Porous Polyethylene for Tissue Engineering Applications in Diabetic Rats Treated with Calcitonin: Histomorphometric Analysis

Flávio Augusto Claro, MD, DDS[1]/José Roberto Sá Lima, PhD, MD, DDS[2]/
Miguel Angel Castillo Salgado, PhD, MD, DDS[3]/Mônica Fernandes Gomes, PhD, MD, DDS[3,4]

Purpose: *The purpose of this work was to study the bone tissue reaction after porous polyethylene (Polipore) implantation into surgical defects in the parietal bones of rats with streptozotocin-induced diabetes, treated with salmon calcitonin.* ***Materials and Methods:*** *Porous polyethylene implants were placed in bone defects created in 36 adult female rats. The rats were divided into 3 equal groups: diabetic treated with calcitonin (DCa), diabetic (D), and control (C). The animals of the DCa group received applications of salmon calcitonin on alternating days immediately after the surgery until sacrifice. The rats were sacrificed after 15, 30, 60, and 90 days, and the defects were examined histologically and statistically through histomorphometric analysis.* ***Results:*** *Histomorphometric analysis showed that there was no statistically significant difference in the mean quantity of inflammatory cells among all study groups after 15 and 90 days. At 30 days, a statistically significant difference was observed between the D and C groups and the D and DCa groups. At 60 days, there was no statistically significant difference between the D and DCa groups.* ***Discussion:*** *Porous polyethylene can be considered an option for implant material when there are investigations that prove its biocompatibility and stability in the host tissues. Salmon calcitonin positively aided the bone repair and attenuated the inflammatory response until 30 days after the surgery.* ***Conclusion:*** *Porous polyethylene was tolerated by the host tissues in all groups, and moderate chronic inflammatory reaction was observed up to the 90-day period. Salmon calcitonin attenuated the inflammatory response up until 30 days.* Int J Oral Maxillofac Implants 2005;20:211–219

Key words: *bone repair, diabetes mellitus, porous polyethylene, salmon calcitonin, streptozotocin*

The esthetic and functional rehabilitation of patients with congenital, pathologic, or post-traumatic facial deformities has been the subject of several clinical and experimental works.[1–9] Various materials have been used for the reconstruction of these deformities, such as biologic materials (autologous, homologous, and heterologous grafts), and synthetic or alloplastic implants (silicone rubber, ceramics, polyamide mesh, methylmethacrylate, hydroxyapatite, polytetrafluorethylene, and porous polyethylene).[10–16]

The use of materials not derived from the patient reduces the morbidity of the surgical act and eliminates the need of a secondary donor site, thus reducing the risk of infection and potential sequelae.[4,11,15,17] The ideal alloplastic implant has been described as a material that is inert, noncarcinogenic, nonallergenic, and easy to shape during the surgery. An ideal alloplastic implant would not produce an inflammatory response. It would resist mechanical trauma, infections, and extrusions; be able to mimic the color and the consistency of the tissue it replaces; promote tissue ingrowth to the interior of the material; and be readily available without additional morbidity to the patient.[13,17–19]

[1]Private Practice in Oral and Maxillofacial Surgery and Prosthodontics, Taubaté, São Paulo, Brazil.
[2]Professor and Chairman, Department of Oral and Maxillofacial Surgery, Periodontology and Radiology, São José dos Campos School of Dentistry, São Paulo State University—UNESP, São José dos Campos, São Paulo, Brazil.
[3]Professor, Department of Biosciences and Oral Diagnosis, São José dos Campos School of Dentistry, São Paulo State University—UNESP, São José dos Campos, São Paulo, Brazil.
[4]Chair, Biosciences Center for Special Health Care Needs and Special Health Care Needs Association, São José dos Campos, São Paulo, Brazil.

Correspondence to: Dr Flávio Augusto Claro, Faculdade de Odontologia de São José dos Campos—UNESP, Departamento de Biociências e Diagnóstico Oral Av Eng Francisco José Longo, 777 / Cep: 12.245-000, São José dos Campos, São Paulo, Brasil. Fax: +55 12 3947 9010. E-mail: flavioaclaro@bol.com.br

Porous polyethylene has been used in orthopedic, auricular, orbital, cranial, and maxillofacial reconstructions because of its biocompatibility and good stability in the interior of the tissues. The presence of pores allows the ingrowth of host tissues, which promotes better anchorage of the polymer to its implantation site.[1–10,12–18,20–25]

A literature review by the authors yielded no research regarding the use of porous polyethylene in the bone tissue of diabetic patients. The bone of these patients generally presents alterations in the formation and mineralization processes, which may delay the bone repair process during the postoperative period. Such alterations could occur because of a reduction in collagen production (which would cause a deficiency in osteoblast function) and/or because of alterations in the mineral metabolism, with consequent osteopenia.[26–28]

Salmon calcitonin has been widely used in patients with pathologies that affect bone metabolism, such as osteoporosis, ostogenesis imperfecta, and Paget's disease, because of its recognized ability to inhibit bone resorption. Several works in vivo and in vitro have demonstrated that this hormone favors bone formation,[29,30] inhibits osteoclastic activity,[31,32] and prevents the development of osteopenia.[33,34]

Because of the need for elucidation concerning the use of alloplastic materials regarding the metabolic alterations of bone tissue observed in diabetic patients and the application of calcitonin for treatment of some bone diseases, the authors' interest in verifying the reactions of bone tissue after porous polyethylene implantation in surgical defects produced in diabetic rats treated with salmon calcitonin emerged.

MATERIALS AND METHODS

Thirty-six female rats (Wistar, *Rattus norvegicus albinus*), 3 to 6 months old, were divided into 3 equal groups: diabetic rats treated with calcitonin (DCa), diabetic rats (D) and control rats (C). These animals were observed in groups of 3 for 1 month, kept in cages, and fed ad libitum. All animals received humane care according to the criteria of the National Research Council, and the study protocol was approved by the Committee for Animal Use of the São José dos Campos School of Dentistry.

Induction of Diabetes

Diabetes was induced in the animals by a single intraperitoneal injection of pancreatic β-cell toxin streptozotocin (Sigma Chemical, St Louis, MO) dissolved in pH 4.5 citrate buffer at a dose of 45 mg/kg bodyweight. Using an Advantage II glucometer (Roche Produtos Químicos e Farmacêuticos, São Paulo, Brazil), the blood glucose level was monitored 1 week after the streptozotocin treatment and throughout the duration of the study to determine the hyperglycemic state of the animals. Most animals developed clinical evidence of diabetes within 2 weeks of the streptozotocin injection. The animals that failed to develop average blood glucose concentrations higher than 300 mg/dL were excluded from the study.

Treatment Protocol

The animals were anesthetized with intramuscular injections of acepromazin (pre-anesthetic, Acepran 1%, Univet Indústria Veterinária, São Paulo, Brazil) and ketamine (anesthetic, Dopalen, Agribands Saúde Animal, São Paulo, Brazil). These drugs were administrated at a dose of 0.1 mL/100 g.

An incision was made in the sagittal plane of the head, followed by muscular dissection, plane to plane, and incision and detachment of the periosteum. Subsequently, a surgical bone defect was created in the parietal bone with the aid of a 3.0-mm trephine activated by a surgical micromotor and irrigated with 0.9% sterile saline solution. The bone defect was round, with a diameter of 3.0 mm and a depth equal to the thickness of the removed cortical bone. An amount of porous polyethylene (Polipore; Makron Biopolímeros, Mogi das Cruzes, SP, Brazil) the size of the surgical defect was placed in the defect (Figs 1a to 1c). Subsequently, the periosteum, muscle, and skin were sutured. The animals were sacrificed by an anesthetic overdose after 15, 30, 60, or 90 days (3 animals per group per time period), and the bone containing the created defect was removed en bloc, fixed in 10% neutral formalin for 48 hours, decalcified in Plank-Rychlo solution, and embedded in paraffin. The histologic sections were approximately 6 μm thick, cut longitudinally at intervals of 70 μm, and stained with hematoxylin-eosin.

Calcitonin Administration

The animals in the DCa group received 16 IU/kg of synthetic salmon calcitonin (Miacalcic; Novartis, Basel, Switzerland) diluted in 0.9% saline solution, administered subcutaneously on alternate days from immediately after the procedure up to the day of sacrifice.[9,33]

Histomorphometric Analysis

The central point of the histologic section randomization and of the selection for histomorphometric analysis was accomplished randomly, thus eliminating the occurrence of sampling bias. An II Zeiss retic-

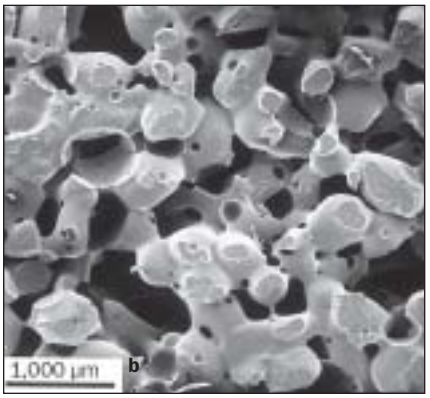


Fig 1 *(a)* Porous polyethylene implant with circular form (3-mm diameter) used in the study. *(b)* Scanning electronic micrograph showing ovoid polyethylene microparticles of different sizes (original magnification ×35). *(c)* The implant after implantation into the surgical defect *(arrows)*.

ule (Carl Zeiss, Göttingen, Germany) was placed over a compensation ocular 8× KPL Zeiss to evaluate the presence of inflammatory cells. The reticule image was superimposed on the desired histologic fields. The reticule points and the total number of points over the bone defect were counted. The presence of acute and chronic inflammatory cells (lymphocytes, neutrophils, macrophages, multinucleated giant cells) was evaluated with the following formula, where IC = inflammatory cells:

$$IC = \frac{\text{Number of reticule points in inflammatory cells}}{\text{Total no. of reticule points in the bone defect}}$$

The chosen bone defect was submitted for examination with serial microscopy sections, from which approximately 100 sections were obtained. From these sections, 5 were randomly chosen for morphometric analysis. Subsequently, 5 histologic fields from each section of the surgical bone defect region were analyzed. During this step, a 40× objective and an ocular 8× KPL with an optical Olympus CH-2 microscope (Tokyo, Japan) were used. The objective showed a 100-point reticule corresponding to 7,840 μm^2 for measuring the bone tissue area.

Statistical Analysis

The histomorphometric results were submitted to analysis of variance (ANOVA) and to the Tukey test with the aid of the GraphPad InStat software version 3.00 for Windows 95 (GraphPad Software, San Diego, CA). The level of significance used was $P < .05$.

RESULTS

15 Days

Group C. The region of the defect was filled by loose connective tissue, with numerous fibroblasts and fibrocytes, as well as large and small engorged blood vessels. This tissue still presented intense and diffused mononuclear inflammatory cell infiltrate with lymphocytes and macrophages. Plasmacytes were scarce; polymorphonuclear cells were absent. Irregular round cavities of various sizes, surrounded by a thin layer of organized granulation tissue with mononuclear inflammatory cells and some multinuclear giant cells were also observed. These cavities, interpreted as negative images of polyethylene particles, were randomly distributed in the formed connective tissue (Fig 2). The giant cells were situated on

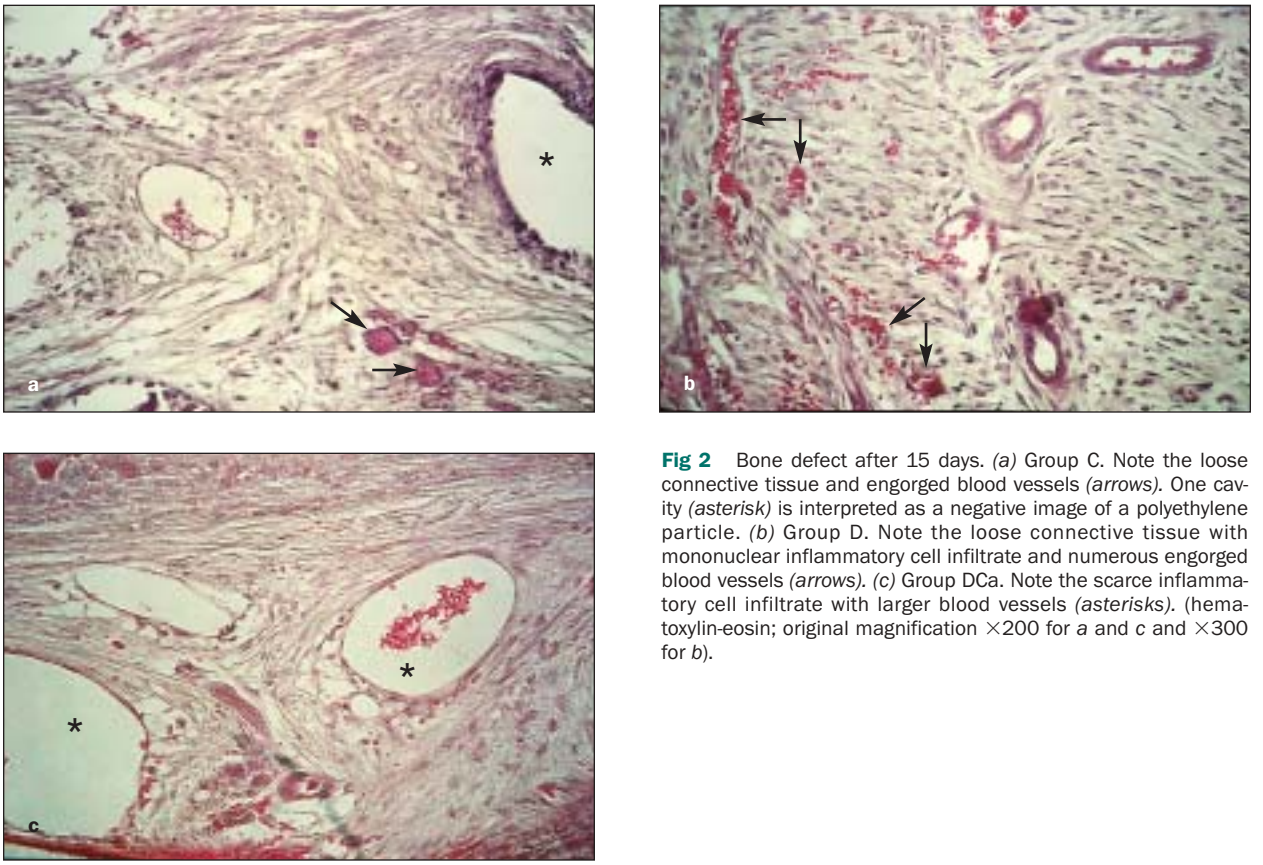


Fig 2 Bone defect after 15 days. *(a)* Group C. Note the loose connective tissue and engorged blood vessels *(arrows).* One cavity *(asterisk)* is interpreted as a negative image of a polyethylene particle. *(b)* Group D. Note the loose connective tissue with mononuclear inflammatory cell infiltrate and numerous engorged blood vessels *(arrows). (c)* Group DCa. Note the scarce inflammatory cell infiltrate with larger blood vessels *(asterisks).* (hematoxylin-eosin; original magnification ×200 for *a* and *c* and ×300 for *b*).

the surface of the implant and in areas near the cavity. The margins of the defect were linear, which characterizes the absence of bone neoformation in this region.

Group D. In group D, the region of the defect was filled with loose connective tissue. However, it exhibited more mononuclear inflammatory cell infiltrate and multinuclear giant cells compared to the control group. In some areas, granulomatous inflammation with epithelioid granulomas composed of epithelioid cells (containing polyethylene particles), lymphocytes, and multinuclear giant cells was seen. The cavities were randomly distributed in the connective tissue and surrounded by a thin layer of granulation tissue, with a greater quantity of multinuclear giant cells, both on the surface of the implant and in the adjacent areas, than was observed in the control group. In some areas of the margins of the defect, the presence of extended areas of bone resorption was observed.

Group DCa. In the DCa group, the bone defect was also filled by loose connective tissue and numerous engorged blood vessels. The quantity of cells and blood vessels seen was similar to that seen in group C and less than that seen in group D. The collagen fibers were denser and more organized than in the other groups. The connective tissue exhibited less mononuclear inflammatory cell infiltrate than group D. It was also observed that the quantity of multinuclear giant cells was similar to that observed in group D. Fewer epithelioid cell granulomas were present in group DCa than in group D. Areas of interstitial hemorrhage were seen in every specimen. The margins of the defect were slightly irregular, with bone formation and, in a few histological specimens, round "nests" of bone tissue adjacent to the implant material were observed. No area of bone resorption was seen in the region adjacent to the defect (Fig 2).

30 Days

Control. In the control group, the region of the defect was filled by well-organized fibrous connective tissue that exhibited moderate and diffused mononuclear inflammatory cell infiltrate. Masses of dystrophic calcification were noticed near the margin of the defect. The cavities were surrounded by a narrow

layer of loose connective tissue exhibiting mononuclear inflammatory cell infiltrate and multinuclear giant cells. Immature bone tissue formation of centripetal growth was observed near the margins of the defect.

Group D. The bone defect was filled by loose connective tissue with numerous engorged blood vessels. The presence of intense and diffused mononuclear inflammatory cell infiltrate was also observed, and in some specimens, areas with abscess formation were observed. The cavities were surrounded by a narrow layer of fibrous connective tissue infiltrated by mononuclear inflammatory cells and multinuclear giant cells. Extended areas of interstitial hemorrhage and edema with rare nests of bone tissue were seen. In some specimens, extended areas of bone resorption and/or necrosis and formation of bone sequestra in the area adjacent to the defect could be detected.

Group DCa. The defect, which was filled with organized fibrous connective tissue, exhibited engorged blood vessels. Moderate and diffused mononuclear inflammatory cells and some epithelioid cell granulomas were seen near the margins of the defect and adjacent to the implant material. Refractive polyethylene microparticles were found in the interior of these granulomas and/or were surrounded by macrophages or multinuclear giant cells.

The presence of well-delineated cavities surrounded by a narrow layer of fibrous capsule with multinuclear giant cells on its surface. Immature bone tissue formation near the cavities was observed. The margins of the defect showed bone neoformation and discrete reabsorption.

60 Days

Control Group. The region of the bone defect was filled by dense fibrous connective tissue and exhibited scarce mononuclear inflammatory cell infiltrate. The cavities were surrounded by a narrow and well-delineated layer of fibrous capsule and exhibited some multinuclear giant cells and engorged blood vessels. Newly formed bone tissue on the margins of the defect and linear dystrophic calcification near the implant were seen.

Group D. The region of the bone defect was filled by fibrous connective tissue, which presented mononuclear inflammatory cell infiltrate and an absence of polymorphonuclear cells. In some areas, the fibrous capsule that surrounded the cavities was thick and infiltrated with mononuclear and multinuclear giant cells. The margins of the defect were irregular and showed areas of bone formation and resorption. Small islands of newly formed bone tissue near the implant material were also seen.

Group DCa. The region of the defect was filled by fibrous connective tissue on the margins and by loose connective tissue in its central portion. The presence of moderate and diffused mononuclear inflammatory cell infiltrate was observed, and in some regions granulomatous inflammation was also seen. The cavities were surrounded by a well-delineated fibrous capsule and exhibited numerous multinuclear giant cells. The presence of newly formed bone tissue formation on the margins of the defect and scarce islands of immature bone tissue dispersed in the formed connective tissue was also observed. No areas of dystrophic calcification were seen.

90 Days

Groups C and DCa. The bone defects were filled with a fibrous connective tissue and exhibited mononuclear inflammatory cell infiltrate only rarely. The cavities were well-delineated, and some multinuclear giant cells on the periphery of its surface were observed. The diabetic group treated with calcitonin showed more vascularization than the control group.

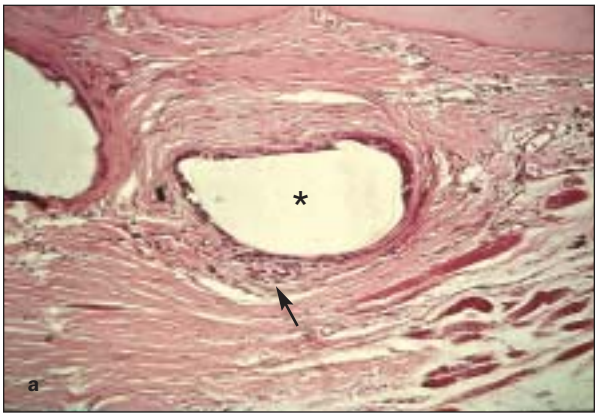
Group D. A greater quantity of mononuclear inflammatory cells and multinuclear giant cells could be seen in the connective tissue compared to the other groups. Discrete foci of dystrophic calcification dispersed in this tissue were observed (Fig 3).

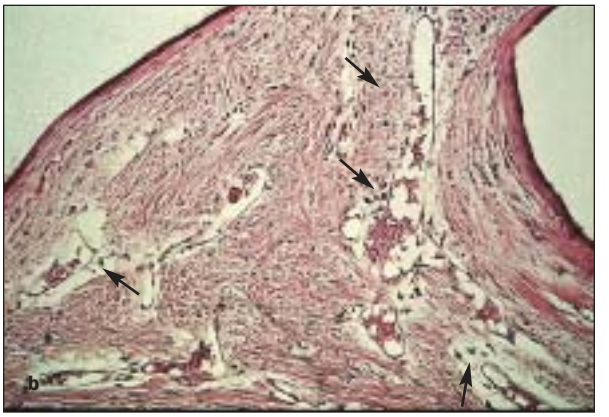
Histomorphometric and Statistical Analyses

The histomorphometric analysis showed variable quantities of inflammatory cells in all the study groups and observation times. After 15 and 90 days, the mean quantity of inflammatory cells in all study groups was not significantly different ($P > .05$). At 30 days, significant differences were observed between groups D and C and groups D and DCa ($P < .001$). At 60 days, significant differences were observed between groups C and D and between groups C and DCa (Table 1). The analysis of the results, shown in Fig 4, demonstrated the evolution of the inflammatory reaction after the implantation of the porous polyethylene into the bone defects at all observation times.

DISCUSSION

Among the several types of alloplastic materials available, porous polyethylene has been used for decades for partial or total ossicular replacement,[20,23] for restorative and esthetic rhinoplasty,[5,18] in orbital implants,[22,25] in auricular reconstruction, in orbital reconstruction, and in the correction of deformities of the cranial base and the frontal, temporal, zygomatic, and mandibular regions.[1,2,4,6,17,19,24] Clinical





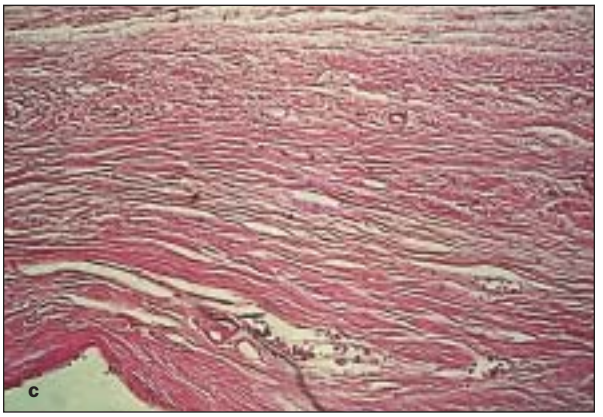


Fig 3 Bone defect after 90 days. *(a)* Group C. Observe the fibrous connective tissue and cavity *(asterisk)* surrounded by well-delineated fibrous capsule and chronic inflammatory cells *(arrow). (b)* Group D. Note the connective tissue. Fewer organized collagen fibers and mononuclear inflammatory cells are dispersed in this tissue *(arrows). (c)* Group DCa. Observe the fibrous connective tissue with little mononuclear inflammatory cell infiltrate (hematoxylin-eosin; original magnification ×300 for *a* and *b* and ×200 for *c*).

Table 1 Statistcal Analysis of the Mean No. of Inflammatory Cells in the C, D, and DCa Groups

Period/group comparison	Mean difference	*P*
15 d		
C vs D	−7.222	NS
C vs DCa	−1.899	NS
D vs DCa	5.333	NS
30 d		
C vs D	−35.444	.001*
C vs DCa	−1.889	NS
D vs DCa	33.556	.001*
60 d		
C vs D	−17.556	.01*
C vs DCa	−14.444	.01*
D vs DCa	3.111	NS
90 d		
C vs D	−1.222	NS
C vs DCa	1.222	NS
D vs DCa	2.444	NS

NS = not significant; $P > .05$.
*Statistically significant difference.

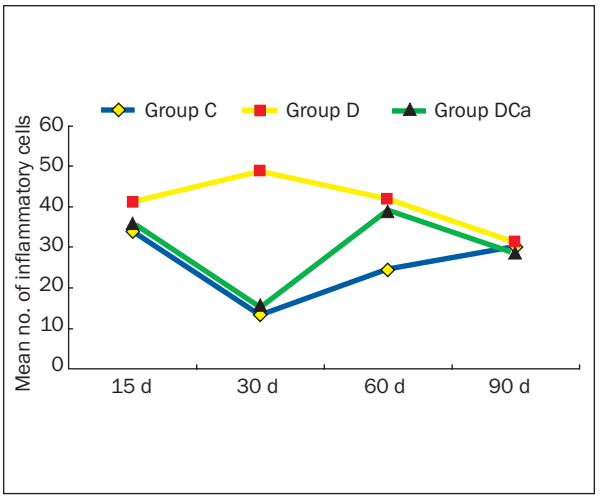


Fig 4 Schematic representation of the evolution of the inflammatory response observed in groups C, D, and DCa.

and experimental studies found in the literature have shown that the presence of porosity in the polyethylene aids tissue invasion, which promotes adhesion and stability in the host tissue.[8,11,12,14,16,21]

In the histologic observations of group C, it could be verified that the polyethylene implant was tolerated by the host tissues, with fast invasion of its pores by well vascularized connective tissue without the occurrence of implant extrusion. These results agreed with the clinical and experimental studies of Yildirim and associates,[8] Rubin and coworkers,[14] and Rossa.[16] The chronic inflammatory reactions and multinuclear giant cells as a foreign body, observed in all periods, interfered in the osseointegration of the implant material. Giant cells were also found in other experiments.[5,12,21,25] Some authors have reported that the presence of these cells is related to the size and/or forms of the polyethylene particles.[11,14,16] The normality of the immune system of the animals and the postoperative care were probably responsible for the lack of infections. Other authors have said that the filling of the pores by the surrounding tissue may reduce the space available for bacterial adhesion and lessen the risk of infection.[10,15]

Areas of dystrophic calcification, which were present in the last 2 observation periods, could be explained by the occurrence of tissue necrosis caused by the chronic inflammatory process in the receptive area. The small statistically significant difference in the mean IC, observed at 15 and 90 days in the control group, suggests that porous polyethylene causes a moderate chronic inflammatory response even when used in normal animals.

According to Boyko and Lipsky,[35] alterations in the immune defense mechanisms of diabetic patients, such as inhibition of the phagocytic capacity of the neutrophils associated with vascular alterations, compromise the local microcirculation and the reparative process. These phenomena could explain the presence of abscess areas and bone sequestra in some specimens of the diabetic group at the 30-day period. On the other hand, the histologic findings for the 90-day period showed that these reactions were transitory. These facts could be related to the postoperative complications of the diabetic animals and to the implant material used in this study.

In this study, areas of bone resorption and neoformation were discretely observed in the latter periods of observation, which is in agreement with the study of Shyng and colleagues,[36] who used polytetrafluorethylene membrane in surgical calvarial bone defects of diabetic rats. In the diabetic group, no extrusion or exposition of the implants was seen; they were tolerated by the host tissue.

In the last few decades, calcitonin has been used in the treatment of several bone pathologies because of its capacity to decrease osteoclastic activity, inhibit the synthesis and liberation of enzymes that promote bone matrix resorption, increase osteoblastic activity, and impair the primitive mesenchymatous cell differentiation in osteoclasts.[29–31,37,38] Its action in bone resorption inhibition seems to be connected to the presence of specific receptors in the cytoplasm of the osteoclasts.[32,39] When this hormone connects with these receptors, the form of these cells is changed. They become smaller and have fewer microvillosities and less cytoplasmic mobility; ie, less functional activity.[39,40]

In the DCa group, the calcitonin was efficient in the inhibition of bone resorption and/or stimulation of bone tissue formation compared to the other groups, a finding that is in agreement with several studies found in the literature.[29–32,37–39]

In the histomorphometric and statistical analysis of this study, the calcitonin decreased the mean IC in the 15- and 30-day observation periods. This fact could be explained by the analgesic and anti-inflammatory properties of this hormone, as described in the studies of Quatraro and coworkers,[41] Zmijewska and associates,[42] and Lyritis and associates.[43]

After implantation of porous polyethylene in bone defects, Wellisz and coworkers[3] and Maas and colleagues[11] observed the presence of bone spicules inside the pores of the implantation material, a finding that was not seen in the present study. This may be related to the small diameter of the pores in the material used, which would have impaired the growth of bone tissue inside the implant.

The use of calcitonin in diabetic patients has been the subject of great controversy in the last years. Some researchers have reported that treatment with calcitonin increases the serum glucose concentration,[44–46] while others have stated that prolonged treatment with the hormone does not promote significant changes in glycemic control.[47–49] In the present results, the glycemic means of the animals in groups D and DCa were similar, which agrees with the findings of Gattereau and colleagues,[47] Sgambato and coworkers,[48] and Giustina and associates.[49]

Although these results were unsatisfactory in relation to surgical practice involving reconstruction of the craniomaxillofacial complex, polyethylene may be considered an option for implant material once there have been investigations to demonstrate its biocompatibility, stability in the host tissues, and ease of shaping in the region.

CONCLUSION

Based on the findings of the present study, it is possible to conclude that porous polyethylene (Polipore) was tolerated by the host tissues in all groups. Moderate chronic inflammatory reaction was observed up to the 90 days postimplantation. The pores of the polyethylene were filled with connective tissue; osseointegration of the implant did not occur. Salmon calcitonin attenuated the inflammatory response up to 30 days after the surgery.

ACKNOWLEDGMENTS

This research was supported by The State of São Paulo Research Foundation (FAPESP; grant number 2000/11940-4). The authors would like to thank Professors José Benedito de Oliveira Amorim and Maria Nadir Gasparoto Mancini of the Department of the Biosciences and Oral Diagnosis of the São José dos Campos School of Dentistry, São Paulo State University—UNESP for helping in the preparation of the diabetic animal model and Roberto Honorio Correa (Director, Quality Assurance & Compliance, MD&D LA-Manufacturing Company Brazil, Johnson & Jonhson, São José dos Campos, São Paulo, Brazil) for the donation of surgical materials.

REFERENCES

1. Wellisz T. Clinical experience with the Medpor porous polyethylene implant. Aesthet Plast Surg 1993;17:339–344.
2. Cenzi R, Guarda-Nardini L. Lútilizzo del polietilene poroso (Medpor) in chirurgia maxillo-facciale. Minerva Stomatol 1995; 44:559–582.
3. Wellisz T, Lawrence M, Jazayeri MA, Golshani S, Zhou ZY. The effects of alloplastic implant onlays on bone in the rabbit mandible. Plast Reconstr Surg 1995;96:957–963.
4. Choi JC, Fleming JC, Aitken PA, Shore JW. Porous polyethylene channel implants: A modified porous polyethylene sheet implant designed for repairs of large and complex orbital wall fractures. Ophthal Plast Reconstr Surg 1999;15:56–66.
5. Niechajev I. Porous polyethylene implants for nasal reconstruction: Clinical and histologic studies. Aesthet Plast Surg 1999;23:395–402.
6. Janecka IP. New reconstructive technologies in skull base surgery: Role of titanium mesh and porous polyethylene. Arch Otolaryngol Head Neck Surg 2000;126:396–401.
7. Soparkar CNS, Wong JF, Patrinely JR, Davidson JK, Appling D. Porous polyethylene implant fibrovascularization rate is affected by tissue wrapping, agarose coating, and insertion site. Ophthal Plast Reconstr Surg 2000;16:330–336.
8. Yildirim G, Haliloglu T, Sapçi T, et al. Tracheal reconstruction with porous high-density polyethylene tracheal prosthesis. Ann Otol Rhinol Laryngol 2000;109:981–987.
9. Li M, Shen Y, Burton KW, et al. A comparison of the skeletal effects of intermittent and continuous administration of calcitonin in ovariectomized rats. Bone 1996;18, 375–380.
10. Brown BL, Neel HB, Kern EB. Implants of Supramid, Proplast, Plasti-Pore, and Silastic. Arch Otolaryngol 1979;105:605–609.
11. Maas CS, Merwin GE, Wilson J, Frey MD, Maves MD. Comparison of biomaterials for facial bone augmentation. Arch Otolaryngol Head Neck Surg 1990;116:551–556.
12. Shanbhag A, Friedman HI, Augustine J, von Recum AF. Evaluation of porous polyethylene for external ear reconstruction. Ann Plast Surg 1990;24:32–39.
13. 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.
14. Rubin PAD, Popham JK, Bilyk JR, Shore JW. Comparison of fibrovascular ingrowth into hydroxyapatite and porous polyethylene orbital implants. Ophthal Plast Reconstr Surg 1994; 10:96–103.
15. Sclafani AP, Thomas JR, Cox AJ, Cooper MH. Clinical and histologic response of subcutaneous expanded polytetrafluoroethylene (Gore-Tex) and porous high-density polyethylene (Medpor) implants to acute and early infection. Arch Otolaryngol Head Neck Surg 1997;123:328–336.
16. Rossa R. Porous Polyethylene: Evaluation of the Tecidual Reactions in Subcutaneous Inclusions [thesis]. São Paulo: University of São Paulo, 1999.
17. Frodel JL, Lee S. The use of high-density polyethylene implants in facial deformities. Arch Otolaryngol Head Neck Surg 1998;124:1219–1223.
18. Romo T III, Sclafani AP, Sabini P. Use of porous high-density polyethylene in revision rhinoplasty and in platyrrhine nose. Aesthet Plast Surg 1998;22:211–221.
19. Duman H, Deveci M, Uygur F, Sengezer M. Reconstruction of contour and anterior wall defects of frontal bone with a porous polyethylene implant. J Craniomaxillofac Surg 1999; 27:298–301.
20. Frootko NJ. Failed ossiculoplasty using porous polyethylene (Plasti-Pore) prostheses. J Laryngol Otol 1984;98:121–126.
21. Kinoshita Y, Kuzuhara T, Kirigakubo M, Kobayashi M, Shimura K, Ikada Y. Soft tissue reaction to collagen-immobilized porous polyethylene: Subcutaneous implantation in rats for 20 weeks. Biomaterials 1993;14:209–215.
22. Karesh JW, Dresner SC. High-density porous polyethylene (Medpor) as a successful anophthalmic socket implant. Ophthalmology 1994;101:1688–1695.
23. Kerr AG, Riley DN. Disintegration of porous polyethylene prostheses. Clin Otolaryngol 1999;24:168–170.
24. Sevin K, Askar I, Saray A, Yormuk E. Exposure of high-density porous polyethylene (Medpor) used for contour restoration and treatment. Br J Oral Maxillofac Surg 2000;38:44–49.
25. Sánchez AN, Sainz JAR, López AS, et al. Integracíon de los implantes orbitarios de polietileno (Medpor). Estudio histológico. Arch Soc Esp Oftalmol 2001;76:25–29.
26. Hough FS. Alterations of bone and mineral metabolism in diabetes mellitus. Part II. Clinical studies in 206 patients with type I diabetes mellitus. S Afr Med J 1987;72:120–126.
27. Spanheimer RG, Umpierrez GE, Stumpf V. Decreased collagen production in diabetic rats. Diabetes 1988;37:371–376.
28. Rico H, Hernandez ER, Cabranes JA, Gomez-Castresana F. Suggestion of a deficient osteoblastic function in diabetes mellitus: The possible cause of osteopenia in diabetics. Calcif Tissue Int 1989;45:71–73.
29. Yamaguchi M, Uto M, Matsui R. Stimulatory effect of calcitonin on bone formation in tissue culture. J Pharmacobiodyn 1989; 12:708–715.
30. Arisawa EAL. Calcitonin Effect on Mandibular Bone Repair of Ovariectomized Rats [thesis]. São José dos Campos: São Paulo State University—UNESP, 2000.
31. Holtrop ME, Raisz LG Simmons HA. The effects of parathyroid colchicine and calcitonin on the ultrastructure and the activity of osteoclasts in organ culture. J Cell Biol 1974;60:346–355.

32. Nicholson GC, Moseley JM, Sexton PM, Mendelsohn FAO, Martin TJ. Abundant calcitonin receptors in isolated rat osteoclasts. Biochemical and autoradiographic characterization. J Clin Invest 1986;78:355–360.
33. Wronski TJ, Yen CF, Burton KW, et al. Skeletal effects of calcitonin in ovariectomized rats. Endocrinology 1991;129:2246–2250.
34. Ichikawa M, Nakamuta H, Hoshino T, Ogawa Y, Koida M. Anti-osteopenic effect of nasal salmon calcitonin in type 1 osteoporotic rats: Comparison with subcutaneous dosing. Biol Pharm Bull 1994;17:911–913.
35. Boyko EJ, Lipsky BA. Infection and diabetes. In: Harris MI (Coord). National Diabetes Data Group. Diabetes in America, ed 2. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1995:485–499.
36. Shyng YC, Devlin H, Sloan P. The effect of streptozotocin-induced diabetes mellitus on calvarial defect healing and bone turnover in the rat. Int J Oral Maxillofac Surg 2001;30: 70–74.
37. Delling G, Glueckselig W. The effect of calcitonin on the regeneration of circumscribed tibia defects and on mineral content of bone in the rat. Isr J Med Sci 1971;7:367–368.
38. Hedlund T, Hulth A, Johnell O. Early effects of parathormone and calcitonin on the number of osteoclasts and on serum-calcium in rats. Acta Orthop Scand 1983;54:802–804.
39. Kallio DM, Garant PR, Minkin C. Ultrastructural effects of calcitonin on osteoclasts in tissue culture. J Ultrastruct Res 1972; 39:205–216.
40. Chambers TJ, Moore A. The sensitivity of isolated osteoclasts to morphological transformation by calcitonin. J Clin Endocrinol Metab 1983;57:819–824.
41. Quatraro A, Minei A, De Rosa N, Giugliano D. Calcitonin in painful diabetic neuropathy. Lancet 1992;339:746–747.
42. Zmijewska M, Imiela J, Ciszek M. Calcitonin in treatment of diabetic foot syndrome [in Polish]. Pol Tyg Lek 1994;49:23–25.
43. Lyritis GP, Ioannidis GV, Karachalios TH, et al. Analgesic effect of salmon calcitonin suppositories in patients with acute pain due to recent osteoporotic vertebral crush fractures: A prospective double-blind, randomized, placebo-controlled clinical study. Clin J Pain 1999;15:284–289.
44. Thomas DW, Frewin DB, Jolley PT. Deterioration in diabetic control during calcitonin therapy [letter]. Med J Aust 1979;2:699, 700.
45. Zofková I. Diabetogenic action of calcitonin in acute experiment in healthy humans. Acta Univ Carol [Med] (Praha) 1986; 32:319–325.
46. Young AA, Wang MW, Gedulin B, Rink TJ, Pittner R, Beaumont K. Diabetogenic effects of salmon calcitonin are attributable to amylin-like activity. Metabolism 1995;44:1581–1589.
47. Gattereau A, Bielmann P, Durivage J, Davignon J, Larochelle P. Effect of acute and chronic administration of calcitonin on serum glucose in patients with Paget's disease of bone. J Clin Endocrinol Metab 1980;51:354–357.
48. Sgambato S, Passariello N, Giugliano D, Torella R, D'Onofrio F. Effect of calcitonin on plasma glucose, C-peptide, glucagon and growth hormone responses to arginine in insulin-dependent diabetic subjects. Acta Diabetol Lat 1981;18:235–241.
49. Giustina G, Cerudelli B, Cimino A, Rigosa C, Rotondi A, Radaeli E. Synthetic salmon calcitonin is not diabetogenic in patients with normal or impaired glucose metabolism. J Endocrinol Invest 1985;8:19–23.