Tissue response to porous high density polyethylene as a three-dimensional scaffold for bone tissue engineering. An experimental study.

Juliana Martínez Rodríguez, Sandra J. Renou, María B. Guglielmotti & Daniel G. Olmedo

Tissue response to porous high density polyethylene as a three-dimensional scaffold for bone tissue engineering. An experimental study.

Juliana Martínez Rodríguez[1], Sandra J. Renou[1], María B. Guglielmotti[1, 2†],

Daniel G. Olmedo[1, 2 *]

[1]Universidad de Buenos Aires. Cátedra de Anatomía Patológica. Facultad de Odontología. Buenos Aires, MT de Alvear 2142, 2° "A", (C1122AAH), Argentina.

[2]National Research Council (CONICET), Buenos Aires (C1122AAH), Argentina.

*Corresponding author: Daniel G Olmedo. Cátedra de Anatomía Patológica. Facultad de Odontología. Universidad de Buenos Aires. MT de Alvear 2142, 2° "A", (C1122AAH), Argentina. e-mail: olmedodg@gmail.com. Phone number: + 549 (11) 5317-0961. Fax number: + 54 (11) 5287-6007

Tissue response to porous high density polyethylene as a three-dimensional scaffold for bone tissue engineering. An experimental study.

High density polyethylene (HDPE) is a synthetic biomaterial used as a three-dimensional scaffold for bone defect reconstruction. Reports differ with regard to its biological response, particularly its osteoconductive capacity. The aim of the present work was to histologically and histomorphometrically evaluate tissue response to porous HDPE. An in vivo study was conducted in rat tibia to evaluate osteogenic capacity, angiogenesis, inflammatory response, and the presence of multinucleated giant cells 14 and 60 days post-biomaterial implantation. Histological examination 14 days post-implantation showed fibrovascular tissue inside pores and on the surface of porous HDPE, acute inflammatory response, scant multinucleated giant cells (MNGCs), and lamellar bone in contact with the biomaterial. An increase in the proportion of lamellar bone tissue, no inflammatory response, and a decrease in the number of MNGCs were observed at 60 days. The histomorphometric study showed a significant time-dependent increase both in the area of bone tissue formed in contact with the porous HDPE (14d: 24.450± 11.623 μm² vs. 60d: 77.104 ± 26.217 μm², p <0.05) and in the percentage of bone tissue in contact with the porous HDPE (osseointegration). A significant decrease in the number of MNGCs was also observed at 60 days post-implantation. Porous HDPE showed adequate osteoconductive properties, and only caused an initial inflammatory response. Although this biomaterial has traditionally been used juxtaosseoulsy, its adequate osteoconductive properties broaden the scope of its application to include intraosseous placement.

Keywords: porous high density polyethylene; biomaterials; bone regeneration; three-dimensional scaffolds; osteogenesis.

1. Introduction

The buccomaxillofacial region is highly complex and vulnerable to trauma, alterations during embryogenesis, and cystic and neoplastic pathologies [1]. Repair of bone defects in this region poses a challenge, and treatment success depends on the size of the defect, the quality of the soft tissue available to cover it, and the choice of the reconstruction method, among other factors [1-3].

Today, autologous bone grafting is considered the gold standard for reconstruction. Nevertheless, this therapy involves an additional surgical procedure to harvest the bone graft from the donor site, increasing surgery associated morbility and potential complications. Bone grafts also undergo a high percentage of bone resorption, often causing functional and esthetic problems [4,5]. Hence, a variety of synthetic biomaterials have been developed as therapeutic alternatives for bone defect rehabilitation. These materials not only aim to serve as skeletal support, but also function as three-dimensional scaffolds to achieve tissue regeneration [6].

In the mid-1980's, porous High Density Polyethylene (HDPE) was introduced on the market. Initially, this biomaterial was used for reconstruction of post-traumatic orbital defects and auricular reconstruction in microtia patients, and was later employed as a complement in facial augmentation in orthognatic surgery [7-9]. At present, it is indicated for use in both cosmetic and reconstructive surgery [7-14]. Porous HDPE is a synthetic polymer made through a sintering process that generates the pores required for vascularized connective tissue ingrowth [15,16]. The presence of fibrovascular tissue in the pores poses an advantage since it stabilizes the bone substitute. Although porous HDPE has been used as a three-dimensional scaffold for bone defect reconstruction, clinical and experimental studies have reported diverse biological responses, particularly with regard to its osteoconductive capacity [17-23].

Biomaterials used as three-dimensional scaffolds must possess certain properties, such as adequate biocompatibility, osteoconduction, and porosity, and optimum mechanical properties, among other characteristics [24-31]. In this regard, and taking into account the different biological responses reported in the literature [15-23], it is essential to objectively analyze tissue response to porous HDPE, evaluating bioindicators such as osteogenic capacity, angiogenesis, inflammatory response, and the presence of multinucleated giant cells (MNGCs) [32,33]. Such evaluation would allow accurately determining the effectiveness of porous HDPE as a three-dimensional scaffold for application in bone defects.

In view of the above, the aim of the present work was to histologically and histomorphometrically evaluate tissue response to porous HDPE at different time points, using a murine experimental model.

2. Materials and Methods

2.1. Experimental Animals

Young male Wistar rats (n=20), weighing ~ 150 g, fed ad libitum were used throughout. The animals were housed in metal cages, and kept on 14:10h light-dark cycles.

2.2. Implants

Samples of porous HDPE sheets (Medpor®, U.S.A.) in the shape of prisms measuring 3 x 0.7 x 0.8 mm were used. Pore shape, size and position were assessed by scanning electron microcopy (SEM Zeiss Supra model 40, Germany). For this purpose, a set of sections was coated with a thin (20-nm) layer of silver in a vacuum evaporator. In addition, the chemical composition of a porous HDPE sample was assessed using Energy- dispersive X-ray spectroscopy (EDS, Oxford Instruments, UK).

2.3. Surgical Procedure

The animals were anesthetized intraperitoneally with a solution of 8 mg of ketamine chlorhydrate (Fort Dodge®, Argentine) and 1.28 mg of Xylazine (Bayer, Germany) per 100 mg of body weight. The skin of both tibiae was shaved prior to performing a 1.5 cm incision along the tibial crest. The subcutaneous tissue, muscles, and ligament were dissected to expose the lateral external surface of the diaphyseal bone. A hole measuring 1.5 mm in diameter was made in the bone with an end-cutting bur, using manual rotating movements to avoid overheating and necrosis of the bone tissue. Porous HDPE implants were placed in the hematopoietic bone marrow compartment of both tibiae (n=40), parallel to their longest axis. A separate-stitch suture was performed. No antibiotic therapy was administered [34]. The animals were euthanized in groups of 10 by an overdose of anesthetic at 14 and 60 days post-implantation. The tibiae were resected, radiographed and fixed in 10% buffered formalin solution.

All procedures were performed in compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication - Guide for the Care and Use of Laboratory Animals: Eighth Edition, 2011) and the guidelines of the School of Dentistry of the University of Buenos Aires (Res. (CD) 352/02 and Res. (CD) 694/02). Adequate measures were taken to minimize animal pain and discomfort. The protocol was approved by the institutional experimentation committee (School of Dentistry of the University of Buenos Aires, Resolution Number 006/2015).

2.4. Histologic Processing

The samples were demineralized in 10% ethylenediaminetetraacetic acid (EDTA, Anhedra, Argentina); the acid solution was renewed every 3 days for 20 days. Following, the samples were embedded in paraffin, to obtain 10μm thick sections at the level of the porous HDPE implant and perpendicular to the longest axis of the tibia. The obtained sections were stained with hematoxylin-eosin, and histologic examination was performed using a light microscope (Leica, DM 2500, Germany).

2.5. Histomorphometric Evaluation

Histomorphometric measures were determined on digitized images of histological sections obtained 14 and 60 days post- implantation of the porous HDPE into the tibia of Wistar rats. The images were obtained using a photomicroscope (Leica, DM 2500, Germany) at 5X magnification, and were analyzed histomorphometrically using LAS EZ software (Leica Application Suite, Germany). The following histomorphometric determinations were performed:

a) Area of bone tissue formed in contact with the porous HDPE.

b) Percentage of bone tissue in contact with the porous HDPE (osseointegration).

In addition, the number of multinucleated giant cells (MNGCs) associated with the porous HDPE was determined.

2.6. Statistical Analysis

The results were compared using Student's *t*-test. Values are expressed as mean and SD; statistical significance was set at $p < 0.05$.

3. Results

3.1. SEM and EDS Analysis

The biomaterial displayed pores of different shape and size, ranging from 80 to 770 μm (Figure 1A,B). Small projections (~10 μm) (Figure 1C) were observed on the surface of the biomaterial. Microchemical analysis using EDS showed the material contained 92.48 weight% carbon and 7.18 weight% oxygen (Figure 1D).

3.2. Radiographic study

The radiographic study of the tibiae at 14 and 60 days post-implantation revealed radiopacity consistent with newly formed bone in the sector where the implant was placed; the radiopaque area increased with time (Figure 2).

3.3. Histologic Analysis

Light microcopy examination at 14 days post-implantation showed the presence of fibrovascular tissue inside the pores (Figure 3A), with an acute inflammatory response and scant MNGCs, some of which were found to contain particulate material (Figure 3B). In addition, lamellar bone tissue in contact with the biomaterial (osseointegration), both inside the pores and on the surface, could be observed (Figure 3C). Sixty-days post-implantation, a greater proportion of lamellar bone tissue in contact with the porous HDPE was observed (Figure 3A,B). There was no inflammatory response, and the number and size of MNGCs decreased. Figure 5 comparatively shows the proportion of lamellar bone tissue at 14 and 60 days post-implantation.

SEM-EDS analysis was performed to evaluate the chemical composition of the particulate material observed in the cytoplasm of MNGCs. The study showed the particulate material contained carbon and oxygen in different weight percentages

compared with the porous HDPE sample (*Particles:* **C**:79.42 weight% and **O**: 11.43 weight% vs. *Porous HDPE*: **C:** 92.48 weight% and **O**: 7.18 weight%). Taking into account that C and O are tissue components, intracellular determinations in regions of the cytoplasm distant from the particles, and extracellular determinations in the fibrovascular tissue were performed in order to determine the difference in the percentage of C and O inside and outside cells. The C and O weight percentages observed inside and outside cells also differed from those observed in the particulate material and in the porous HDPE sample (Figure 6). Thus, EDS analysis was not sufficient to ascertain that the particles observed inside the MNGCs were porous HDPE particles. However, examination under polarized light showed that the particulate material inside the MNGCs had the same birefringence as the remnants of porous HDPE observed in the histological samples after processing.

3.4. Histomorphometric Analysis

The area of bone tissue formed in contact with the porous HDPE differed significantly between the studied time points (Figure 7A) (14d: 24.450±11.623 μm² vs. 60d: 77.104 ± 26.217 μm², (p <0.05). The percentage of bone tissue in contact with the porous HDPE (osseointegration) (Figure 7B) differed significantly between both groups (14d: 32.6±6% vs. 60d: 74.3±10%, p <0.05).

A significant time-dependent decrease in the number of MNGCs was observed (14d: 7.2±1.9 vs. 60d: 1.5±0.5, p <0.05, (Figure 7C).

4. Discussion

Alloplastic biomaterials are increasingly considered as alternative materials for use as three-dimensional scaffolds in regenerative bone medicine today [17,19,23,28]. Porous

high density polyethylene has been used for decades in the maxillofacial region to reestablish the facial contour and attain adequate volume in bone defect areas not requiring subsequent dental implant rehabilitation [7-11]. Nevertheless, their use as a three-dimensional scaffold has only been investigated more recently [18-24].

Clinical and experimental reports published in the literature evaluated tissue response to porous HDPE. The reported experimental studies were conducted in dogs, monkeys, rabbits, and rats [20-23]. Studies by Spector et al. [15] and Klawitter et al. [16] in dog femur, reported bone ingrowth in pores as small as 40μm, and found optimum pore size to be 100 to 135 μm. However, other *in vivo* studies in experimental animals reported bone growth far from the biomaterial. Such is the case of a study by Sabini et al. in which bone ingrowth was not found to occur around porous HDPE discs implanted in Sprague-Dawley rats in a subperiosteal location [22].

As to the clinical reports, Tark et al. histologically evaluated porous HDPE implants placed in children with craniosynostosis, and removed immediately after the distraction and consolidation period. The authors found no evidence of osteogenesis [19].

In view of the above, the histological finding of bone tissue inside the pores and on the surface of porous HDPE implants, and the integration of the material with the host bone remain a matter of discussion [19-23].

Hence the need to quantitatively evaluate the osteoconductive capacity of porous HDPE, using histological and histomorphometric studies. In this regard, the present study assessed porous HDPE osteoconductivity in osteogenic hematopoietic bone marrow of rat tibia, using an experimental model developed by our research group[34]. The model poses the advantage that it provides a microenvironment that is isolated from microbial colonization and mechanical forces, and allows quantitating tissue response,

ruling out confounding variables. In the present work, bone tissue response was assessed using histomorphometric studies, which were not performed in most of the clinical and experimental works reported in the literature.

One of the key factors to achieving adequate osseointegration is immobilization and/or fixation of the biomaterial to the bone surface. In the studies conducted by Sabini et al. [22], Spector et al. [15] and Klawitter et al. [16], the implants were placed in contact with the surface of the host bone but were not fixed, which could have affected bone tissue response to the biomaterial. This variable was controlled in the experimental model used here, since the implant was placed inside the medullary compartment where it was not subjected to forces and did not move.

Infection of the biomaterial is one of the major causes of porous HDPE failure in the clinical setting [7]. This variable was also controlled in our experimental model, since the biomaterial was isolated from the external milieu.

The histological results obtained here confirm that porous HDPE has adequate osteoconductive properties, serving as a scaffold for osteogenesis inside the pores and on the surface of the biomaterial. In addition, the histomorphometric studies provided quantitative results that showed a significant time-dependent increase in both the area and the percentage of newly formed bone in contact with the biomaterial (osseointegration).

Another important factor that must be taken into account is that most of the published reports used a small number of animals [15,16,20-23]. In the present study, the number of animals was the minimum number required to guarantee a uniform biological response and to obtain reliable statistical results.

A biomaterial that can be used as a three-dimensional scaffold in the maxillofacial region should favor biological integration with the bone tissue. In the case

of porous biomaterials, like porous HDPE, the newly formed tissue inside the pores is responsible for providing a biological anchor, generating physical and biological integration, enhancing implant stability, thus optimizing long-term outcomes, which are the ultimate goals when esthetic results are required. The size and position of the pores are also critical factors for vascularization, fibrovascular tissue ingrowth, and formation of bone tissue inside a biomaterial [17,25,26,29,30,35,36]. The combination of pore size, porosity volume fraction, pore inter-connections, and permeability, would be determining factors to biomaterial-host integration [36]. According to Karageorgiou et al [17], high porosity materials with an average pore size >300 μm are recommended for successful osteogenesis *in vivo*. However, the first experimental studies reported osteogenesis in 40 μm pores [15,16]. The characterization of porous HDPE performed in the present study showed interconnected pores ranging in size from 80 to 770 μm, which would favor tissue ingrowth. The latter was observed histologically as formation of fibrovascular tissue and bone tissue inside and on the surface of the biomaterial at both 14 and 60 days post-implantation.

Another essential prerequisite for porous HDPE-host tissue integration is rapid vascularization of the scaffold. Early vascularization can reduce the window during which an implant is susceptible to extrusion, migration and infection [30]. The histological results of the present study (fibrovascular growth and subsequent lamellar bone formation) are an indication of good angiogenic response.

As shown by the biocompatibility analysis performed here, porous HDPE induced an acute inflammatory response at the early stage post-implantation. Our observations are in line with previous reports by Klawitter et al. [16], Niechajev et al. [17], and Gosau et al. [31]. Furthermore, the present histological results showed the presence of MNGCs close to the biomaterial. Some authors have termed these cells

biomaterial-associated multinucleated giant cells (BMGCs), though their involvement in the inflammatory and healing events of the foreign body response remains unclear [32,33,37]. These BMGCs have most often been associated with the cell type of the foreign body giant cell. They form by the fusion of monocytes and macrophages through a process termed "frustrated phagocytosis", which is usually associated with the degradation of the biomaterial. For a long time, these cells were considered pro-inflammatory cells per se [32,33]. However, *in vitro* and *in vivo* studies have shown that BMGCs have different phenotype profiles, depending on the physical-chemical characteristics of the biomaterials that induce their formation with pro- and anti-inflammatory cytokine expression [32,38-40].

In the past, MNGCs were thought to have a negative effect on the healing process. However, it is well documented today that these cells can secrete growth factors favoring repair, and that the presence of BMGCs does not necessarily imply an adverse host response to the biomaterial. Furthermore, the presence of BMNGs can have a positive effect, since these cells can release different chemical mediators such as vascular endothelial growth factor (VEGF), which promotes vasculogenesis and angiogenesis and thus benefits healing [37-39]. Barbeck et al. showed that labeling with molecules like hydrolytic enzyme tartrate-resistant acid phosphatase (TRAP) allows distinguishing pro- and anti-inflammatory subforms of MNGCs. However, the application of marker molecules such as TRAP and its role in the inflammatory tissue reaction to biomaterials need to be examined in greater depth [33].

In addition, it is of note that the quantitative results obtained here showed a decrease in the number of MNGCs from day 14 to day 60 post-implantation. This time-dependent decrease is in line with a study by Barbeck et al. on biological response to small Bio-OssTM granules, which showed the presence of multinucleated giant cells

10-15 days post implantation, a decrease in MNGCs number at 30 days, and very few MNGCs 60 days post-implantation [32]. In the present study, very few samples exhibited MNGCs containing material, which would indicate phagocytic clearance of the biomaterial, likely in response to the projections (~10 μm) observed on the surface of the biomaterial. Because the weight% of C and O as determined by EDS differed among all intra- and extracelular measurements, it cannot be affirmed that the material observed inside MNGCs corresponded to the porours HDPE derived particles. Evaluation by EDS is not an adequate quantitative measure to discern the origin of the intracellular particles, given that C and O are constituents of tissue. Nevertheless, the particulate material inside the MNGCs had the same birefringence as the remnants of porous HDPE observed in the histological samples after histological processing. This observation lends strong support to the conclusion that the particles observed inside the MNGCs were porous HDPE particles.

The results obtained with the experimental model used here showed that porous HDPE has adequate osteoconductive properties, though it initially causes an inflammatory reaction. The HDPP showed adequately sized inter-connected pores, which favor tissue ingrowth. Although this biomaterial has traditionally been used juxtaosseoulsy to restore bone volume in regions not requiring subsequent dental implant rehabilitation, its adequate osteoconductive properties broaden the scope of its application to include intraosseous placement. The present results lend support to its potential use in skeletal reconstruction, such as fractures with bone loss not involving the alveolar ridge, and maxillary sinus augmentation and/or labial alveolar palatal fissures.

Disclosure statement

No potential conflict of interest was reported by the authors

References

[1]   Neovius E, Engstrand T. Craniofacial reconstruction with bone and biomaterials: review over the last 11 years. J Plast Reconstr Aesthet Surg. 2010;63:1615-1623.

[2]   Andrades P, Militsakh O, Hanasono MM, et al. Current strategies in reconstruction of maxillectomy defects. Arch Otolaryngol Head Neck Surg. 2011;137:806-812.

[3]   O'Connell DA, Futran ND. Reconstruction of the midface and maxilla. Curr Opin Otolaryngol Head Neck Surg. 2010;18:304-310.

[4]   Mertens C, Decker C, Seeberger R, et al. Early bone resorption after vertical bone augmentation-a comparison of calvarial and iliac grafts. Clin Oral Implants Res. 2013;24:820-825.

[5]   Herford AS, Dean JS. Complications in bone grafting. Oral Maxillofac Surg Clin North Am. 2011;23:433-442.

[6]   Goldsmith D, Horowitz A, Orentlicher G. Facial skeletal augmentation using custom facial implants. Atlas Oral Maxillofac Surg Clin North Am. 2012;20:119-134.

[7]   Niechajev I. Facial reconstruction using porous high-density polyethylene (Medpor): long-term results. Aesthetic Plast Surg. 2012;36:917-927.

[8]   Romano JJ, Iliff NT, Manson PN. Use of Medpor porous polyethylene implants in 140 patients with facial fractures. J Craniofac Surg. 1993;4:142-147.

[9]   Romo T 3rd, Morris LG, Reitzen SD, et al. Reconstruction of congenital microtia-atresia: outcomes with the Medpor/bone-anchored hearing aid-approach. Ann Plast Surg. 2009;62:384-389.

[10]   Yaremchuk MJ. Facial skeletal reconstruction using porous polyethylene implants. Plast Reconstr Surg. 2003;111:1818-1827.

[11] Niechajev I. Porous polyethylene implants for nasal reconstruction: clinical and histologic studies. Aesthetic Plast Surg. 1999;23:395-402.

[12] Gosau M, Schiel S, Draenert GF, et al. Gesichtsschädelaugmentationen mit porösen Polyethylenimplantaten (Medpor®) [Craniofacial augmentation with porous polyethylene implants (Medpor: first clinical results]. Mund Kiefer Gesichtschir. 2006;10:178-184. German

[13] Rai A, Datarkar A, Arora A, et al. Utility of high density porous polyethylene implants in maxillofacial surgery. J Maxillofac Oral Surg. 2014;13:42-46.

[14] Mericli AF, Gampper TJ. Treatment of postsurgical temporal hollowing with high-density porous polyethylene. J Craniofac Surg. 2014;25:563-567.

[15] Spector M, Flemming WR, Kreutner A. Bone growth into porous high-density polyethylene. J Biomed Mater Res. 1976;10:595-603.

[16] Klawitter JJ, Bagwell JG, Weinstein AM, et al. An evaluation of bone growth into porous high density polyethylene. J Biomed Mater Res. 1976;10:311-323.

[17] Karageorgiou V, Kaplan D. Porosity 3 D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26:5474-5491.

[18] Bhatnagar A, Kumar B, Rai A. Growing skull fracture repair by porous polyethylene sheet. Saudi J Health Sci. 2013;2:217-219.

[19] Tark WH, Yoon IS, Rah DK, et al. Osteoconductivity of porous polyethylene in human skull. J Craniofac Surg. 2012;23:78-80.

[20] Dougherty WR, Wellisz T. The natural history of alloplastic implants in orbital floor reconstruction: an animal model. J Craniofac Surg. 1994;5:26-32.

[21] Dos Santos PL, Santiago JF Jr, Ponzoni D, et al. Maxillary bone defect reconstruction using porous polyethylene implants. J Craniofac Surg. 2011;22:2337-2340.

[22] Sabini P, Sclafani AP, Romo T 3rd, et al. Modulation of tissue ingrowth into porous high-density polyethylene implants with basic fibroblast growth factor and autologous blood clot. Arch Facial Plast Surg. 2000;2:27-33.

[23] Oliveira RV, de Souza Nunes LS, Filho HN, et al. Fibrovascularization and osteogenesis in high-density porous polyethylene implants. J Craniofac Surg. 2009;20:1120-1124.

[24] Sclafani AP, Romo 3rd, Ukrainsky G, et al. Modulation of wound response and soft tissue ingrowth in synthetic and allogeneic implants with platelet concentrate. Arch Facial Plast Surg. 2005;7:163-169.

[25] Gaalen Sv, Kruyt M, Meijer G, et al. Tissue Engineering of bone. In: Van Blitterswijk C, Thomsen P, Lindahl A, Hubbel J,Williams D, Cancedda R, De Bruijn J, Sohier J, editors: Tissue Engineering. Burlington: Academic Press; 2008:pp 560-609.

[26] Ward BB, Brown SE, Krebsbach PH. Bioengineering strategies for regeneration of craniofacial bone: a review of emerging technologies. Oral Dis. 2010;16:709-16.

[27] Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008;29:2941-2953.

[28] Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 2012;30:546-554.

[29] Harris GM, Rutledge K, Cheng Q, et al. Strategies to direct angiogenesis within scaffolds for bone tissue engineering. Curr Pharm Des. 2013;19:3456-3465.

[30] Kanczler JM, Oreffo RO. Osteogenesis and angiogenesis: the potential for engineering bone. Eur Cell Mater. 2008;15:100-114.

[31] Gosau M, Draenert FG, Ihrler S. Facial augmentation with porous polyethylene (Medpor)-histological evidence of intense foreign body reaction. J Biomed Mater Res B Appl Biomater. 2008;87:83-87.

[32] Barbeck M, Udeabor SE, Lorenz J, et al. Induction of multinucleated giant cells in response to small sized bovine bone substitute (Bio-OssTM) results in an enhanced early implantation bed vascularization. Ann Maxillofac Surg. 2014;4:150-157.

[33] Barbeck M, Motta A, Migliaresi C, et al. Heterogeneity of biomaterial-induced multinucleated giant cells: Possible importance for the regeneration process? J Biomed Mater Res A. 2016;104:413-418.

[34] Cabrini RL, Guglielmotti MB, Almagro JC. Histomorphometry of initial bone healing around zirconium implants in rats. Implant Dent. 1993;2:264-267.

[35] Patel M, Fisher JP. Biomaterial scaffolds in pediatric tissue engineering. Pediatr Res. 2008;63:497-501.

[36] Hing KA. Bioceramic bone graft substitutes: Influence of porosity and chemistry. Int J Appl Ceram Technol. 2005;2:184-199. https://doi.org/10.1111/j.1744-7402.2005.02020.x

[37] Barbeck M, Booms P, Unger R, et al. Multinucleated giant cells in the implant bed of bone substitutes are foreign body giant cells-New insights into the material-mediated healing process. J Biomed Mater Res A. 2017;105:1105-1111.

[38] Martínez C, Fernández C, Prado M, et al. Synthesis and characterization of a novel scaffold for bone tissue engineering based on Wharton's jelly. J Biomed Mater Res A. 2017;105:1034-1045.

[39] Ghanaati S, Unger RE, Webber MJ, et al. Scaffold vascularization in vivo driven by primary human osteoblasts in concert with host inflammatory cells. Biomaterials. 2011;32:8150-8160.

[40] Brodbeck WG, Anderson JM, Giant cell formation and function. Curr Opin Hematol. 2009;16:53-57.
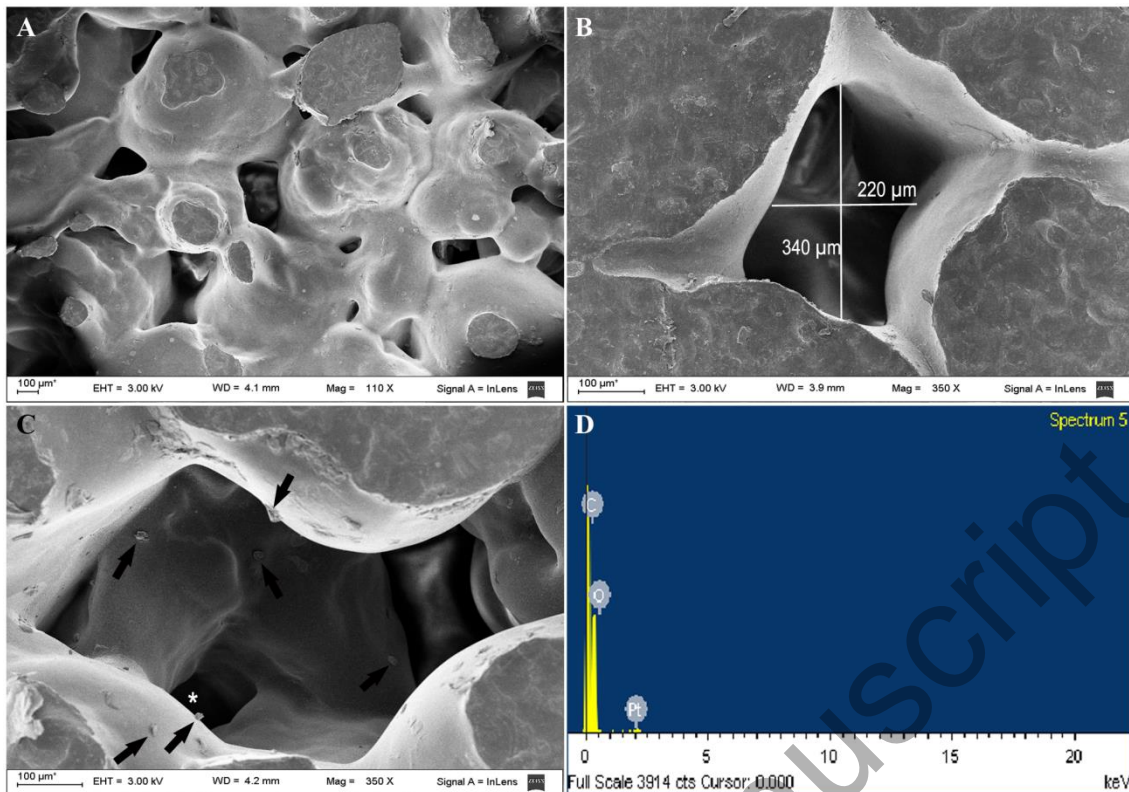
Figure Legends

Figure 1. Characterization of porous HDPE (SEM and EDS). **A)** note pore location and different pore size and shape. **B)** the dimension of one of the pores is shown as an example. **C)** pore inter-connection (*) as well as small projections on the surface of the biomaterial (→) can be observed. **D)** spectrum corresponding to carbon and oxygen, as shown by EDS.
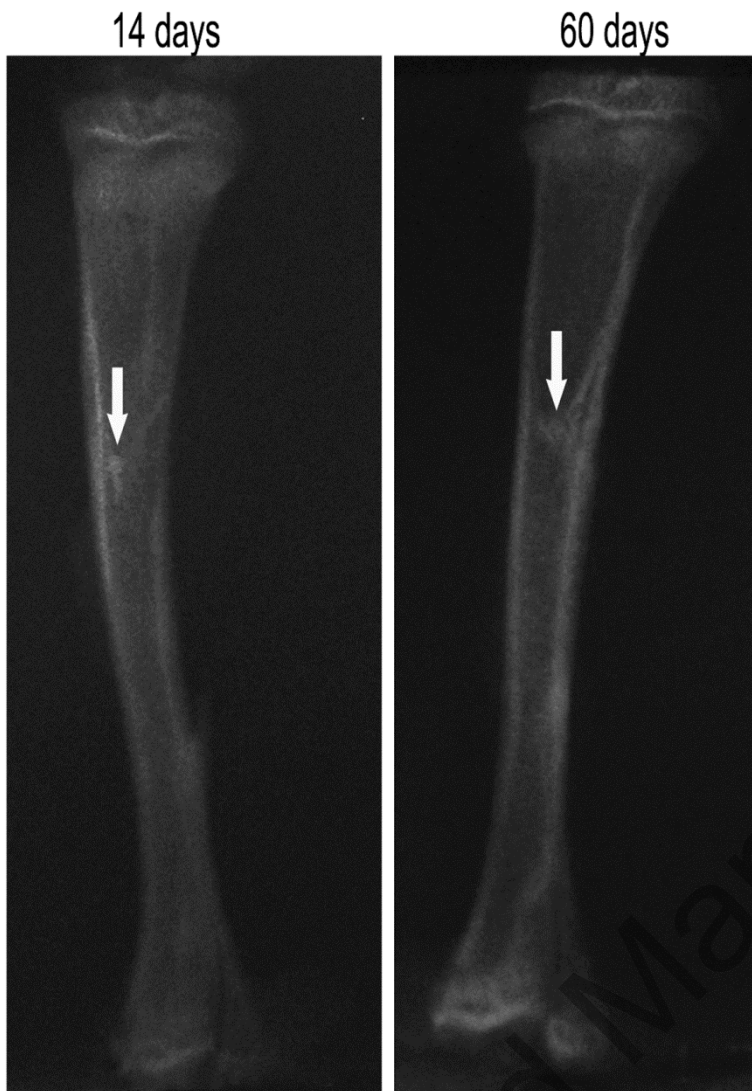
Figure 2. Radiographic study of the tibia 14 and 60 days post-implantation of the biomaterial. Radiopacity (→) in the implantation sector can be seen to increase with time.
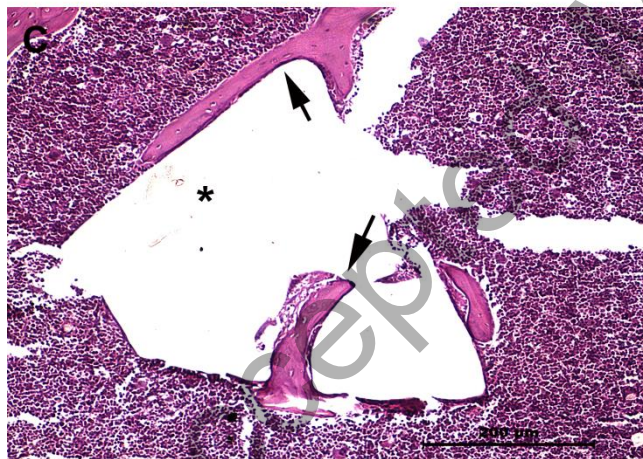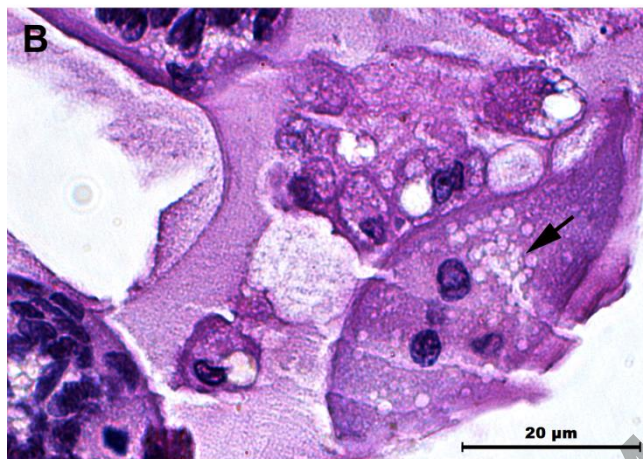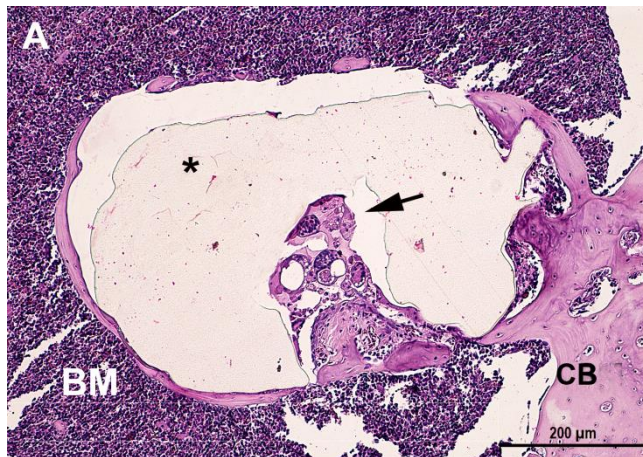
Figure 3. Histological study 14 days post-implantation of porous HDPE. Microphotograph **A** shows fibrovascular tissue growth (→) into one of the pores. Remnants of porous HDPE can be seen after histological processing (*). At higher magnification **(B)**, MNGCs containing particulate material (→) in their cytoplasm can be observed. Microphotograph **C** shows lamellar bone tissue (→) inside a pore and on

the surface of the negative spaces (*) corresponding to the biomaterial. (BM) Bone marrow; (CB) cortical bone. **A,C**: Orig. Mag. X50. **B**: Orig. Mag. X1000. Demineralized sections; H-E stain.
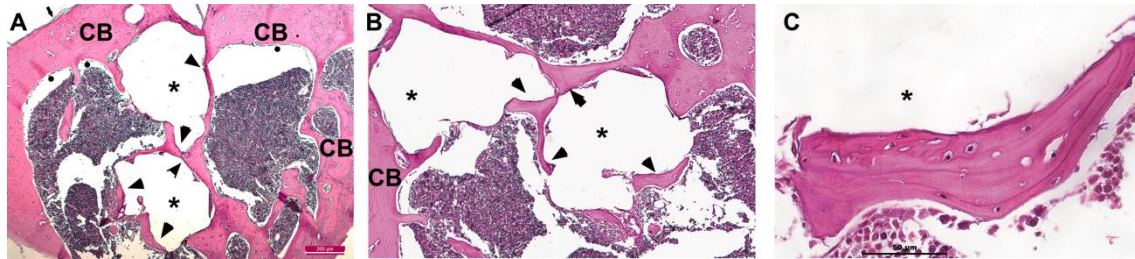
Figure 4. Histological study 60 days post-implantation of porous HDPE. (**A**) Cross-section of the tibia showing the cortical bone (CB),the endosteal (●) and periosteal (→) sides, and newly formed tissue in contact with the biomaterial(◀).(**B**) At higher magnification, lamellar bone tissue in contact with the porous HDPE can be observed. Note the presence of lamellar bone tissue inside the pores (◀). **C**) Figure A at higher magnification, a lamellar bone trabecula can be seen in detail. Demineralized section. **A**) Orig. Mag. X 25. **B**) Orig. Mag. X50. **C**) Orig. Mag X 400. H-E Stain. (*) Negative space corresponds to the biomaterial after histological processing.
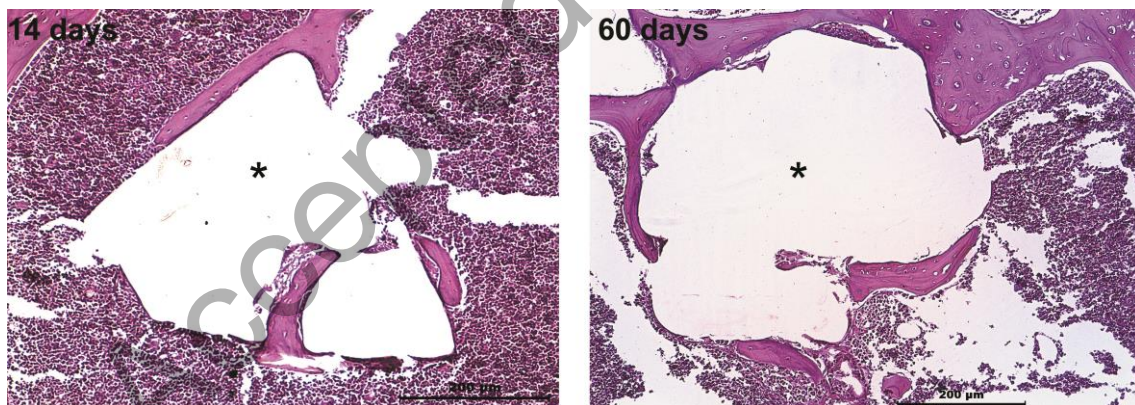

Figure 5. Comparative histological study 14 vs. 60 days post-implantation. Note the increasing proportion of lamellar bone tissue in contact with the biomaterial, with time. Orig. Mag. X50. Demineralized sections. H-E Stain. (*) Negative space corresponds to the biomaterial after histological processing.
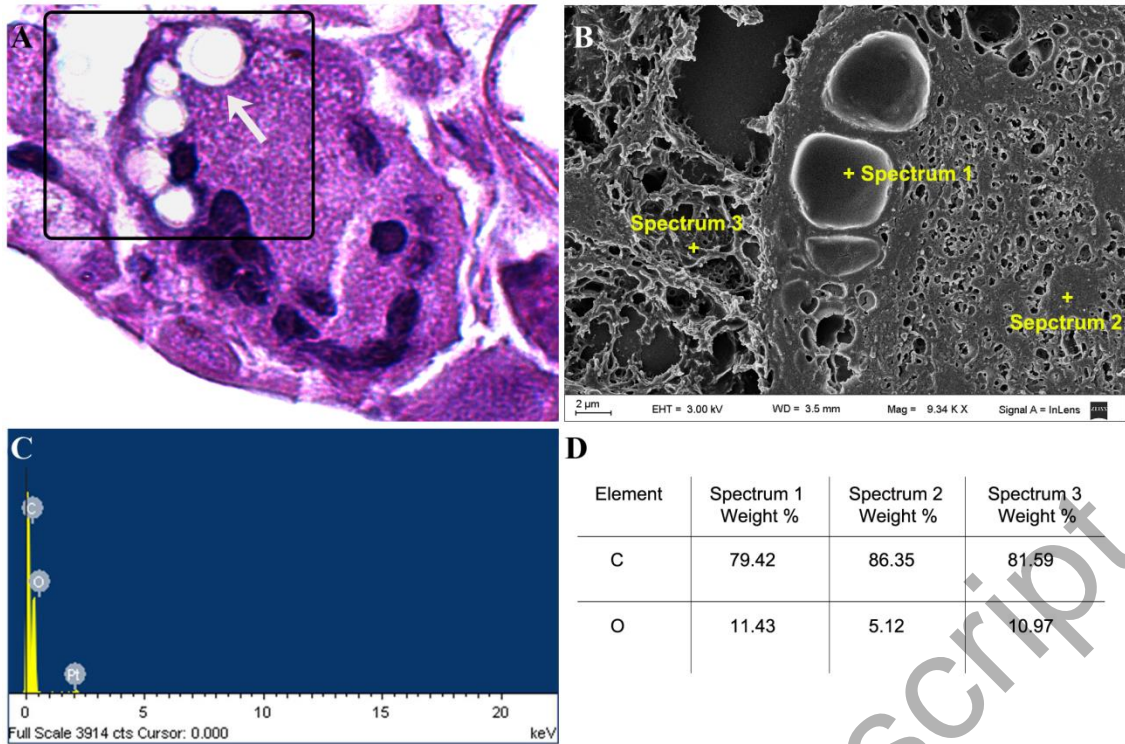
Figure 6. SEM-EDS analysis. Microphotograph **A** shows a MNGC containing porous HDPE particles (→), as seen by light microscopy. Figure **B** is a microphotograph obtained by SEM showing the area marked in Figure **A** in greater detail. **C)** the EDS spectrum show carbon and oxygen in the biomaterial inside the MNGC (spectrum 1). Figure **D** shows weight% determinations of C and O inside and outside cells (spectrums 1,2 and 3). **A)** demineralized section. H-E. Orig. Mag. X1000.

Regions analyzed by EDS: **Spectrum1**: porous HDPE particles inside the MNGCs; **Spectrum 2**: MNGCs cytoplasm; **Spectrum 3:** Fibrovascular tissue.
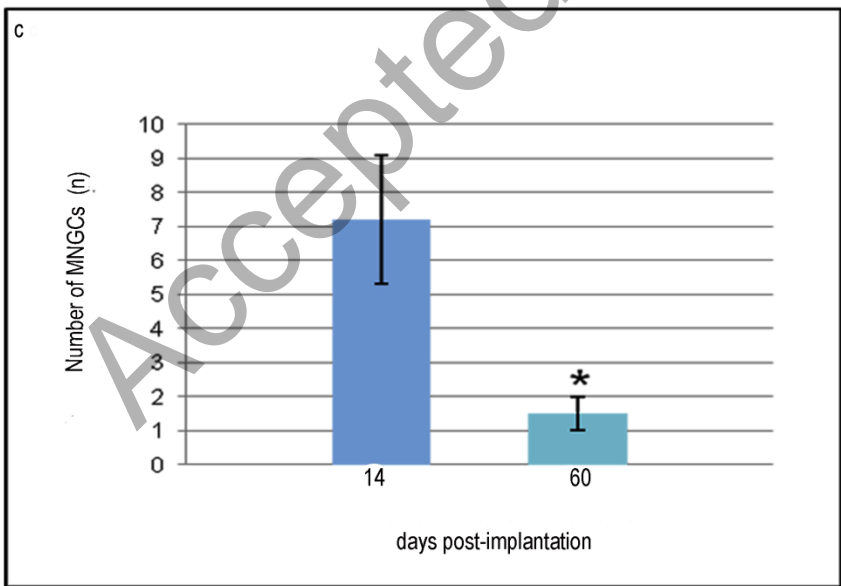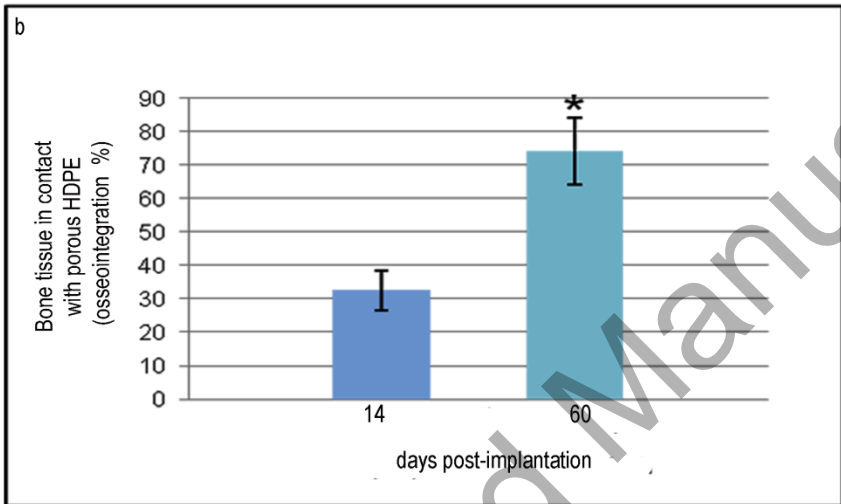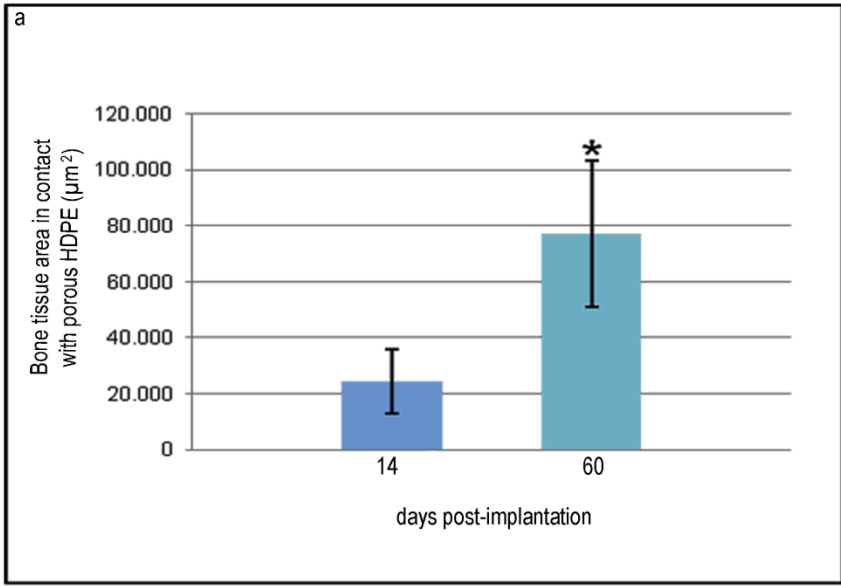
Figure 7. Comparative histomorphometric study of the biomaterial 14 vs. 60 days post-implantation. **A)** area of bone tissue in contact with the biomaterial; **B)** percentage of bone tissue in contact with the porous HDPE (osseointegration); **C)** number of MNGCs. The histograms show the mean ± SD, *P* < 0.05 compared to the 14 day post-implantation group.