USE OF HUMAN FASCIA LATA IN RAT CALVARIAL BONE DEFECTS

Mariano A.R. Amer[1], Pablo A. Rodríguez[2], Sandra J. Renou[3], María B. Guglielmotti[3,4]

[1] Department of Anatomy, School of Dentistry, University of Buenos Aires, Argentina.
[2] Department of Endodontics, School of Dentistry, University of Buenos Aires, Argentina.
[3] Department of Oral Pathology, School of Dentistry, University of Buenos Aires, Argentina.
[4] National Research Council (CONICET), Argentina.

ABSTRACT

Tooth loss leads to a decrease in alveolar bone volume, and consequently to the need for guided bone regeneration (GBR) techniques to restore bone anatomy, and the adequate choice of therapy. Fascia lata membrane (FLM) has been used in surgical procedures in neurology, orthopedics, otorhinolaryngology, cardiology, vascular surgery, gynecology, and dentistry for guided tissue regeneration.

The aim of the present preliminary study was to evaluate bone tissue response in rat calvarial bone defects covered with human fascia lata membrane (FLM).

Eight Wistar rats, 230g body weight, were subjected to bone surgery to create a 5x5mm long/ 1mm deep calvarial bone defect on either side of the median suture, using a piezoelectric scalpel and irrigation. The animals were treated according to the following protocol: Group I (GI): placement of a single layer of FLM (Biotar, Rosario, Prov. de Santa Fe, Argentina) to cover the defects; Group II (GII): double layer of FLM to cover the defects; Group III: no membrane; Group IV: control. All the animals were euthanized 60 days post-surgery; the heads were resected, radiographed, decalcified, and processed for embedding in paraffin and Hematoxylin-Eosin and Masson's trichrome staining.

All bone defects covered with a single or double layer of FLM showed adequate osteogenesis, and none exhibited an inflammatory response. Groups III and IV Control showed scant osteogenesis and no alterations in soft tissues.

The results obtained with this experimental model show biocompatibility of FML with the surrounding tissues at the studied time points. No alterations were observed in osteocytic lacunae or osteocytes in the bone after osteotomy using a piezoelectric scalpel.

Further studies need to be conducted to assess bone tissue response to FLM in combination with bone substitutes.

Key words: *fascia lata; skull; bone regeneration; piezosurgery; rats.*

FASCIA LATA HUMANA EN DEFECTOS ÓSEOS DE CALOTA DE RATA

RESUMEN

La pérdida de piezas dentarias conlleva la disminución volumétrica del hueso alveolar y la necesidad de recurrir a técnicas de regeneración ósea guiada (ROG) para restablecer las condiciones anatómicas y aplicar las terapéuticas adecuadas. La membrana de fascia lata (MFL) ha sido utilizada en intervenciones quirúrgicas del área neurológica, ortopédica, otorrinolaringológica, cardiológica, vascular, ginecológica y odontológica para regeneración tisular guiada.

El objetivo de este trabajo preliminar fue evaluar la respuesta tisular de defectos óseos en calota de rata recubiertos con MFL.

Se utilizaron 8 ratas Wistar de 230 gr de peso, a las que se les realizó en la calota 2 defectos óseos de 5 x 5 mm de lado por 1 mm de profundidad, con bisturí piezoeléctrico e irrigación, a ambos lados de la línea media, según técnica estandarizada. Se realizó el siguiente protocolo: Grupo I (G I): colocación de una sola capa de MFL (Biotar, Rosario, Prov. de Santa Fe, Argentina) para cubrir el defecto; Grupo II (G II): colocación de MFL en doble capa para cubrir el defecto; Grupo III (G III): sin membrana; Grupo IV (G IV): control. Se les provocó la eutanasia a los 60 días. Las calotas fueron resecadas, radiografiadas y procesadas, previa descalcificación, para su inclusión en parafina y coloración con Hematoxilina-Eosina y Tricrómico de Masson.

En todos los defectos óseos recubiertos con MFL simple o doble se evidenció una adecuada osteogénesis y ausencia de respuesta inflamatoria y macrófagos. El G III y el G Sham evidenciaron escasa osteogénesis y no mostraron alteraciones en el tejido blando. La MFL en el modelo experimental utilizado evidenció una respuesta compatible con el tejido circundante en los períodos estudiados. El tejido óseo remanente a la osteotomía con bisturí piezoeléctrico presentó las lagunas osteocíticas ocupadas con osteocitos y sin alteraciones.

En estudios futuros se evaluará la respuesta tisular con MFL y utilizando un sustituto óseo.

Palabras clave: *fascia lata; calota; regeneración ósea; piezocirugía; ratas.*

INTRODUCTION

The use of autologous bone, and heterologous and alloplastic bone substitutes is widespread in surgery rooms and dental offices at present. According to estimates, 2.2 million bone grafts were placed in 2013, including procedures to repair defects in maxillofacial surgery, neurosurgery, orthopedics, and dentistry[1]. Nevertheless, autogenous bone harvesting is associated with complications such as hematoma and infection, a second surgical site where the graft is harvested, and potential damage to neighboring anatomic structures[3]. Interestingly, the use of a heterologous or alloplastic graft avoids the consequences associated with autologous bone harvesting, as regards morbidity of the donor site and the limited volume of bone available for grafting.

Barrier membranes used in guided tissue regeneration procedures can be either resorbable or non-resorbable. The membrane forms a barrier that prevents migration of soft tissue into the graft site, and thus enhances bone healing[3]. Fascia lata membranes (FLM) have been used as a resorbable biomaterial[4] in a number of surgical specialties, including dentistry. The structure of the membrane fibers, its ease of harvesting, and clinical manageability have made FLM a predictable biomaterial, which has been successfully used in numerous surgical fields.

Traditionally, experimental models of bone defects involved the use of conventional rotating burs to create the defect. However, these traditional methods pose disadvantages, such as overheating and damage to bone tissue. Piezosurgery avoids the complications associated with conventional rotary drilling methods, and has become a method of choice for cutting bone in a number of procedures, including maxillary sinus floor augmentation, crestal bone splitting, autogenous bone harvesting, and orthognathic procedures [5].

Clinical and preclinical studies combined with *in vitro* studies have shown that piezosurgery produces clean and precise osteotomies, and decreases bleeding[6]. Studies evaluating micromorphological differences after using a rotary tool, an oscillating saw, and piezoelectric osteotomy, showed that piezosurgery preserved the original structure of the bone[7], resulting in a lower number of inflammatory cells and lower expression of pro-inflammatory cytokines, 7 and 14 days after surgery[8].

The aim of the present study was to perform a qualitative evaluation of bone tissue response in rat calvarial bone defects made using a piezoelectric device and treated with guided bone regeneration with and without placement of FLM, using histological studies.

MATERIALS AND METHODS

Eight adult male Wistar rats, 230 g body weight, were randomly divided into four groups. The animals were housed in galvanized wire cages, four animals per cage, at 22°C to 24°C temperature and 52% to 56% humidity, under 12 hour-light/dark cycles.

All experiments were performed following the Guide for the Care and Use of Laboratory Animals of the National Research Council (US)[9], and the protocols were approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires.

On day 0, all the animals were anesthetized by intraperitoneal injection of 2% xylazine hydrochloride (5mg/kg body weight) and 5% ketamine hydrochloride (50 mg/kg body weight). The surgical area was infiltrated with 0.2 ml lidocaine (1:200.000). Using a scalpel (Bard-Parker scalpel, 15C blade, Swam Norton, England), a 15 mm sagittal incision was made in the parietal region, from the frontal bone to the occipital protuberance. The calvarium was exposed by blunt dissection with a Molt curette with atraumatic non-sharp edges (Hu-Friedy, USA). Bone defects measuring 5x5mm and 1mm deep were performed on both sides of the median suture, avoiding perforation of the inner bone plate. Bone surgery was performed using a piezosurgery device (Variosurg, NSK, NAKANISHI INC., Japan) and a SG3 scaler (NSK, NAKANISHI INC., Japan). Animals in Group I (GI) underwent surgery and placement of a single layer of human fascia lata membrane (FLM) (Biotar, Rosario, Prov. de Santa Fe, Argentina) on both defects (Fig. 1). Those in Group II (GII) were subjected to surgery and placement of a double

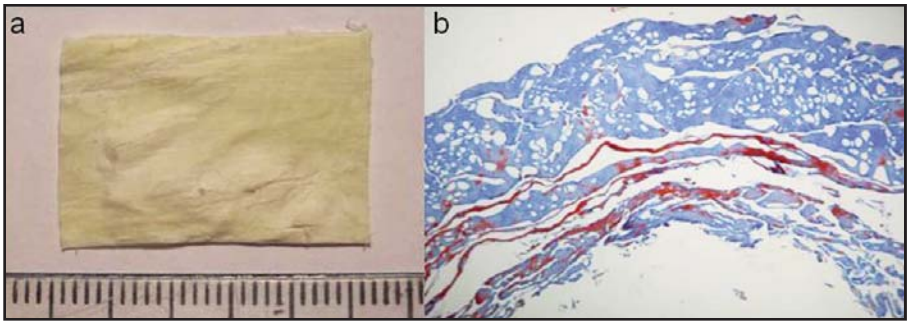

Fig. 1: Fascia Lata Membrane; a: Macroscopic appearance of FLM; b: Light microscopy of FLM stained with Masson's Trichrome. Note the fibrous tissue. Orig. Mag. X 2.5.

layer of FLM on both defects. Animals in Group III underwent surgery without placement of the barrier membrane, and Group IV served as control.

The wound was closed with single knot sutures using 5-0 nylon (Ethicon.USA). The rats were maintained on standard chow and tap water *ad libitum*, and were administered antibiotic therapy and a daily subcutaneous dose of 2 mm^3 of Dipenisol. In addition, they received a 1ml dose of Dexamethazone the first two days post-surgery.

All the animals were euthanized at 60 days; the heads were resected and fixed in 10% formalin solution. The skulls were radiographed, decalcified in formic acid, and processed for embedding in paraffin. Cross-sections oriented sagittally to the bone defect were obtained and stained with H-E and Masson's Trichrome.

RESULTS

None of the bone defects covered with a single (G I) (Fig. 2) or double (G II) (Fig. 3) layer of FLM showed an inflammatory response or macrophages, and all exhibited adequate osteogenesis with lamellar bone formation.

It is of note that no alterations were observed in the osteocyte lacunae containing osteocytes or in the

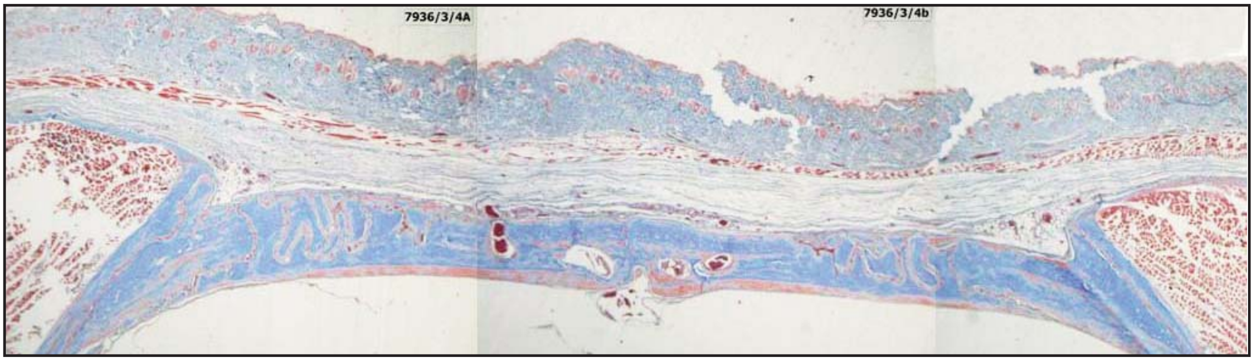

Fig. 2: Microphotograph of a histological section corresponding to Group I (60 days post surgery). The defect was covered with a single layer of human fascia lata membrane. Note the presence of lamellar bone tissue in the bone defect. Also note that the bone of the surgical bed is of adequate quality, and contains an adequate number of osteocytes. Orig. Mag. X 2.5; Masson's Trichrome stain.

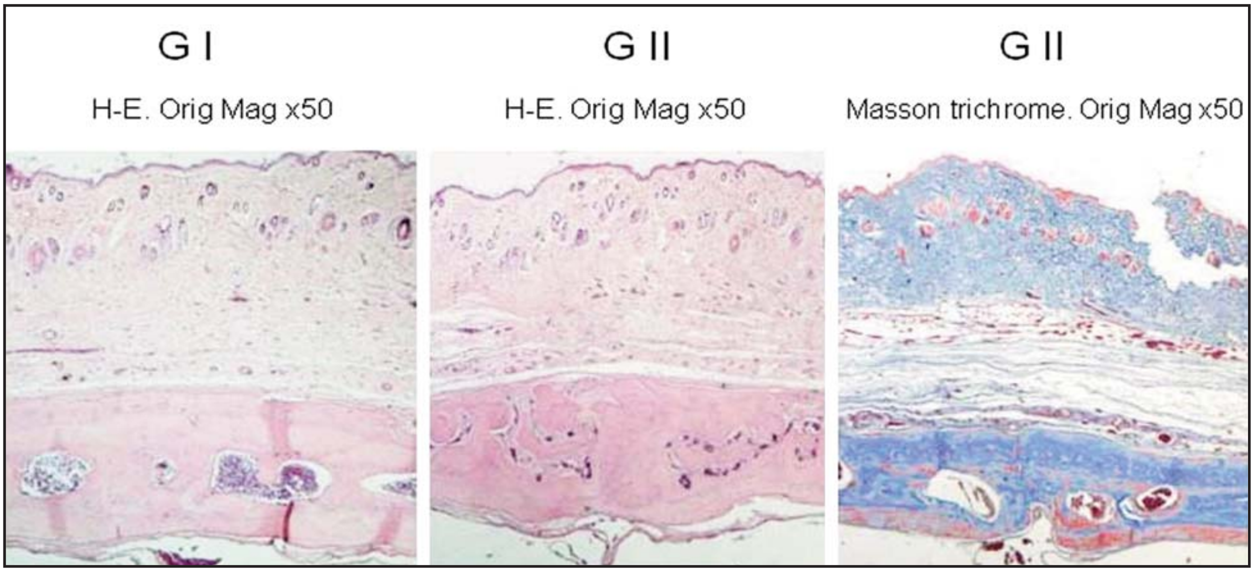

Fig. 3: Microphotographs of histological sections corresponding to Groups I and II (60 days post surgery). The defects were covered with a single layer of human fascia lata membrane, and G II defects were covered with a double layer of human fascia lata membrane. Both groups showed newly formed bone tissue in the bone defect. The fascia lata membranes exhibited no alterations in their structure, which consisted mainly of a collagen matrix. Orig. Mag. X 50; H-E stain.

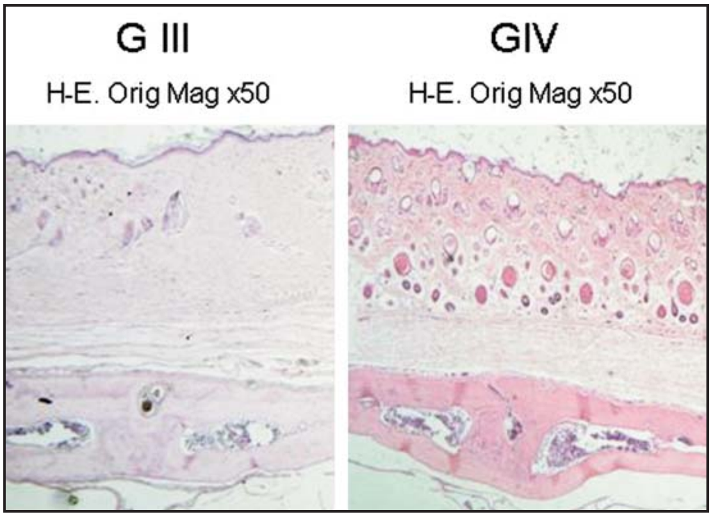

Fig. 4: Microphotographs of histological sections corresponding to Groups III and IV (60 days post surgery). No membrane was placed in G III defects, and GIV served as control. No alterations and scant osteogenesis were observed in both these groups. Orig. Mag. X 50; H-E stain.

surgical bed, where the bone defect was created by osteotomy using a piezoelectric scalpel.
No alterations and scant osteogenesis were observed in G III and G IV (Fig. 4).
The fascia lata membranes showed no evidence of alterations in their structure, which consisted mainly of a collagen matrix. No sign of inflammation and no macrophages or giant multinucleated cells were observed at the periphery of the membranes.

DISCUSSION

Treatment of bone defects created by trauma, infection, tumor resection, and skeletal abnormalities poses a great challenge[4]. Although there are numerous methods for bone reconstruction, each has specific indications and limitations. Well established methods include bone grafting and placement of bone substitutes or growth factors, among others [10-17].
The concept of GBR has been used in reconstructive surgery since the mid-1950s.
In 1964, P.J. Boyne published a study on the regeneration of alveolar bone beneath cellulose acetate filter implants[18].
As stated by Retzepi M et al (2010)[19], bioresorbable membranes were developed to avoid the need for surgical removal.
The results of the present study showed adequate wound healing of bone defects treated_with a single or double layer of human fascia lata membrane (FLM; Biotar, Rosario, Prov. de Santa Fe, Argentina) (Group I and Group II respectively), as shown by the finding of osteogenesis with lamellar bone formation. The defects in Group III, which were not treated with the FLM, exhibited less lamellar bone at the surgical defect. Attention must be drawn to the biocompatibility of FLM, as evidenced by the absence of inflammation, macrophages, and giant multinucleated cells.
The bone of the surgical bed created by osteotomy using a piezoelectric scalpel showed lamellar bone tissue of adequate quality, and contained an adequate number of osteocytes, demonstrating that piezosurgery does not alter the structure of the bone at the surgical site.
The results obtained with the experimental model used here, involving osteotomy with a piezoelectric scalpel to create a bone defect in rat calvaria and treatment of the defect with GBR using FLM, showed adequate osteogenic repair and adequate quality of the bone at the surgical site, both when using a single layer and a double layer of FLM.

CONCLUSION

Tissue response to FLM observed 60 days post-surgery using the experimental model presented here, provides further evidence of the biocompatibility of human fascia lata, as well as of its enhancing effect on osteogenesis during the repair process of experimentally created bone defects.
Further studies need to be conducted to assess bone tissue response to FLM in combination with bone substitutes.

ACKNOWLEDGEMENTS
The authors acknowledge the collaboration of Marcela Álvarez and Ricardo Orzusa, technical assistants at the Departments of Oral Pathology and Biochemistry respectively, of the School of Dentistry, University of Buenos Aires, Argentina.
This work was supported by research Grants UBACYT 2014- 2017 20020130100332 and PIP CONICET 2014-2016 11220130100091.

CORRESPONDENCE
Dr. Mariano Axel Ramón Amer.
Department of Anatomy.
School of Dentistry. University of Buenos Aires.
Marcelo T. de Alvear 2142 1B, (C1122AAH),
Buenos Aires, Argentina.
e-mail: marianoamer@gmail.com

REFERENCES

1. Cunha MJ, Esper LA, Sbrana MC, et al. Effect of low-level laser on bone defects treated with bovine or autogenous bone grafts: in vivo study in rat calvaria. Biomed Res Int 2014; 2014:104-230.
2. Prolo DJ, Rodrigo JJ. Contemporary bone graft physiology and surgery. Clin Orthop Relat Res 1985; 200:322-342. Review.
3. Herford AS, Dean JS. Complications in bone grafting. Oral Maxillofac Surg Clin North Am 2011; 23:433-442.
4. Dimitriou R, Mataliotakis GI, Calori GM, Giannoudis PV. The role of barrier membranes for guided bone regeneration and restoration of large bone defects: current experimental and clinical evidence. BMC Med 2012; 26:81-105. Review.
5. Esteves JC, Marcantonio E Jr, de Souza Faloni AP, et al. Dynamics of bone healing after osteotomy with piezosurgery or conventional drilling - histomorphometrical, immunohistochemical, and molecular analysis. J Transl Med 2013; 23: 221-234.
6. Claire S, Lea SC, Walmsley AD. Characterization of bone following ultrasonic cutting. Clin Oral Investig 2013; 17:905-912.
7. Maurer P, Kriwalsky MS, Block Veras R, et al. Micromorphometrical analysis of conventional osteotomy techniques and ultrasonic osteotomy at the rabbit skull. Clin Oral Implants Res 2008; 19:570-575.
8. Preti G, Martinasso G, Peirone B, et al. Cytokines and growth factors involved in the osseointegration of oral titanium implants positioned using piezoelectric bone surgery versus a drill technique: a pilot study in minipigs. J Periodontol 2007; 78:716-722.
9. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.
10. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Othop Relat Res 2000; 371;10-27
11. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury 2005; 36:S20-27. Review.
12. Pederson WC, Person DW. Long bone reconstruction with vascularized bone grafts. Othoped Clin North Am 2007; 38:23-35
13. Gorustovich A, Rosenbusch M, Guglielmotti MB. Characterization of bone around titanium implants and bioactive glass particles: An experimental study in rats. Int J Oral Maxillofac Implants 2002; 17:644-650.
14. Stvrtecky R, Gorustovich A, Perio C, Guglielmotti MB. A histologic study of bone response to bioactive glass particles used before implant placement: A clinical report. J Prost Dent 2003; 90:424-428.
15. Gorustovich A, Veinsten F, Costa O, Guglielmotti MB. Histomorphometric evaluation of the effect of bovine collagen granules on bone healing. An experimental study in rats. Acta Odontol Latinoamer 2004; 17:9-13.
16. Gorustovich AA, López JM, Guglielmotti MB, Cabrini RL. Biological performance of boron-modified bioactive glass particles implanted in rat tibia bone marrow. Biomed Mater 2006; 1:100-105.
17. Redondo MA, Renou SJ, Puia SA, Costa OR, Guglielmotti MB. Use of anorganic bovine bone matrix in an experimental model of bone healing. Acta Odontol Latinoam 2012; 25:306-311.
18. Boyne PJ. Regeneration of alveolar bone beneath cellulose acetate filter implants. J Dent Res 1964; 43:827-829.
19. Retzepi M, Donos N. Guided bone regeneration: biological principle and therapeutic applications. Clin Oral Implants Res 2010; 21:567-576.