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Radiographic evaluation of the effect of porcine submucosa membrane graft and bioactive glass on bone filling in rat calvaria

Avaliação radiográfica do efeito de membrana de submucosa suína e vidro bioativo no preenchimento ósseo em calvária de rato

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Abstract

Objectives: The aim of this study was to radiographically evaluate the filling of standardized bone defects in rat calvaria treated with bioactive glass and with porcine submucosa membrane, alone or in combination, when compared to defects filled with clot. **Materials and methods**: Eighty male Wistar rats, weighing 250 to 300 g, were divided into four groups, with trial period of two and eight weeks. Standardized bone wounds were surgically created in the calvaria measuring 6 mm in diameter and treated with bioactive glass, porcine submucosa membrane, combination of both or only clot. After two or eight weeks, the animals were euthanized and bone specimens were collected for evaluation with standard radiographs and digitized for analysis with ImageJ image analyzer to compare the percentage of filling of the defects by measuring the variations of levels of gray. Data were scored and analyzed statistically (ANOVA and Tukey HSD, $p < 0.05$). **Results**: The association of bioactive glass and submucosa membrane showed the best result of filling of bone cavities, followed by the single use of bioactive glass. Membrane alone showed similar results with the clot group. **Conclusion**: It was possible to conclude that the association of bioactive glass and porcine submucosa membrane could be good option for bone filling of bone defects.

Keywords: Bioactive glass. Porcine submucosa membrane. Bone repair.

Resumo

Objetivos*: O objetivo deste estudo foi avaliar radiograficamente o preenchimento de defeitos ósseos padronizados em calvária de ratos, tratados com vidro bioativo e com membrana de submucosa suína, isolados ou em associação, quando comparados com defeitos preenchidos com coágulo.* ***Materiais e métodos****: Oitenta ratos Wistar machos, pesando de 250 a 300 g, foram separados em quatro grupos, com períodos de avaliação de duas ou oito semanas. Defeitos ósseos padronizados foram cirurgicamente criados na calvária medindo 6 mm de diâmetro e tratados com vidro bioativo, membrana submucosa suína, combinação de ambos ou apenas com coágulo. Após duas ou oito semanas, os animais foram mortos e os espécimes ósseos foram coletados para avaliação com radiografias padronizadas e digitalizadas para análise com programa ImageJ, visando a comparar a porcentagem de preenchimento dos defeitos por meio da variação dos níveis de cinza. Os dados foram obtidos e avaliados estatisticamente (ANOVA e Tukey HSD, p < 0,05).* ***Resultados****: A associação do vidro bioativo com a membrana submucosa suína demonstrou os melhores resultados de preenchimento das cavidades ósseas, seguido pelo uso isolado do vidro bioativo. A membrana isolada demonstrou resultados semelhantes ao grupo coágulo.* ***Conclusão****: Foi possível concluir que a associação do vidro bioativo e da membrana submucosa suína pode ser uma boa opção para o preenchimento de defeitos ósseos.*

Keywords*: Vidro bioativo. Membrana submucosa suína. Reparo ósseo.*

Introduction

The indications of bone grafts are the reduction of probing depth in periodontal defects, gain of attachment level, bone defects filling and regeneration of bone, periodontal ligament and cementum and treatment of bone defects in edentulous areas (1). Bone replacement grafts have been extensively used in periodontal therapy and can be separated into three categories: autogenous, allogeneic, and synthetic (2). Although autogenous intraoral bone graft has shown very favorable results, potential pain after surgery and sites that are not readily available in adequate quantities may limit the use of this technique (3).

Allogeneic bone grafts have been tested in periodontal defects with controversial findings, with some investigations suggesting that allogeneic bone grafts do not have enough bone-growth inducing activity, failing to induce ectopic bone formation (4). Other disadvantages of allogeneic grafts are that wound healing around graft material is mediated by encapsulation of the graft particles (5) and that the patients may express serious concerns about the potential risk of disease transmission (6). All these disadvantages of the previously described grafts have turned the attention to alloplastic materials where some studies have shown significantly more clinical attachment gain when compared to open flap debridement (7, 8).

Other studies show the use of bioactive glass in areas of osseous repair (9) and in areas of immediate implant in extraction socket associated with the use of acellular dermal matrix graft with good results (10), but there are reports showing histological evidences of the healing being almost exclusively repaired with minimal or no regeneration (11, 12). The use of membranes as physical barriers helps the stability of the graft and the isolation of unwanted cells to heal in periodontal and osseous defects (13, 14). The membranes should contain the following characteristics or criteria for use: provide tissue integration, occlusive to cells, easy clinical management, create space between defect and the membrane, and biocompatibility (15).

The submucosa layer of the small intestine of pigs is a xenogenic membrane, acellular, biodegradable; after preparation it has an approximate thickness of 0.1 mm, and properties allowing its use as a biomaterial (16, 17). The porcine small intestinal submucosa does not trigger responses in antigen receptor and is capable to work as a scaffold to induce the repair of the native tissue in which it was deployed (18).

There is no report in the literature comparing the use of bioactive glass and porcine submucosa membrane in osseous replacement of osseous defects. The aim of this study was to evaluate bone filling in defects created in rat calvaria, using standardized radiograph.

Materials and methods

This study followed the ethical guidelines of research in animals submitted to and approved by the Ethics Committee on Animal Use (CEUA/PUCPR) under the protocol #255/07.

Eighty Wistar rats weighing approximately 250 g and divided into four groups of 20 each were used. The surgical protocol (19) included anesthesia performed by the combination of Xylazine 5 mg/kg (Rompun™, Bayer HealthCare, São Paulo, Brazil) intraperitoneally and ketamine hydrochloride 5 mg/kg (Vetaset™, FortDodgeSaude, Campinas, Brazil).

After anesthesia, a semilunar incision was made and a total flap was diplaced in the skin of the calvaria, exposing the bone of the skull. After that, a standardized circular incision of 6 mm was performed using a trephine drill (Nobelbiocare, Kloten, Switzerland) with abundant cooling (saline) coupled with a contra-angle dental handpiece with speed between 2.500-3.000 rpm. After careful removal of the incised portion of the calvaria with a molt curette, the bioactive glass (Perioglas™, Block Drug Co., Jersey City, USA) was applied in 20 animals, filling the bone cavity completely. In another group of 20 animals it was applied only porcine submucosa membrane, where the membrane covered bone trepanation completely, being placed beyond the borders of the lesion around 2 mm. In another group of 20 animals bioactive glass was used associated with membrane, following the sequence of the two previous groups. In the last group of animals (control group) no application of any material was made, only blood clot formation was observed on wound, thus serving as untreated controls.

In all animals, the flaps were sutured with single sutures with mononylon 4.0 (Jonhson and Jonhson, São José dos Campos, Brazil). The animals were euthanized with a lethal dose of anesthetic, and the evaluation period of two weeks (ten animals in each group treated and ten in the control group) and eight weeks (the same number of animals in the previous evaluation period).

After euthanasia, the bone of the skull was carefully removed and stripped of soft tissue, and the samples were placed in 10% formaldehyde for subsequent radiographic analysis. Radiographs were performed using X-ray Periapical film (Kodak Ektaspeed™, São Paulo, Brazil) with the beam direction perpendicular to the skull, with exposure time in 0.2 seconds in standard radiographic dental equipment.

After obtaining the radiographs, radiographic development was performed respecting the binomial time/temperature, and all radiographic films were processed simultaneously in the same development solutions. After that, the films were scanned with a resolution of 300dpi (Figure 1) for further evaluation in an image analyzer program ImageJ (Figure 2). The scanned images were evaluated according to the percentage of filling of the cavity treated, using an assessment area with the standard diameter for all samples, using as parameter the area around the bone defect as the absolute value of gray levels (100%) and comparing it with the gray levels measured by the program in the wounded area. The following the formula was used:

$$X\% = Z. 100 / Y$$

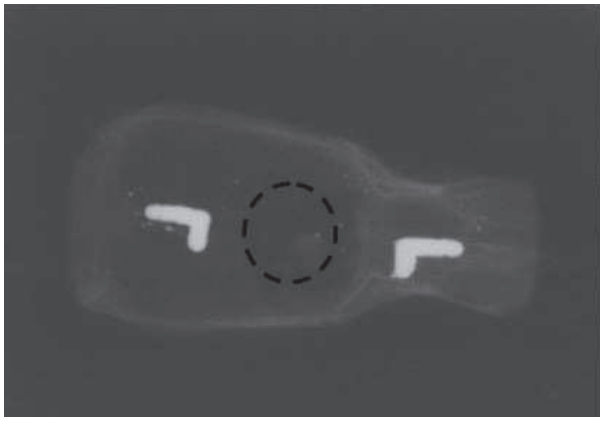


Figure 1 - Scanned periapical radiograph showing the wounded area (circle)

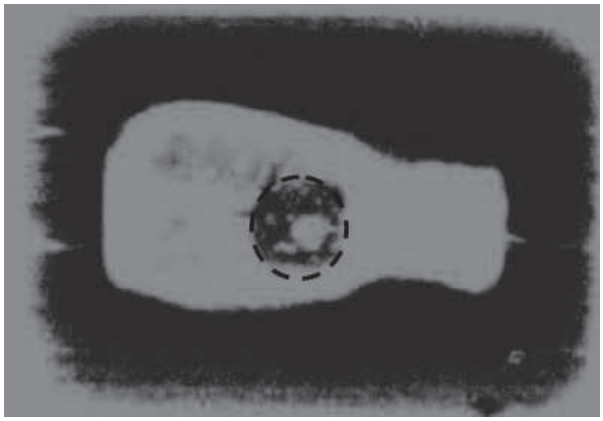


Figure 2 - Same scanned image analyzed by the program ImageJ, showing the wound partially filled (dashed circle)

Where X% is the percentage of completion of the wound area, Z is the value of gray levels measured by the program in the wound area, 100 is the total percentage and Y is the value of gray levels in the normal area adjacent to area of bone defect.

The measured results were tabulated and the mean and standard deviation were calculated for all groups and periods. After these data, statistical analysis was applied, comparing the existence of difference in the average percentage of filled according to groups.

It was initially tested the normality of the data for each group using the Kolmogorov-Smirnov ($p < 0.05$) and homogeneity of variance between groups using the Levene test. The results showed that only one group was not normally distributed, but there was homogeneity of variance between groups, so it was used ANOVA with a criterion of class to identify which groups differed. Since there was homogeneity of variance between groups, ANOVA accused difference in mean filling between the groups ($p < 0.05$), and the power of the test was greater than 98%, it was used then multiple comparison test (Tukey HSD) for parametric homogeneous variance, in order to identify which groups differed among others.

Results

The results demonstrated a strong indication of the highest percentages of filling in the groups containing bioactive glass (Perioglas™) and the association of Perioglas™ and porcine submucosa membrane (Table 1).

The submucosa group and the control group showed similar results, being lower than other groups. The highest percentage of filling were therefore observed with the combination of bioactive glass membrane and porcine submucosa with two and eight weeks (55,91% and 60,16%) with no statistical differences between the bioactive glass group within eight weeks (52,19%).

The bioactive glass used alone had significant difference in comparison to the control group and submucosa membrane alone at two or eight weeks ($p < 0.05$) but no significant difference was found in the membrane group with respect to the evaluation periods (two or eight weeks).

At eight weeks, the percentage of radiographic filling in the Perioglas™ group showed, statistically, the same levels that the group associated with bioactive glass membrane during periods of two and eight weeks ($p < 0.05$) (Graphic 1).

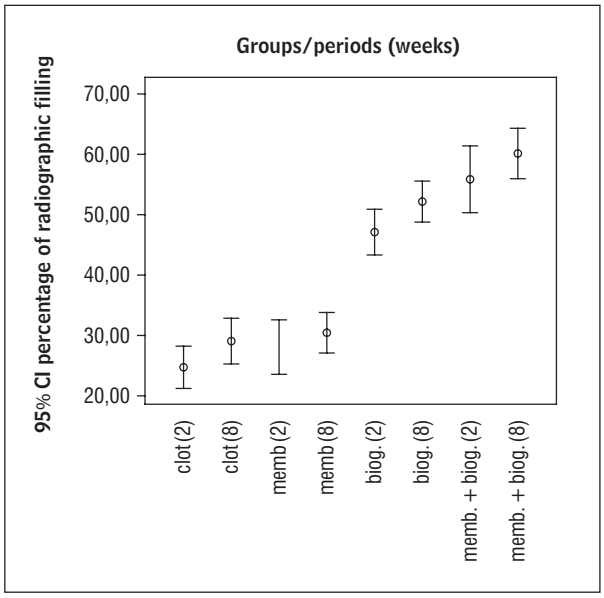
Discussion

The application of biomaterials in periodontics and implant dentistry has been a clinical reality. The use of synthetic materials for bone filling of periodontal defects and in areas of edentulous ridges

Table 1 - Average percentage of filling according to the groups (N = number of animal per group; $\dot{X}$ = average percentage of bone filling; SD = standard deviation)

Groups	N	$\dot{X}$	SD
Clot (2 weeks)	8	24,76	4,22
Clot (8 weeks)	8	29,12	4,50
Membrane (2 weeks)	9	28,11	5,88
Membrane (8 weeks)	10	30,46	4,72
Bioactive glass (2 weeks)	10	47,13	5,28
Bioactive glass (8 weeks)	8	52,19	4,10
Bioactive glass + membrane (2 weeks)	9	55,91	7,20
Bioactive glass + membrane (8 weeks)	8	60,16	5,02

Source: Research data.



Graphic 1 - Interval of confidence (95%) for the mean percentage of filling for groups and periods

Source: Research data.

have received special attention for its ease of acquisition and disposal of biological hazards and cross-infection. Among these biomaterials, bioactive glass is a choice that has filled several of the requirements in the regenerative area.

The bioactive glass helps to stabilize the clot, which acts as osteoconductive biomaterial in the process of osteogenesis (20), remaining as a template or scaffold for the replacement by new bone tissue after material absorption. According to Shapoff, Alexander, Clark (21), the resorption of this material occurs by ionic dissolution, where the replacement time varies with the amount and location of the material, but usually occurs over a period of three months to two years. In this study, it was associated the use of bioactive glass (Perioglas™) to a membrane derived from porcine submucosa, which has been used for the treatment of esophageal perforations in the medical area (18) but no description use for the purpose of physical barrier in periodontal or osseous defects.

One method to evaluate the effect of biomaterials in the treatment of periodontal defects is the use of radiographs to analyze the filling of bone defects (22). This method gives us a radiographic indication of bone filling of the defects, but in a subjective manner, without specific knowledge of the type of tissue that is formed in the affected area. It is only possible to demonstrate radiographically an increase in bone density by increasing radiopacity. This evaluation only suggests bone formation or the presence of mineralized tissue or graft. When possible, clinical and histological evaluations should be performed to confirm radiographic results.

It is possible to state that the objective of the present evaluation was achieved since it was found differences in the filling of osseous defects during bone healing assessed radiographically when using bioactive glass (Perioglas™), alone or in combination with intestinal submucosa of pigs, compared to untreated controls. The results showed bone filling in all treatment and periods. The highest percentage of bone filling was found in the group where it was associated bioactive glass and membrane (within two and eight weeks) and the group of bioactive glass used alone at eight weeks showed, statistically, no difference with the association group.

The explanation for the fact that in the early period of two weeks, the association group had already high percentage of bone filling might be due to the presence of the membrane that helped the stability of the bioactive glass and, consequently, of the initial stabilization of the wound.

The use of bioactive glass as a bone filler showed good results, being significantly higher than the isolated use of the submucosa membrane and blood clot (control).

The use of porcine submucosa membrane has no clinical application in Dentistry yet, but further studies are needed to evaluate the tissue response histologically, its biocompatibility with periodontal tissues, and its actual effect as a physical barrier in the process of bone and periodontal regeneration.

Conclusion

Within the limits of this study, it was possible to conclude that the use of bioactive glass associated with membrane derived from porcine submucosa showed the best results of bone filling analyzed radiographically, followed by the single use of bioactive glass, and that the membrane used alone showed similar results to the ones observed in the control group.

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