Fibrovascularization and Osteogenesis in High-Density Porous Polyethylene Implants

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Abstract: High-density porous polyethylene (HDPP) has been extensively used in craniofacial reconstructions with high-level success and minimal complications. It is known for its biocompatibility and satisfactory stability in the receptor bone area, presenting only a few reports of mobility and infection. In the current study, attention was given to the interface area between HDPP and bone surface to analyze fibrous and bone tissue formation and ingrowth into the pores of the material placed in the mandible of rabbits. Twelve male New Zealand rabbits underwent surgical procedure to receive bilateral HDPP implants in buccal face of dentate mandibular alveolar process, fixed with titanium screws. After 7, 14, 45, and 90 days, the animals were killed, and the specimens were retrieved for histologic and immunohistochemical analyses. No implant loss or infection was detected at the retrieval of the specimens. The microscopic analysis presented satisfactory integration of the material to the bone surface, with new bone formation from the receptor bed and inside the pores of the material, observed from the 15th day. After 90 days, remodeling bone and fibrous tissue was seen in the interface region. Among some of the pores, mature lamellar bone was present. Immunohistochemistry pointed out a moderate expression either to Core binding factor protein 1/RUNX2 or to vascular endothelial growth factor for early periods evaluated, that is, 7 and 15 days after surgery. These results confirm the osteoconductive behavior and high biocompatibility of the material, associated to its adequate immobilization, leading to its lifelong presence in human biologic system.  

Key Words: High-density porous polyethylene, immunohistochemistry, rabbit, Cbfa-1, VEGF

Biomaterials represent a wide spectrum of materials intended to interact with biologic systems, largely used in medicine and dentistry. Among them there are materials from synthetic and non-synthetic origin, such as metals, polymers, ceramics, and glasses, with specific application. Despite these possibilities, when it comes to craniofacial reconstruction, autogenous bone grafts are still considered the criterion standard for satisfactory tissue recovery and repair, mainly for the possibility of future rehabilitation with endosseous implants. However, if only volume and contour are desired, a particular nondegradable and highly biocompatible synthetic material has been extensively used for craniofacial contour recovery, the high-density porous polyethylene (HDPP). For this purpose, adequate stabilization of the material to the receptor bed is desired to avoid dislocation and contamination and to ensure a long-term permanence. It is known that the porosity of the material permits fibrous tissue ingrowth and bone formation into pores, although fibrovascularization has also been reported as the main biologic response for this stabilization. The current study aimed to describe the contact interface HDPP/bone tissue in an animal model by means of histologic analysis and immunohistochemical technique, focusing on local vascularization and osteoblasts differentiation.

MATERIALS AND METHODS

Animals and Experimental Design

The experimental protocols were approved by the Animal Committee of the Sacred Heart University (USC), School of Dentistry. Twelve adult male New Zealand rabbits weighing approximately 3 kg were randomly distributed into 4 groups containing 3 animals each to be killed at the following periods: group 1, 7 days;
group 2, 15 days; group 3, 45 days; group 4, 90 days, after receiving HDPP implants in the mandible.

**Surgical Procedure**

High-density porous polyethylene implants (Medpor; Porex Surgical, College Park, GA) were prepared in a round shape presenting 1 × 0.3 cm to be implanted in the rabbits. At the beginning of the experimental design, all animals underwent general anesthesia obtained by intramuscular administration of 0.25 mL/kg of 1% ketamine (Francotar; Virbac, Ltd, Roseira, Sao Paulo, Brazil) associated with sedative, 1 mL/kg of 2% chlorohydrate of xylazine (Virbaxyl 2%; Virbac, Ltd). A 1-cm dermoperiostal submandibular incision was performed bilaterally, and the periosteum of alveolar mucosa was elevated to the molar region to expose the bone surface of the mandible and not to lay down the incision over the implanted site. All implants were fixed using 1.5 ´ 6-mm titanium screws (Neoortho; Curitiba, Paraná, Brazil; Fig. 1). No food or water restriction was adopted after surgical procedures.

**Histologic Procedures**

After 7, 15, 45, and 90 days, 3 animals at each period were randomly selected and killed with an overdose of anesthetic solution for histologic and immunohistochemical analyses. The mandibles were retrieved en bloc and fixed in 10% buffer formalin (Merck). The implants were identified in both sides of the mandible and not to lay down the incision over the implanted site. All implants were fixed using 1.5 ´ 6-mm titanium screws. After histologic procedures, and its presence is identified by the receptor bone could be visualized and were stained with hematoxylin-eosin and Masson trichrome.

**Immunohistochemistry**

Paraffin was removed with xylene from serial sections of 4 µm, and the sections were rehydrated in graded ethanol, then pretreated in a microwave with 0.01 mol/L citric acid buffer (pH 6) for 3 cycles of 5 minutes each at 850 W for antigen retrieval. The material was preincubated with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) solution for 5 minutes for inactivation of endogenous peroxidase and then blocked with 5% normal goat serum in PBS solution for 10 minutes. The specimens were then incubated with anti-Runx2 polyclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a concentration of 1:200 or anti–vascular endothelial growth factor (VEGF) polyclonal primary antibody (Santa Cruz Biotechnology) at a concentration of 1:200. Incubation was carried out overnight at 4°C in the refrigerator. This was followed by 2 washes in PBS for 10 minutes. The sections were then incubated with biotin-conjugated secondary antibody anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA) at a concentration of 1:200 in PBS for 1 hour. The sections were washed twice with PBS followed by the application of preformed avidin-biotin complex conjugated to peroxidase (Vector Laboratories) for 45 minutes. The bound complexes were visualized by the application of a 0.05% solution of 3-3’-diaminobenzidine solution and counterstained with Harris hematoxylin. For control studies of the antibodies, the serial sections were treated with rabbit immunoglobulin G (Vector Laboratories) at a concentration of 1:200 in place of the primary antibody. In addition, internal positive controls were performed with each staining bath.

**RESULTS**

**General Findings**

Neither postoperative complications nor behavioral changes were observed in the animals. The rabbits returned rapidly to their normal diet and showed no loss of weight during the experiment (data not shown). None of the animals died during the experiment, and no infection in the surgical site was observed.

**Histopathologic Analysis**

High-density porous polyethylene implant did not remain after histologic procedures, and its presence is identified by the spaces left by the material. Seven days after the implant surgery, organized and vascular fibrous connective tissue was seen between bone surface and the implant (Fig. 2), and also among HDPP pores, with a few mononuclear leukocytes. On the 14th day, an intense vascularization was observed in the interface region, and a focus of new bone formation was evident, coming from the receptor area and inside the pores, in

**FIGURE 2.** Connective tissue (CT) is observed between HDPP (*) and receptor bone bed. Discrete areas of osteogenesis were noted (arrows). Masson trichrome; bar, 250 µm.

**FIGURE 3.** On the 14th day, A, Primary bone (arrow) is in contact with the material (*), interposed by numerous blood vessels (v). B, New bone formation is also present among the pores (arrows). Masson trichrome; bar, 250 µm.
contact with the space left by the material (Fig. 3A and B). Foreign-body multinucleated giant cells were visualized associated with the implant. On day 45, areas of fibrous connective tissue could still be seen filling the interface region and inside the pores and a number of multinucleated giant cells (Fig. 4). By the 90th day, areas of remodeling bone were observed in the interface region, and lamellar mature bone was predominant inside the pores (Fig. 5A and B).

**Immunohistochemistry**

Core binding factor protein 1 (Cbfa-1) expression was detected predominantly in the cytoplasm. After 7 days of the surgery, Cbfa-1 immunoreactivity could be seen in circumjacent areas to the biomaterial with a moderate pattern (Fig. 6A). A similar pattern occurred in the group after 14 days of surgery (Fig. 6B). After the last periods established in this study, that is, 45 or 60 days, a weak Cbfa-1 immunoreactivity was noticed (Fig. 6C).

Regarding VEGF immunohistochemistry, this could be detected in cells from blood walls inside the defect only. Moderate immunoreactivity was noticed in all periods established in this study, mainly after 14 days of surgery (Fig. 6D). As expected, this period corresponds to an intense vascularization in the interface region.

**DISCUSSION**

In dental medicine, a number of biomaterials have been experimented and indicated for various situations from tooth restorations to jaw reconstruction. Most of them are intended to remain for a long-lasting period or even permanently in direct contact with living tissues. This situation is very clear when it comes to rehabilitation using endosseous implants. Because of their success, which directly depends on the quantity and quality of local bone, most reconstructive biomaterials are developed to interact with and substitute this tissue, many of them containing calcium and phosphate permitting bioactive fixation between bone and material. However, when the necessity is to recover and maintain shape and contour of the injured area, synthetic nonabsorbable implants that permit a biologic fixation of bone on their surface are preferred, such as some kind of polymers. For these situations, the HDPP (Medpor) has been extensively used, and long-term results are reported with a few complications or morbidity. Besides being a highly biocompatible material, its physical characteristics such as the presence of pores and their specific size are equally important. Klawitter et al stressed the relevance of size pores approximately 100 to 250 μm for optimal tissue ingrowth, using the same material of the current study, in a canine model. A similar experiment was reported by Spector et al in the same year, revealing initial penetration of bone tissue inside the material by day 7, also using microradiographs such as those used by the previous authors, with a gradual replacement of fibrous connective tissue by bone in the subsequently analyzed periods being complete after 8 weeks. In our study, we also found localized areas of osteogenic activity coming from the receptor bed by day 7 but with a predominance of fibrous tissue in the interface area and inside the pores. New bone formation and osteoblast activity in the material pores were evident on the 14th day, and after 90 days, remodeling bone in the interface area and inside the pores confirmed agreement with the cited authors.

The reported biologic behavior of HDPP led some authors to test its possible osteogenic capacity. Bilkay et al compared the biochemical levels of alkaline phosphatase and osteocalcine of hydroxyapatite, HDPP, and bone collagen positioned in tibial periosteal sacs of rabbits in the periods of 1, 2, 4, and 8 weeks. Despite results pointed out HDPP as presenting the highest osteogenic ability, it is known that this is not an osteoinductive material, as recognized by the authors, but its appropriate architecture permitted better tissue response and ingrowth, improving periosteum osteogenicity.

Also, Carinci et al identified a number of genes expression in osteoblastlike cells MG63 exposed to HDPP using microarray techniques, related to signal transduction, transcription, translation, cell cycle regulation, vesicular transport and production of cytoskeletal elements, cell adhesion molecules, and extracellular matrix components, although the exact interaction among remained unclear.

To further elucidate putative mechanisms of action involving HDPP on rabbit bone repair, we designed additional experiments by means of immunohistochemistry to observe the expression of Cbfa-1/RUNX2 and VEGF. Interestingly, our results demonstrated a moderate Cbfa-1/RUNX2 expression at initial periods established in this study, that is, 7 and 14 days after HDPP implantation. In the same way, VEGF displayed immunoreactivity in the interface area, as depicted in dental medicine, a number of biomaterials have been experimented and indicated for various situations from tooth restorations to jaw reconstruction. Most of them are intended to remain for a long-lasting period or even permanently in direct contact with living tissues. This situation is very clear when it comes to rehabilitation using endosseous implants. Because of their success, which directly depends on the quantity and quality of local bone, most reconstructive biomaterials are developed to interact with and substitute this tissue, many of them containing calcium and phosphate permitting bioactive fixation between bone and material. However, when the necessity is to recover and maintain shape and contour of the injured area, synthetic nonabsorbable implants that permit a biologic fixation of bone on their surface are preferred, such as some kind of polymers. For these situations, the HDPP (Medpor) has been extensively used, and long-term results are reported with a few complications or morbidity. Besides being a highly biocompatible material, its physical characteristics such as the presence of pores and their specific size are equally important. Klawitter et al stressed the relevance of size pores approximately 100 to 250 μm for optimal tissue ingrowth, using the same material of the current study, in a canine model. A similar experiment was reported by Spector et al in the same year, revealing initial penetration of bone tissue inside the material by day 7, also using microradiographs such as those used by the previous authors, with a gradual replacement of fibrous connective tissue by bone in the subsequently analyzed periods being complete after 8 weeks. In our study, we also found localized areas of osteogenic activity coming from the receptor bed by day 7 but with a predominance of fibrous tissue in the interface area and inside the pores. New bone formation and osteoblast activity in the material pores were evident on the 14th day, and after 90 days, remodeling bone in the interface area and inside the pores confirmed agreement with the cited authors.

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by immunopositive cells on cells on the capillary walls. Therefore, it seems that HDPP is able to adequately permit osteoblasts differentiation and neovascularization into the material.

Different from previous studies, Jordan et al9 observed fibrovascularization of the HDPP (Medpor) used as orbital implants in a rabbit model. Five animals (50%) of their experimental group received the implants encased in polyglactin 910 mesh, and the other 5 was left unwrapped. No differences were noted between the groups, presenting 100% of fibrovascularization of the implants after 12 weeks. Also, Mavrikakis et al,10 observing retrieved HDPP implants used as lower eyelid spacers owing to exposure, poor stability, and contour after 6 months to 2 years, found fibrovascular tissue in the analyzed specimens.

In this way, fixation and immobilization of the material to the receptor bed seems to play an important role in HDPP biointegration. Especially in our study, at the beginning of tissue repair, it sure prevented the implant to move, once no diet restriction was imposed postoperatively, which could disrupt new vascularization, leading to tissue ischemia and necrosis in the interface area.

CONCLUSIONS

Our results along with the literature confirmed that the ideal architecture of HDPP, its optimal osteoconductive behavior and biocompatibility along with an adequate immobilization, permitted adequate vascularization and uniform and continuous osteoblasts cell differentiation along tissue repair period, leading to its lifelong presence in human biologic system.

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