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RESEARCH ARTICLE

MESH INDUCED BIOMEMBRANE: THE CONCEPT OF SYNTHETIC PERIOSTEUM

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ABSTRACT

Repair of segmental bone defects is an enormous challenge for reconstructive surgeons. Available techniques are fraught with difficulties. In view of these critical concerns, a novel technique of bone grafting for management of long bone defect in rabbit model was proposed. Primary objective was to ensure the feasibility of proposed technique and tested the hypothesis that the Polyglycolic acid mesh induced bio-membrane imitates periosteum both mechanically and biologically. The cohort comprises of ten adult rabbits (Lepus negricolis) of either sex weighing between 2.5 and 3.5 kg. The proposed single stage technique using the principle of induced membrane and GTR (Guided Tissue Regeneration) was done to manage the long bone defect. Radiological assessments were performed at 4, 8, and 12 weeks. At 4 and 12 week an open biopsy of induced membrane so called "synthetic periosteum" was done for histomorphological evaluation. The normal periosteum of contra-lateral limb was used as control. At 12 weeks, Radiographs revealed a successful osseous union. A vascularized induced bio-membrane, closely adherent to the bone was appreciated in place of mesh. No sign of infection was detected in any rabbit. Histological outcome demonstrate mesh induced bio-membrane so-called "synthetic periosteum" is likely to function as original periosteum that has mechanical and biological advantages. The induced membrane is hypervascularized promote revascularization and favors the corticalization of the autograft. The technique proposed here will open new dimensions in the field of management of segmental long-bone defects both in animals and human.

INTRODUCTION

Bone defects, traumatic or caused by congenital malformation, or following tumor excision remains a critical challenge in orthopedic surgery. Repair of segmental bone defects is a long and arduous task for the surgeon. Fibrous healing of bone defects instead of osseous regeneration causing functional disturbances (Hjorting-hansen. & Anderson J O, 1971). Multiple surgeries are often required to manage segmental bone defect owing to complex mechanics and biology involved in reconstruction. A procedure such as distraction osteogenesis which has steep learning curve requires sophisticated armamentarium; associated with complication such as pin tract infection and impaired fracture healing (DeCoster TA *et al*,2004; Ilizarov GA and Ledyaev VI, 1992; Trigui M *et al*, 2008). The osteomyocutaneous flap (Yazar S *et al*,2004) or vascular fibula (Levin LS,2006; Legré R *et al*, 1998; El-Gammal TA *et al, 2008*) requires microvascular anastamoses usually associated with significant donor site morbidity. More recently, a two stage French technique (Masquelet AC *et al*, 2000; Masquelet AC and Begue T,2010) of bone-grafting within induced bio-membranes using antibiotic impregnated polymethylmethacrylate spacer offers a potential treatment alternative. Toxic effects of the non-reacted monomers, highly exothermic reaction during polymerization and associated complications of polymethylmethacrylate (Dahl OE *et al* 1994; Deramon H *et al*, 1999; Leeson MC and Lippitt SB 1993; Memtsoudis SG,2007; Salles AG *et al*, 2008) long term hardware complications along with staged procedure are major issue of concern to surgeons. Furthermore, periosteum is vital to bone; stripping/denudation of periosteum in high energy injuries like open fracture (Gustilo RB, Anderson JT, 1976) will lead to bone necrosis the formation of sequestra; difficulty in graft containment, increased resorption of graft contained and also allows diffusion of growth and osteoinductive factors to external environment. As a result, a solution that resolves these issues must be provided. To overcome these pitfalls, present technique of "synthetic periosteum" using Polyglycolic acid mesh in rabbit model is proposed. The present research study has two major aims. First is to investigate the feasibility

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of the proposed technique of bone grafting so as to modify the previous staged technique to novel single stage technique of bone grafting. The study's second aim is to test the hypothesis that the proposed concept of "synthetic periosteum" of induced bio-membrane imitates natural periosteum both mechanically and biologically. The present model, however, is of basic interest because it comes close to the situation of gap non-union where the surgeon's attempts union by armamentarium other than simply bone grafting and/or stabilization. To the best of our knowledge such concept of "synthetic periosteum" has not been reported until now.

METHOD AND MATERIAL

In current research, we used rabbits as an animal model to develop a cost-effective approach to manage the long bone defects. The proposed technique should be suitable for future clinical application. The present research is approved by the Dean, Director Research Services (No. 879/DRS/NDUSU; Dated 08.07.2013) Institutional Animal Ethical Committee (No.46/IAEC/CWFH/2013; Dated 23.08.2013), College of Veterinary Science and Animal husbandry Jabalpur and Dean and Chairperson IAEC NSCB medical college Jabalpur (No. 114/Ortho; Dated 19.11.2013; No. 14608; dated 04.12.2013; 13.01.2014) after screening thoroughly keeping in mind the guidelines of CPCSEA.

The cohort comprises of ten adult rabbits (Lepus negricolis) of either sex weighing between 2.5 and 3.5 kg; with complete closure of epiphysis plate. The rabbits were housed in individual cages and accustomed to handling and animal housing environment for 2 weeks. During pre-and post operative period standard diet and water were made ad libitum to them. Animals were anesthetized with a combination of 6-8 mg/kg xylazine and 50 mg/kg ketamine administered intramuscularly.

Surgical procedure (Figure 1)

The single stage technique of mesh induced membrane of bone grafting comprises of: 1) Creation of bicortical bone defect; 2) Placement of VICRYL (polyglactin 910) mesh over the defect; 3) Stabilization of defect; 4) Harvesting of cortico-cancellous graft; 5) Packing of the defect with cortico cancellous graft; 6) Suturing of mesh with the graft just like a sleeve; 7) closure.
The hind limb were shaved and washed with chlorhexidine gluconate. Under aseptic precaution after draping, the bone was exposed through antero-lateral approach. The soft tissue was carefully dissected, periosteum elevated. Complete bicortical defect was created with micro drill in meta-diaphyseal region of tibia measuring 5-8mm. The periosteum was removed along with the bone. Also approximately 5mm of periosteum was further stripped from both proximal and distal fragment. The defect was stabilized with stainless steel plates (2 mm; Sky) and cortical screws (6-8 mm) maintaining the length of the limb. The plate was applied on lateral surface. The VICRYL (polyglactin 910; Ethicon) mesh was then placed in such a way that it encircles the entire defect both proximally and distally just like a sleeve. The osteotomized bone that that was removed was used as a source of bone graft. Also from the proximal third through a small cortical window cancellous graft was harvested. After placing mesh, the defect created was press packed with cortico-cancellous graft. The mesh then sutured with 4-0 resorbable sutures. The graft was very well contained within the mesh sleeve. Muscle fascia and skin were closed separately over the defect with 4-0 resorbable sutures. The limb was immobilized with posterior splint. All surgeries were conducted by the same investigator.

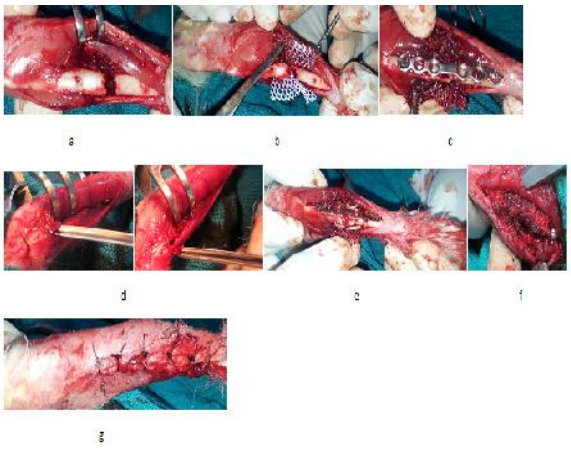

Figure 1 Steps of proposed technique

Figure 1 a) Creation of bicortical bone defect; b) Placement of VICRYL (polyglactin 910) mesh over the defect; c) Stabilization of defect; d) Harvesting of cortico-cancellous graft; e) Packing of the defect with cortico cancellous graft; f) Suturing of mesh with the graft just like a sleeve; g) closure.

Post-procedure protocols: Animals were handled according to the standard procedures of laboratory animals. A broad spectrum antibiotic (Amoxicillin-Sulbactam combination; ~20 mg/kg twice daily for five days) and analgesic (meloxicam; 0.1mg/kg once a day for three days) were given. X-ray assessments were performed (at least two planes) at 4, 8, and 12 weeks: to record the orientation of implants, and also it provide a basis for the evaluation of how fracture healing is progressing (Ohgushi H *et al*, 1989). At 4 and 12 week an open biopsy of induced membrane so called "synthetic periosteum" was done for histomorphological evaluation. The normal periosteum of contra-lateral limb was used as control.

RESULT

All the rabbits recovered well from anesthesia. The post-operative course was uneventful in eight of the rabbit; one of the rabbit died 4 weeks after the procedure due to respiratory problem. The other one underwent second surgery after 1 week due to implant failure.

Figure 2 Radiological evaluation

Figure 2 Radiological evaluation: after 4 week documented a sufficient bone formation with a complete bone bridge area. At 8 week, x-rays demonstrated continuous radio-dense area in the defect. At 12 weeks, bone union was completely accomplished and medullary cavity was nearly recanalized.

Radiological and histopathological findings

X-ray (Figure 2): The radiological investigation revealed the implant to be in position; X-ray analysis after 4 week documented a sufficient bone formation with a complete bone bridge in the defect area. At 8 weeks, x-rays demonstrated continuous radio dense area in the defect. At 12 weeks, bone union was completely accomplished and medullary cavity was nearly recanalized. The created osteoperiosteal defect was repaired successfully.

Gross examination (Figure 3): in general, the findings were in accordance with the results of x-rays. Macroscopic evaluation revealed the experimental tibia- mechanically stable and to consist of a bone-like tissue. No sign of implant dislocation was observed. The autogenous cortico-cancellous graft was integrated well with the parent bone, both proximally and distally. A vascularized induced bio-membrane, closely adherent to the bone was appreciated in place of VICRYL (polyglactin 910) mesh. No sign of infection was detected in any rabbit.

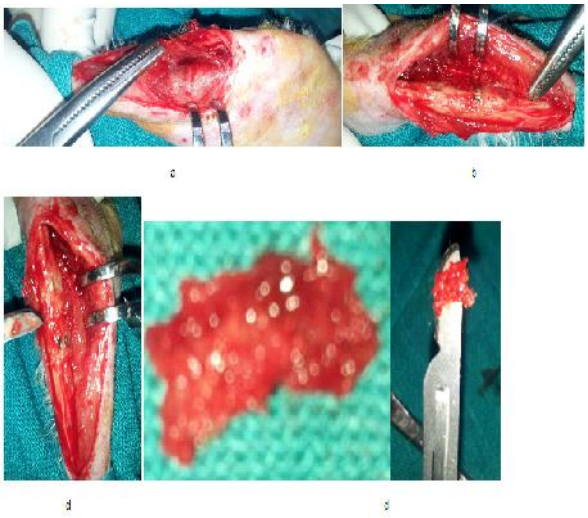

Figure 3 Gross evaluation

Figure 3: Gross evaluation: a) & b) A vascularized induced bio-membrane, closely adherent to the bone was appreciated n place of VICRYL (polyglactin 910) mesh; c) autogenous cortico-cancellous graft was well integrated with the parent bone, both proximally and distally; d) the synthetic periosteum (the mesh is replaced by vascularized bio-membrane)

Histology (Figure 4)

Control: The normal periosteum that acts as a control showed fibro-collagenous cells with blood vessels.

Mesh induced bio-membrane (synthetic): Histological analysis of mesh induced biomembrane the so called "synthetic periosteum" showed:

At 4 week, fibro-collagenous tissue showing marked granulomatous reaction consist of inflammatory cells and giants cells. Small, clear spaces are seen. The surrounding area shows granulomatous reaction. Few osteocytes round bodies present with eosinophillic cytoplasm. The histological picture imitates the reparative phase of fracture healing. At 12 weeks, histological evaluation revealed periosteal reaction with osteoclast like giant cell. Bands of fibro-collagenous tissue with minimal inflammatory cells are seen. Few fat cells surrounded by fibro-collagenous tissue and inflammatory cells and few osteoclasts like cells. Periosteal reaction with reduced inflammatory cells and increased collagen with appearance of osteoclast showed healing/remodeling phase. At 12 weeks, the histological picture of induced bio-membrane very nearly imitates that of control i.e. with fibro-collagenous tissue.

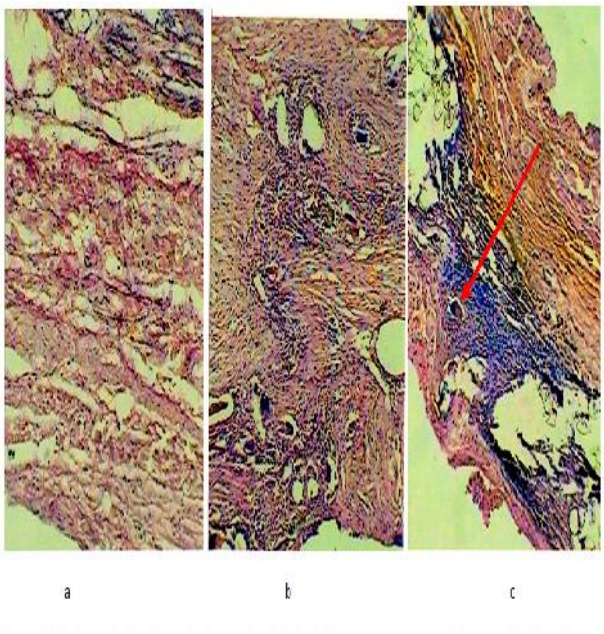

Figure 4 Histological evaluation

Figure 4: histological evaluation: a) normal periosteum (control) showing fibro-collagenous cells with blood vessels. b) Synthetic membrane (at 4 week) fibro-collagenous tissue showing marked granulomatous reaction consist of inflammatory cells and giants cells. Small, clear spaces are seen. The surrounding area shows granulomatous reaction. Few osteocytes round bodies present with eosinophillic cytoplasm; c) Synthetic membrane (at 12 week) periosteal reaction with osteoclast like giant cell (arrow)

DISCUSSION

Segmental long bone defects in high energy injuries are a challenging cocktail of bone and soft-tissue damage and loss. Current soft tissue reconstructive procedures now make it possible to obtain cover of large defects. However, Reconstruction of an osteoperiosteal defect remains a challenge while maintaining acceptable mechanical alignment and limb length. Varieties of techniques are available, but all are fraught with difficulties [2].

During the past few years, several studies (Woon CY *et al*, 2010; Pelissier P *et al* ,2004; Viateau V *et al*, 2006; Biau DJ *et al*, 2009; Huffman LK *et al*, 2009; Apard T *et al*, 2010; . Rezzouk J *et al*, 2005) based on technique of inducing a biomembrane at the site of an osseous defect with staged grafting have demonstrated successful outcome. Furthermore, these induced membranes are vascularized and have high concentrations of growth and osteoinductive

factors (Pelissier P *et al* ,2004). The two-stage approach of Masquelet (Masquelet AC *et al,* 2000; Masquelet AC, Begue T, 2010)is long technique, requiring 6 months without weight-bearing ;associated with complications attributed to bone cement (Dahl OE *et al* 1994; Deramon H *et al*, 1999; Leeson MC and Lippitt SB 1993; Memtsoudis SG,2007; Salles AG *et al*, 2008) hardware and increased morbidity.

Furthermore, Cement polymerization results in expansion in the initial volume of cement positioned. As a result, if too much cement is placed, it can be difficult to remove during the second stage. It has been our experience that during the second stage, enbloc and/or piecemeal removal of cement is difficult and at times damages the induced membrane.

The literature (Caffesse RG and Quinones CR, 1992; Nyman, S *et al*, 1987; Dahlin C *et al*, 1990; Karring T *et al*, 1993; Aaboe M *et al*, 1993; Dahlin C *et al*, 1998) does contain reports of several successful intervention studies based on the principle of guided tissue regeneration (GTR) using a membrane technique.

The rationale is to create a physical barrier by placing a membrane so as to produce mechanical hindrance to undesirable cells and giving preferences to specific cells which are capable to regenerate the desired therapeutic tissue. To the best of our knowledge, the current research is the first to combine the principle of induced membrane and GTR in long bones. GTR can be accomplished using degradable or non-degradable membranes (Aaboe M *et al*, 1993).

The non-degradable membrane requires a secondary procedure for its removal [Nyman R *et al*, 1995, Fleisher, N *et al*, 1988]. In the present research the authors have used the absorbable Vicryl mesh as for inducing bio-membranes, thus eliminating the need for second procedure.

Nyman *et al* concluded that the bone union of segmental long-bone defects can be accomplished with the aid of a physical membrane barrier. When the bone defect is large, the membrane alone is inadequate to heal the defect (Viateau V *et al*, 2006).

As a result, the authors of present research used cortico-cancellous bone graft within the mesh sleeve so has to enhance the regenerative process. The segmental defect can be stabilized with an extra- or intramedullary implant. With intra-medullary and newer implants like locking plates does not delay the mobilization.

In the current research the authors have used the extramedullary implant for internal fixation. The proposed technique is a single stage procedure of bone grafting based on the principle of mesh induced membrane and GTR with stabilization of long bone defects.

In the initial stage the vicryl mesh that is placed in form of a sleeve act as physical barrier preventing the in-growth of soft tissue at the osseous defect, thus permitting bone tissue exclusively to occupy the defect. Also, the sleeve retained the graft well. This vicryl mesh induce the bio-membrane around 4 week that prevents the resorption of the graft placed, augment corticalization, providing a barrier to diffusion of growth and osteoinductive factor to external environment; thus supporting revascularization and consolidation of the defect. At 12 weeks, the vicryl mesh is completely degraded and was replaced by a vascularized membrane that imitates like periosteum histologically; the defect shows complete osseous union. In the present research the periosteum of the contra-lateral normal limb was used as a control. The normal periosteum showed fibro-collagenous cells with blood vessels .At 12 weeks, on histological evaluation, the experimental "synthetic membrane" showed similar cellular picture as that of control i.e. with fibro-collagenous tissue.

The critical finding of present research support potential applications of proposed technique to achieve bone union in long bone osteoperiosteal defects with the aid of a mesh that behave as "like-periosteum" both mechanically and biologically. These mesh induced biomembrane prevents the ingrowths of soft-tissue; augment the desired cell to repopulate the defect; hastens the integration of bone graft and are biocompatible.

The radiological union and histomorphological evaluation of mesh induced bio-membrane lead the author to coin the term "synthetic periosteum" for such bio-membrane.

Besides small cohort the other weakness of present research is that no biomechanical tests were conducted on our constructs. Therefore, we cannot say with certainty whether the physical properties of our regenerated bone match those of native bone.

"Ethical standards" disclosure: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

Conflict of interest: nil/none

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