. 6 Histological pattern of TEHB and autologous bone graft. **A** Scaffold tissue, area of new bone formation towards the proximal end. Transverse section of the bone showing thickness towards the lumen. **B** Histology pattern of autologous bone graft, this bone formation in the control tissue appears similar to the changes seen in the scaffold element. Scale is: magnification—10x

test. The evaluation was made clinically as the radiological analysis also show signs of union based on serial X-ray being done. We assume that TEHB scaffold reabsorption by osteoclast may be affected by several factors as explained above. However, the bone union rate observed in scaffold group is almost like autologous bone graft group. Bone formation at the defect site only displayed bone union and the bone is not fully remodelled.

We conclude that bone union is autograft group sheep is faster compared scaffold group based on the serial radiological X-ray and shear test. We assume that TEHB scaffold reabsorption by osteoclast may be affected by several factors as explained above. However, the bone union rate observed in scaffold group is almost like autologous bone graft group. Bone formation at the defect site only displayed bone union and the bone is not fully remodelled.

Compliance with ethical standards

References

1. Baghaei K, Hashemi SM, Tokhanbigli S, Asadi Rad A, Assadzadeh-Aghdaei H, Sharifian A, et al. Isolation, differentiation, and characterization of mesenchymal stem cells from human bone marrow. Gastroenterol Hepatol Bed Bench. 2017;10:208–13.
2. Polo-Corrales L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold design for bone regeneration. J Nanosci Nanotechnol. 2014;14:15–56.
3. Sohn HS, Oh JK. Review of bone graft and bone substitutes with an emphasis on fracture surgeries. Biomater Res. 2019;23:9.
4. Mareschi K, Ferrero I, Rustichelli D, Aschero S, Gammaitoni L, Aglietta M, et al. Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. J Cell Biochem. 2006;97:744–54.
5. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials. 2018;180:143–62.
6. Zhu H, Guo ZK, Jiang XX, Li H, Wang XY, Yao HY, et al. A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone. Nat Protoc. 2010;5:550–60.
7. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: a review. Bioact Mater. 2017;2:224–47.
8. Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, et al. Scaffolds for bone tissue engineering: state of the art and new perspectives. Mater Sci Eng C Mater Biol Appl. 2017;78:1246–62.
9. Kattimani VS, Kondaka S, Lingamaneni KP. Hydroxyapatite—past, present, and future in bone regeneration. Bone Tissue Regen Insights. 2016;7:9–19.
10. Ficai A, Andronescu E, Voicu G, Ficai D. Advances in collagen/hydroxyapatite composite materials. In: Attaf B, edior. Advances in composite materials for medicine and nanotechnology, IntechOpen; 2011. Chapter 1. https://doi.org/10.5772/13707.
11. Zhou H, Lee J. Nanoscale hydroxyapatite particles for bone tissue engineering. Acta Biomater. 2011;7:2769–81.
12. Lim J, Razi ZRM, Law JX, Nawi AM, Idrus RBH, Chin TG, et al. Mesenchymal stromal cells from the maternal segment of human umbilical cord is ideal for bone regeneration in allogenic setting. Tissue Eng Regen Med. 2018;15:75–87.
13. Zhou J, Xu C, Wu G, Cao X, Zhang L, Zhai Z, et al. In vitro generation of osteochondral differentiation of human marrow mesenchymal stem cells in novel collagen–hydroxyapatite layered scaffolds. Acta Biomater. 2011;7:3999–4006.
14. Whelan DB, Bhandari M, Stephen D, Kreder H, McKee MD, Zdero R, et al. Development of the radiographic union score for tibial fractures for the assessment of tibial fracture healing after intramedullary fixation. J Trauma. 2010;68:629–32.
15. Mirzasadeghi A, Narayanan SS, Ng MH, Sanaei R, Cheng CH, Bajuri MY, et al. Intramedullary cement osteosynthesis (IMCO): a pilot study in sheep. Biomed Mater Eng. 2014;24:2177–86.
16. Petite H, Viateau V, Bensaïd W, Meunier A, de Pollak C, Bourguignon M, et al. Tissue-engineered bone regeneration. Nat Biotechnol. 2000;18:959–63.
17. Izzawati B, Daud R, Afendi M, Majid MA, Zain NAM, Bajuri Y. Stress analysis of implant-bone fixation at different fracture angle. J Phys Conf Ser. 2017;908:012019.