

## **Polyurethane and PTFE barriers for guided bone regeneration: A histomorphometric study in rabbits parietal bone**

### **Barreiras de poliuretano e PTFE para regeneração óssea guiada: Estudo histomorfométrico no osso parietal de coelhos**

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### **ABSTRACT**

The purpose of this study was to evaluate the process of bone repair in surgical defects created in parietal bone of rabbits by the guided bone regeneration technique, using polyurethane (PUr) and PTFE barriers. The surface characteristics of the barriers in scanning electronic microscopic were also evaluated. In this research, 24 adult rabbits were used, 12 were in control group (C) and 12 were in experimental groups (right parietal – PUr group and left parietal – PTFE group). In the C group, the defect was filled only by blood clot. In the experimental groups, the PUr and PTFE barriers were positioned on the floor and on the surface of each bone defect. After 15, 30, 60 and 90 days, 3 animals in the C and 3 in the experimental groups were sacrificed and the defect bones were submitted to microscopic analysis. The results of the study showed no significant differences in the experimental groups, demonstrating quantitative and qualitative superiority bone fill and faster bone regeneration when compared to the C group. The physical barriers presented homogenous surface and no porosity. The PUr was biocompatible, osteoconductive and was not absorbed during the process of bone repair.

### **UNITERMS**

Guided bone regeneration; membranes; polyurethanes; polytetrafluoroethylene.

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### **INTRODUCTION**

The treatment of the defects in the maxillary bone is a complex process influenced by age, bone structure, blood supply, defect morphology and adjacent soft tis-

sue. Guided bone regeneration (GBR) was introduced to facilitate bone growth. The use of a membrane as a mechanical barrier prevents bone defect undesirable cell invasions during its repair, allowing preferential repopulation of this area with specific cells<sup>9,11,18</sup>.

Prerequisites of a barrier membrane include biocompatibility, cellular occlusive, tissue integration, stiffness and easy handling<sup>18,19</sup>. The PTFE barrier has been successfully used in experimental and clinical studies, once for not presenting porosity it hinders the bacterial colonization, allowing its exhibition to the oral cavity, besides the fact of presenting biocompatibility, low cost and easy manipulation<sup>15-17</sup>.

Polymers used as biomaterials can be naturally occurring, synthetic, or a combination of both. A natural polyurethane (PUr) resin obtained by polymerization of the polyester polyol, derived from *Ricinus communis*, a tropical castor bean, has been developed for bone repair<sup>4,12</sup>. PUr barrier was developed to be applied in GBR and this polymeric had their properties described as being biocompatible, osteoconductor, antimicrobial, absorbable and having osseointegrate properties<sup>2,3,5,6,13</sup>. However, in the barrier form few works were accomplished<sup>1,17</sup>.

The purpose of this study was, therefore, to evaluate the tissue behavior of PUr and PTFE barriers in bone repair.

## MATERIALS AND METHOD

Twenty-four New Zealand adult rabbits with an average weight of 3.5kg were divided into 3 groups: control (C, n=12), and experimental groups (PTFE and PUr, n=12). All animals received human care according to the National Research Council's criteria and the study protocol had been previously approved by the Committee for Animal Use of the São José dos Campos Dental School of the São Paulo State University – UNESP.

The animals were anesthetized intramuscularly with Rompum® 0,1mg/dl (Bayer SA, São Paulo, SP, Brazil) as a preanesthetic solution and Ketalar® 0,25mg/dl (Aché Laboratórios Farmacêuticos S. A., Parke-Davis, Guarulhos, SP, Brazil) for complete anesthesia. An incision was made in the sagittal plane of the head, followed by muscular dissection, plane to plane. Subsequently, a surgical bone defect was created in each parietal bone, with the aid of 8mm trephine and irrigated with saline solution. The bone defect had a circular form, with its depth equal to the thickness of the removed cortical bone. In the PTFE and PUr groups, the PTFE (nonporous PTFE barrier 0.13mm thick - Tecnoflon-Brasflon, Ind. & Com. Plásticos, São Paulo, SP, Brazil) and PUr (*Ricinus communis* derived barrier 0.13mm thick - Augment-M, Ricinix Biomateriais – Poliquil, Belo Horizonte, MG,

Brazil) barriers were placed on the floor of the defect and on the surface of the surgical bone defect. In C group, the bone defects contained very soon a blood clot. Subsequently, the periosteum and muscle were sutured as well as the skin.

The animals were treated with benzilpenicillin, 0,1ml/Kg (Pentabiotic, Fort Dodge Saúde Animal LTDA, São Paulo, SP, Brazil) and Celecoxib 2,8mg/kg, (anti-inflammatory, Pfizer Pharmacia, São Paulo, SP, Brazil) 1h before the surgery. These drugs were injected intramuscularly in all animals. Fifteen, 30, 60 and 90 days after surgery, 3 defects of each group were obtained after each observation-time. The bone content of the created defect was removed in bloc, fixed in 10% formalin for 72h, decalcified in Plank-Rychlo solution and embedded in paraffin. The histological sections were cut approximately with 5µm of thickness and were stained with Hematoxylin-Eosin.

## Scanning Electron Microscope

The surface aspect of PUr and PTFE barriers was evaluated by the use of MEV (JMS 5310-JEOL with 15 kV, National Institute for Space Research - INPE, São José dos Campos, São Paulo, Brazil) in increases of 35X, 500X, 2000X, 7500X.

## Statistical and histomorphometric analyses

The central point of histological section randomization and selection for histomorphometric analysis was accomplished randomly, eliminating the occurrence of sampling bias<sup>8</sup>. A Zeiss II reticule was placed over a compensation ocular 10X Zeiss microscope (W-PI, Carl Zeiss, Gottinger, Germany) to evaluate the bone density. The reticule image was superimposed on the desired histological fields. The reticule points and the total number of points over the bone defect were counted. The chosen bone defect was submitted for examination with serial microscopic sections, from which approximately 100 sections were obtained. From these sections, 4 were randomly chosen for histomorphometric analysis. Subsequently, 8 histological fields from each section, in the surgical bone defect region, were analyzed. At this step, a 20X objective (A-Plan, Carl Zeiss, Gottingen, Germany) and an ocular 10X (W-PI, Carl Zeiss, Gottingen, Germany) of an optical microscope (Axioskop 40, Carl Zeiss, Gottingen, Germany) were used. The objective showed a 100-point reticule corresponding to 7840µ<sup>2</sup> for measuring the bone tissue area.

The histomorphometric results were submitted to analysis of variance ANOVA and linear regression (STATISTIX 8.0 for Windows; Analytical Software; Tallahassee, FL, USA). The level of significance used was  $p < 0.05$ .

## RESULTS

### *Scanning electronic microscopy*

The PTFE barrier showed plane and regular surface, and PUr barrier presented irregular surface showing areas of depression. Besides, granular structures of varied sizes were observed, mainly in the areas among the areas of depression. Both structures did not present porosity (Figure 1a and 1b).

### *Microscopic feature*

#### *15 days*

The bone defect of the C group showed bone defect filled by osteogenic connective tissue and for immature bone trabeculae. There were the more newly formed bone trabeculae in the PUr and PTFE groups than in the C group. Hemorrhage interstitial areas and infiltrated of mononucleares inflammatory cells were also present in the experimental groups

(Figure 2). Besides, we conclude that the bone cortical showed larger thickness in experimental groups when compared to C group.

#### *30 days*

In all groups, the area of the defect was filled with immature bone tissue and osteogenic connective tissue. The bone trabeculae were mature in the extremities and immature and irregular arrangement in the central portion. In the PTFE and PUr groups, thick and homogeneous bone trabeculae were evidenced exhibiting lineal arrangement, extending from the peripheral portion to central. Besides, discrete and diffuse infiltrated of mononuclear inflammatory cells were observed in PTFE and PUr, while it was moderate in the C groups. Newly formed bone marrow was evidenced mainly in the extremities of the defect in all groups. The bone cortical of the surgical defect limits showed thickness similar to the original cortical bones.

#### *60 days*

In the C group, the defect area was filled by mature bone trabeculae, with irregular arrangement and form, and osteogenic and fibrous connective tissue. The newly formed bone tissue exhibited large Havers channels. In the PTFE and PUr groups, the defect was filled by mature and immature bone trabeculae

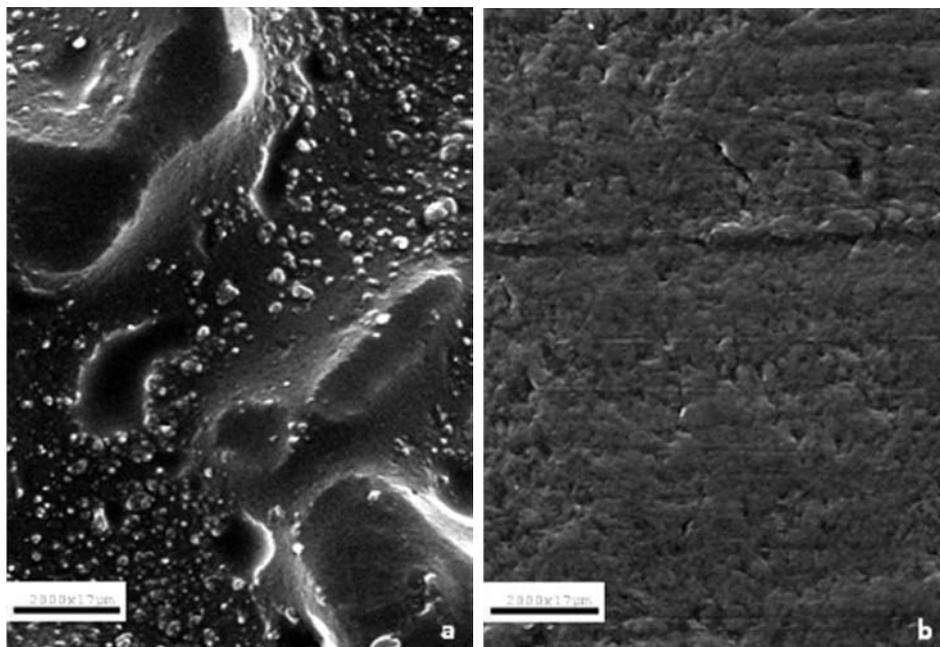


Figure 1 - MEV images: a) PUr barrier exhibiting surface with saliencies of irregular outline and granules of varied size and b) PTFE barrier showing plane and regular surface with absence of pores, increase  $\times 2000$ .

and osteogenic connective tissue. The mature bone trabeculae were located in the extremities of the defect region, and the immature ones in the central portion. The bone cortical in the defect region showed thickness similar to the original cortical bones, that it was not observed in the C group.

*90 days*

In the C group, the defect region was filled by mature bone trabeculae and fibrous connective tissue. The formed bone trabeculae showed irregular arrangement and form. The thickness of the bone defect area of the C group was less than that of the experimental groups. The fibrous connective tissue was permeated by bone formed bone trabeculae. In the PTFE and PUr groups, the surgical defect region still presented osteogenic

connective tissue, located mainly in the central portion (Figure 3). This tissue showed numerous osteoprogenitor and osteoblasts cells and no mononuclear inflammatory cells. The PTFE and PUr barriers were not resorbed in all observation periods.

**Histomorphometric analysis**

The histomorphometric analysis aimed at measuring the volume density of the newly formed bone matrix in the bone defects of the C, PTFE and PUr groups, as well as to provide the necessary data for the statistical analysis of these measurements. The mean values and standard deviation of the bone defect histomorphometry of the studied groups for the different periods are shown in Table 1.

**Table 1 - Optical density of newly formed bone (Mean + SD)**

Days after surgery	C	PTFE	PUr
15	0,3131*±0,11	0,5113±0,07	0,4381±0,06
30	0,3913*±0,06	0,5406±0,09	0,4702±0,05
60	0,4273*±0,11	0,6356±0,09	0,5740±0,04
90	0,4792*±0,14	0,9256±0,03	0,8583±0,09

\*p<0.05 (interaction between C, PTFE and PUr)

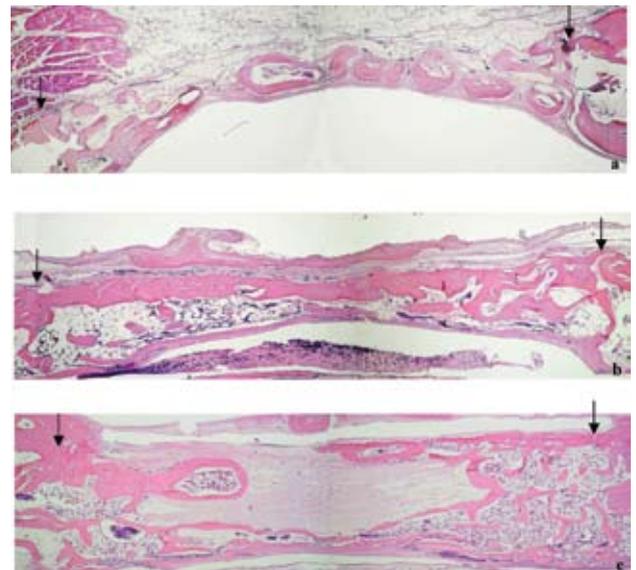
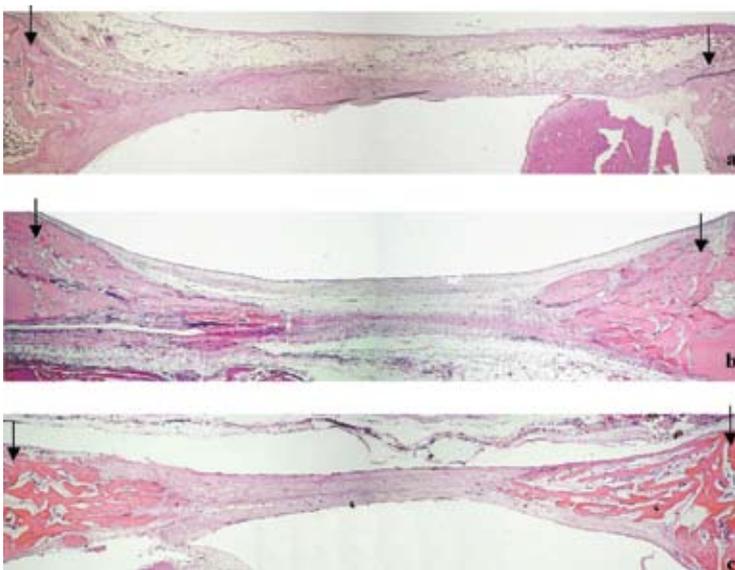


Figure 2 - Photomicrographs of the surgical defect region after periods of 15 days. Limits of the surgical bone defect are indicated by arrows: (a) control, (b) PTFE and (c) PUr groups, HE x100.

Figure 3 - Photomicrographs of the surgical defect region after periods of 90 days. Limits of the surgical bone defect are indicated by arrows: (a) control, (b) PTFE and (c) PUr groups, HE x100.

## DISCUSSION

The literature on GBR is vast, however, there are still so many other points to be researched and discussed. An ideal physical barrier does not exist. Some researchers<sup>9</sup> give preference to the physical barriers in PTFE because they promote larger growth of bone tissue with denser quality, unlike the absorbable ones where the products from their degradation can produce a local inflammatory process taking to a smaller bone formation. The need of a second surgical time for removal of non-absorbable barriers represents one of the main disadvantages of its use. On the other hand, some absorbable membranes can fail in keeping the space or in the needed time of enduring to allow bone growth. Therefore, longevity and capacity for keeping the space are the greatest challenges of the absorbable membranes in GBR<sup>15</sup>.

Some works demonstrated that the new bone formation can take place in the GBR technique with the exclusion of the periosteum placing a totally occlusive barrier<sup>16,18</sup>. Confirming this fact, we verified in the present study that the amount of bone tissue was smaller in the C group demonstrating that periosteum, in spite of supplying nutrition and growth factors for the beginning and progression of osteogenesis, did not have any important influence in the final result.

The porous physical barrier was structured to allow the passage of fluids and nutrients, creating an appropriate compartment for osteogenesis, integration and anchorage of the soft tissue, avoiding its exposure to the oral cavity<sup>18,19</sup>. The membrane pores exposed in oral cavity, however, allow the colonization and larger retention of microorganisms causing consequent local infection and reduction of bone growth<sup>14,19</sup>, therefore they should be removed as soon as possible. According to the present work findings in the MEV porosity was not observed in the PUr or PTFE barriers. That fact would present relevant advantages regarding the bacterial retention when compared to physical barriers with porosity.

The biocompatibility and the effectiveness of the use of a PTFE nonporous physical barrier were demonstrated in recent clinical and experimental studies and in the treatment of several kinds of bone defects<sup>15-17</sup>, what hugely supports the present study results. Besides the biocompatibility, the PTFE application as physical barrier is sustained due to the capacity of maintenance of appropriate space for the blood clot. Our study also demonstrated that the permeability and the integration of the membrane are not necessary for bone formation in the GBR technique<sup>14,18</sup>.

The derived polyurethanes of the castor oil plant presented good results of biological compatibility<sup>4,10</sup>. The observations, regarding the inflammatory infiltrate in the samples treated with PUr barrier, showed an initial inflammatory reaction which decreased along the time. Inflammatory multinuclear giant cells (IMGCs) were not found in bone defect area of PUr group. However, in the area where the PUr mini screws were set aiming the fixation of the barriers, the presence of thin fibrous conjunctive tissue capsule, outlining the referred material, were noticed. Mononuclear inflammatory cell infiltrate was also present, with lymphocytes, plasmocytes, macrophages and IMGCs in direct contact with the small particles of PUr spread in the adjacent tissue of bone defect area.

The cytotoxicity absence observed in Ignácio et al.<sup>10</sup> study was interpreted as a result of the use of an improved polymeric one. According to these authors necrosis areas and cellular alterations were not observed, as hydropic or hyaline degeneration and triglycerides and cholesterol accumulation, which would be cytotoxicity indications.

The antimicrobial action of PUr was evaluated both *in vitro*<sup>13</sup> and *in vitro*<sup>6</sup> studies using the castor oil plant detergent. In clinical study<sup>1</sup>, in the places where the exhibition of the barrier happened there was no significant deposition of biofilm, not even inflammatory reactions were observed in the tissues or infections in the surrounding areas. In none of the observation periods of PUr group infection signs were evidenced. We believed that the porosity absence, as demonstrated in MEV, could be an important factor to contribute for the decreasing of microorganism retention and colonization. However, posterior analyses of the antimicrobial properties of the PUr barrier would contribute to explain the real effectiveness in the GBR technique.

Despite the evidences concerning PUr absorbing characteristics<sup>2,4,7,10</sup>, in clinical study<sup>1</sup>, the non absorption was justified due to the thickness of the material (0.20mm), suggesting that if the barrier presented smaller thickness it could be eliminated more quickly. However, in previous study<sup>17</sup>, using 0.13mm thickness barriers in the subcutaneous of mice, no absorption indications were present in a seventy-day period. In our results, any signs of PUr absorption were evidenced during all the observation periods. This experiment demonstrated that, initially absorbable rigid materials are conducive for regeneration and bone formation. However, in many clinical situations its degradation, not superior to 12 months, is extremely important so

that it will not lose the advantages of being absorbable<sup>9</sup>.

The presence of osteogenic tissue in the PUr surface and its incorporation in the new bone tissue formed has been related<sup>4</sup>. In the other hand, implanting the polymeric in soft tissue<sup>5,17</sup>, the formation of osteogenic tissue was not observed, discarding the osteoinductor property of the implant material. In the present study, the barrier osteointegration was not observed, but the constant presence of a connective capsule, infiltrated by inflammatory cells.

Nevertheless, more researches with specific methodologies to prove other relevant properties of the PUr barrier can complement the obtained results of this work, searching for the explanation of the behavior and their effects in the different tissue types, when applied in reconstructive surgeries.

## CONCLUSIONS

The methodology applied and the results obtained in this study lead us to the conclusion that:

- the amount of new bone tissue formed was larger in PTFE and PUr than in C groups;
- there was better bone structural quality in PTFE and PUr than in C group and quality similarity among the experimental groups;
- the bone repair was faster in PTFE and PUr than in C group and there was not significant difference in the speed of the repair process among the groups PTFE and PUr;
- both barriers present homogeneous surface, regular and porosity absence;
- the PUr barrier presented biocompatibility, osteoconductor property and was not absorbed during the process of bone repair.

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## RESUMO

O propósito deste trabalho foi avaliar o processo de reparação óssea em defeitos cirúrgicos confeccionados em osso parietal de coelhos tratados pela técnica de regeneração óssea guiada, utilizando as barreiras de poliuretano (PUr) e de PTFE. As características de superfície das barreiras em microscopia eletrônica de varredura também foram avaliadas. Nesta pesquisa foram utilizados 24 coelhos adultos, sendo 12 animais do grupo controle (C) e 12 dos grupos experimentais (parietal direito - grupo PUr e parietal esquerdo - grupo PTFE). No grupo C, o defeito ósseo foi preenchido apenas por coágulo sangüíneo. Nos grupos experimentais, as barreiras de PUr e PTFE foram posicionadas no assoalho e na superfície da loja cirúrgica. Decorridos 15, 30, 60 e 90 dias, 3 animais de cada grupo foram sacrificados e as peças contendo os defeitos foram submetidas à análise microscópica. Os resultados obtidos possibilitaram concluir que não houve diferença significativa entre os grupos experimentais, demonstrando superioridade quantitativa e qualitativa do preenchimento ósseo, e reparação óssea mais rápida quando comparados com o grupo C. As barreiras físicas apresentaram superfície homogênea e ausência de porosidade. A barreira PUr apresentou biocompatibilidade, propriedade osteocondutora e não foi absorvida durante o processo de reparação óssea.

## UNITERMOS

Regeneração óssea guiada; membranas; poliuretanas; politetrafluoretileno.

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