Biocompatibility analisys of Bioglass® 45S5 and Biosilicate® cone in rabbit eviscerated cavity

Análise da biocompatibilidade de cones de biovidro e biovitrocerâmico (Biosilicato®) em cavidade eviscerada de coelho

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ABSTRACT

Objective: *To evaluate bioglass, bioglassceramic biocompatibility in rabbit's eviscerated cavities.* ***Methods:*** *Forty- five rabbits were submitted to right eye evisceration, followed by the inclusion of bioglass and bioglassceramic I e II prosthesis in the escleral cavity. The animals were sacrificed at seven, 90 and 180 days after surgery. The animals had daily clinical exam; biochemical exam, histological analysis and morphometric evaluation.* ***Results:*** *The animals stayed healthy during the expriment, with good cone integration to the host tissue. None cone extrusion were observed. Histologically, it was observed pseudocapsule formation around the cones and the inflammatory reaction was higher at M1, getting progressively lower while getting at M3, being the lowest in rabbits which received bioglass cones (GA) than at any other groups.* ***Conclusion:*** *Bioglass and bioglassceramic I e II cones can be useful to repair anophthalmic cavity.*

Keywords: *Rabbits; Anophthalmic cavity; Biocompatible materials; Implants, experimental*

RESUMO

Objetivo: Avaliar experimentalmente a biocompatibilidade de cones de biovidro e biovitrocerâmico em cavidades evisceradas de coelhos. **Métodos:** Foram utilizados 45 coelhos albinos submetidos à cirurgia de evisceração do olho direito, seguida da inclusão de cones de biovidro e dois tipos de biovitrocerâmicos (chamados de FI e FII) na cavidade escleral. Os animais foram sacrificados em três momentos (7, 90 e 180 dias). Os parâmetros avaliados foram: peso, exame clínico diário, exames bioquímicos, avaliação histológica, exame morfométrico. **Resultados:** Os animais mantiveram-se saudáveis durante o experimento, não tendo ocorrido extrusão do implante em nenhum animal. O exame morfológico mostrou que houve a formação de pseudocápsula ao redor dos cones, com superioridade dos cones de biovidro e biovitrocerâmico FI, os quais apresentaram menor reação inflamatória e menor formação da pseudocápsula ao redor dos cones que os demais. A reação inflamatória foi mais intensa após 7 dias da colocação dos cones, diminuindo em direção aos 180 dias, sendo menos intensa nos coelhos que receberam cones de biovidro. **Conclusão:** Os cones de biovidro e biovitrocerâmico FI e FII podem ser úteis para a reparação da cavidade anoftálmica, com melhor resposta quando se usa cones de biovidro e de biovitrocerâmico FI.

Descritores: Coelhos; Cavidade anoftálmica; Materiais biocompatíveis; Implantes experimentais

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Introduction

Repairing the loss of volume that occurs after enucleation and evisceration is necessary to preserve the appearance of patients with anophthalmic sockets.[1,2] This is done through implants in the orbital cavity, with subsequent adaptation of external prostheses.[3]

The first implants were done in 1885 with glass spheres used to repair eviscerations and then enucleations.[4] Glass was the primary material used until the 1940s[5], when new materials were introduced such as polymethylmethacrylate (PMMA) and silicone, also called non-integrated implants.

At that time a different type of sphere was developed, made of a metallic material resembling a "sieve" whose anterior part was exposed, allowing integration with the host, as the host tissue could grow in its interior.[4] In the 1980s this concept re-emerged with the use of integrated spheres initially made of natural hydroxyapatite and then of synthetic hydroxyapatites and porous polyethylene.[6,7]

The risk of complications related to the material and the surgical technique[8], such as extrusion, wound dehiscence, mechanical trauma, and severe inflammation led to the study of new materials[9-11]. It can be safely said that no ideal material has yet been found for repairing the anophthalmic socket.

Bioglass was discovered in 1969, but it was only approved for use and registered as Bioglass™ in 1985, with the development of the third generation of this biomaterial, which then started to be used in many areas of medicine.[3,12-14] Thermal treatment can lead to the crystallisation[4] of a special type of glass based on 45S5 bioglass, thus forming the bioglass-ceramic composite *Biosilicato*™, which has been showing good results in animals and humans and has been used to replace ossicles in the human ear, fill the dental alveoli of dogs,[13-17] and as a polymer matrix to reconstruct the zygomatic complex in humans. [18] The use of this material in anophthalmic sockets needs to be validated.

The aim of this study was to use an animal model to verify whether 45S5 bioglass cones — bioglass-ceramic (Biosilicato™) with a crystalline phase called FI, and bioglass-ceramic with two crystalline phases called FII — can be used to repair anophthalmic sockets.

Methods

This study followed the ethical principles for animal experimentation adopted by the Research Ethics Committee of the Botucatu Medical School, UNESP, São Paulo, Brazil.

This was a blind, experimental, randomised phase II study that used 45 male Norfolk (albino) *Oryctolagus cuniculus* rabbits aged between three and six months. The groups differed by the type of biomaterial: 45S5 bioglass cones, Biosilicato™ FI cones, and Biosilicato™ FII cones. The researcher was unaware of the type of cone used in each rabbit until the end of the study, when the groups were revealed.

Fabrication of the cones used in the study: Cones with an anterior diameter of 10 mm, a posterior diameter of 3 mm and a length of 12 mm were made from a high-precision graphite mould in the Laboratory of Vitreous Materials (LAMAV) of the Federal University of São Carlos, SP. The cones were made of 45S5 bioglass as developed by Hench[19,20] and Biosilicato™ FI and FII produced from the same chemical composition based on Na_2O, CaO, SiO_2, and P_2O_5 — the only difference between the latter two was the thermal treatment to induce crystallisation. For Biosilicato™ FI cones the thermal treatment produced only the crystalline phase $1Na_2O.2CaO.3SiO_2$, while P_2O_5 remained as a solid solution. For Biosilicato™ FII cones, the thermal treatment cycle went beyond the $1Na_2O.2CaO.3SiO_2$ phase, allowing phosphorus ions to form a crystalline phase with calcium and yielding calcium phosphate, i.e. apatite (Figure 1). All cones were individually sterilised with ethylene oxide before use.

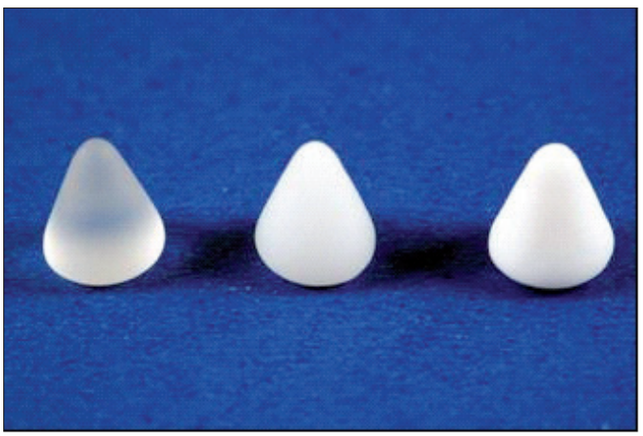

Figure 1. The three types of cones made of biomaterials: **A)** Bioglass; **B)** Biosilicato™ FI; **C)** Biosilicato™ FII

Experimental time points: The study was divided into a baseline and three observation time points called M0 (baseline), which included biochemical tests and surgery; M1, in which 15 animals were sacrificed 7 days after M0; M2, in which 15 animals were sacrificed 90 days after M0; and M3, in which 21 animals were sacrificed 180 days after M0. That is, five animals from each group were sacrificed at each experimental time point.

Study variables: Daily medical evaluation; assessment of potential systemic toxicity through biochemical tests to evaluate the hepatic function (ALT, AST, LDH, ALP), heart function (CPK), and renal function (urea, creatinine); morphological examination, with histological (haematoxylin and eosin [HE] at M1, M2, and M3) and morphometric evaluation of the pseudocapsule and the inflammatory reaction standardised at four positions — anterior, posterior, 3 o'clock, and 9 o'clock (using HE slides) —, and the amount of collagen in the pseudocapsule (using slides stained with picrosirius red).

Experimental steps: After a period of adaptation to the environment, the animals had a blood sample collected from the auricular vein for biochemical tests and were then anaesthetised with Zoletil™ at a dose of 15mg/kg. The rabbits' right eyes were eviscerated and the lost volume was replaced with one of the three different types of cones. Once the cones had remained in the orbital cavity for the expected time period, the animals were anaesthetised, had their blood collected again for biochemical tests, and were then immediately sacrificed with an overdose of intramuscular ketamine hydrochloride. The contents of the orbit were then removed and prepared for morphological examination with fixation in 10% formaldehyde, dehydration in an ascending series of alcohols, embedding in paraffin and staining with haematoxylin-eosin and picrosirius red for light microscopy. After fixation, the cones were removed from the scleral cover for preparation of the histological sections, as the biomaterial cannot be sectioned. Analysis was performed on the inner part of the scleral cover, where there was direct contact of the host sclera with the cone.

Statistical analysis: Biochemical and morphometric findings were entered into Excel spreadsheets and analysed statistically

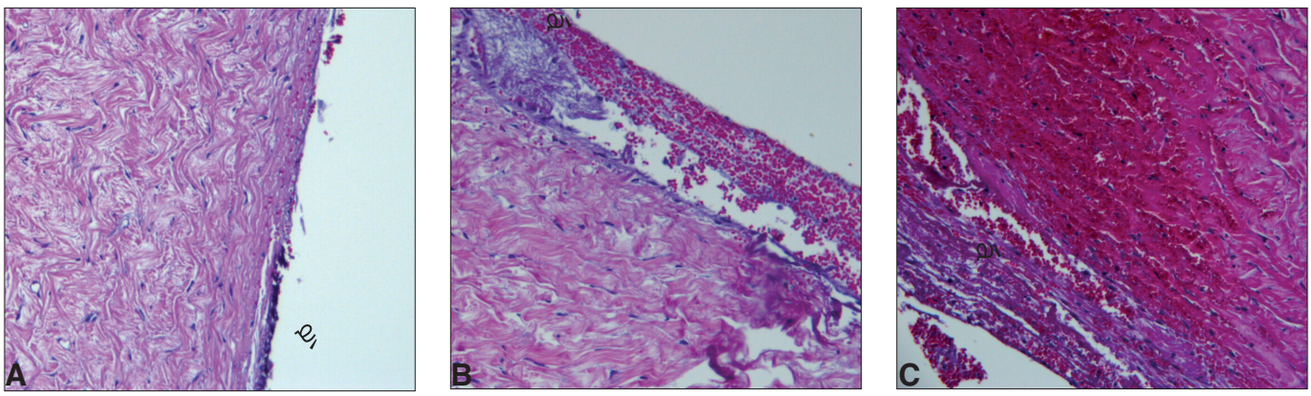

Figure 2. Histological sections showing tissue repair between the sclera and the cones at M1 (arrow). **A)** Bioglass. **B)** Biosilicato™ FI. **C)** Biosilicato™ FII. All sections show the formation of a pseudocapsule made of fibroblasts, red blood cells and inflammatory cells (HE 40X)

through Parametric and Non-Parametric Variance, median and maximum and minimum values or the mean and standard deviation, supplemented by Dunn's and Tukey's tests, according to the variable under study. A significance level of .05 was used.

Results

Medical examination: All animals remained well, feeding normally and gaining weight during the study. Daily observation of the orbital cavity showed no conjunctival hyperaemia, dehiscence, or cone extrusion.

Assessment of systemic toxicity: The animals showed no signs of systemic toxicity, as evidenced from the results of biochemical tests which remained within the normal range, even though statistically higher levels of ALT, alkaline phosphatase, LDH, and CPK were observed at the early stages of the experiment, compared to M3.

Morphology:

1) Histology: The same pattern of tissue reaction described below was observed in all three study groups. After seven days (M1), necrotic tissue and the formation of regenerative tissue were observed at the interface between the inner part of the sclera and the outer part of the studied material, including spindle-shaped cells with rounded nuclei consistent with the morphology of young fibroblasts, as well as red blood cells and inflammatory cells (primarily neutrophils) in a meshwork of fibrin and oedema, corresponding to acute inflammation. Small fragments of biomaterial as well as neovascularisation were observed within the tissue reaction. The sclera was swollen, especially in its anterior portion, in sites coinciding with the sclero-scleral suture. At this point it was already possible to observe the formation of a pseudocapsule made of fibroblasts and inflammatory cells arranged in a circular fashion and involving most of the inner portion of the sclera (Figure 2). After 90 days (M2), the number of inflammatory cells had decreased. The pseudocapsule was more dense and was made of fibroblasts containing smaller nuclei than those observed in M1; it was apparently thinner, with fewer red blood cells and less oedema. Neovascularisation with red blood cells was observed. After 180 days (M3) no oedema was observed and the inflammatory reaction was mild. Neovascularisation with red blood cells was observed. Fragments of bioglass or bioglass-ceramic, identified as areas of birefringence, were surrounded by reparative connective tissue without inflammatory reaction (Figure 3).

2) Morphometry: Although no statistically significant differences were observed for most comparisons between the bioglass, bioglass-ceramic FI, and bioglass-ceramic FII groups, the thickness of the pseudocapsule tended to be smaller in animals with bioglass and bioglass-ceramic FI cones in all measuring sites and especially in the anterior area (Table 1). The amount of collagen in the pseudocapsule was also similar in all three experimental groups, both at M1 and M3. Comparing the values within each group at M1 and M3, statistical differences were observed only in the bioglass-ceramic FI group, where the amount of collagen was higher at M1. With regard to cellularity, comparison between the time points within each group showed a significant reduction in the number of inflammatory cells from M1 to M2 to M3. Inflammation was also more intense for the bioglass-ceramic FII group at M1 compared to the other groups.

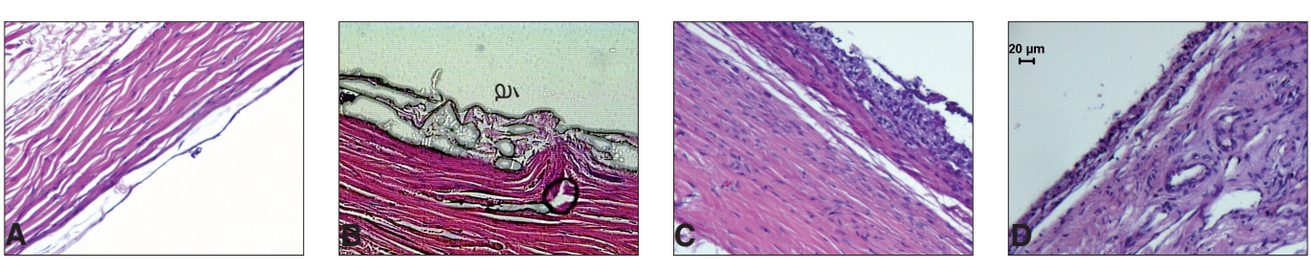

Figure 3. Histological sections showing tissue repair between the sclera and the cones at M3 (arrow). **A)** Bioglass. **B)** Biomaterial (arrow) surrounded by regenerative tissue **C)** Biosilicato™ FI. **D)** Biosilicato™ FII. Note the pseudocapsule made of fibroblasts, the absence of oedema and the mild inflammatory reaction (HE x 40)

Table 1

Median and maximum and minimum values for the thickness of the pseudocapsule formed between the sclera and the cones in rabbit eviscerated cavities according to the group, the experimental time point and the site examined

Group	Site	Time point		
		M1	M2	M3
A	Anterior	21,945 (12,397; 37,596)	10,833 (4,714; 25,710)	18,828 (11,209; 35,136)
	Posterior	20,230 (10,292; 23,320)	12,730 (7,004; 19,503)	15,652 (8,522; 19,007)
	3 o'clock	18,282 (13,444; 23,797)	10,222 (6,451; 20,041)	12,451 (10,735; 32,262)
	9 o'clock	25,206 (20,693; 51,633)*	14,481 (5,358; 19,485)	13,792 (10,537; 30,120)
B	Anterior	93,854 (23,634; 108,932)* # α	12,746 (5,533; 30,881)	14,948 (10,419; 21,911)
	Posterior	49,759 (18,125; 59,581)*	12,836 (7,036; 60,532)	17,456 (9,993; 44,070)
	3 o'clock	15,094 (11,974; 65,345) α	22,132 (10,312; 28,586)	19,446 (12,899; 73,155)
	9 o'clock	30,894 (18,155; 47,194)*	12,235 (9,134; 27,000)	16,236 (14,223; 31,315)
C	Anterior	47,138 (24,291; 128,852)	20,341 (10.090; 67,763)	73,796 (20,112; 126,212)#Δ
	Posterior	51,970 (34,444; 137,236)* Δ	19,397 (6,833; 47,915)	17,075 (12,158; 48,799)
	3 o'clock	35,644 (13,905; 51,501)	16,371 (5,147; 43,442)	23,530 (17,135; 37,503)
	9 o'clock	37,538 (13,861; 235,228)	17,343 (5,593; 40,870)	33,352 (10,059; 40,795) Δ

A: bioglass; B: Biosilicato™ FI; C: Biosilicato™ FII
* ($p < 0.05$) M1 x (M2,M3)
# ($p < 0.05$) Anterior x (Posterior, 3 o'clock, 9 o'clock)
Ä ($p < 0.05$) C x (A, B)
á ($p < 0.05$) B x A

Discussion

The medical examination and the weight of the animals showed that the procedures did not interfere with homeostasis, as the rabbits gained weight during the experiment and developed normally. For biochemical tests, the values at M0 were used as a baseline and were then compared to the values at the time of sacrifice. Most biochemical results were in agreement with those found by other researchers,[9,17,18,21,22] with only physiological variations without clinical significance, as the final values were lower than the initial ones.

Morphological analysis showed that tissue repair following cone implantation was of the reparative-cicatricial type, with an influx of fibroblasts, inflammatory cells and neovasculature, and formation of a pseudocapsule around the cones, starting with an early reparative process and then evolving into a chronic phase, with reduction of oedema and inflammatory cells. These changes characterise a healing process[23], with replacement of damaged intraocular tissue by connective tissue.[9,22,23]

The formation of a pseudocapsule was observed, and a reduction of its thickness was shown through morphometry, probably due to a reduction in oedema and in the number of inflammatory cells during the experiment. Comparison of the pseudocapsule's thickness with different biomaterials showed that the bioglass-ceramic FII group had a thicker pseudocapsule both 7 and 180 days after implantation, indicating that bioglass-ceramic FII caused a greater inflammatory reaction throughout the experiment. The thicker pseudocapsule at M1 in bioglass-ceramic FI and FII groups is probably due to bioreactivity, as cones submitted to crystallisation become more bioreactive and their inflammatory response occurs earlier than with bioglass cones.[24,25] Thus, values were higher at M1 and decreased towards M3, when no statistical difference in biocapsule thickness was found between of the bioglass and bioglass-ceramic FI groups.

However, differences in pseudocapsule thickness were visible to the naked eye in different areas. Therefore, we chose to evaluate the thickness of the pseudocapsule in four different regions: Anterior, posterior, three o'clock, and nine o'clock. Thus, it was possible to demonstrate significant variations in the thickness of the anterior pseudocapsule, possibly due to the more exacerbated inflammatory response around the surgical site and the suture site. Moreover, the anterior region is more exposed to environmental factors, with a higher risk of contamination.

Collagen assessment showed statistical differences within the bioglass-ceramic FI group between M1 and M2, but values were not high and were not very different from those seen in the bioglass and bioglass-ceramic FII groups. These observations show that the implanted cones did not cause an exacerbated inflammatory response and produced no abnormal healing or significant fibrosis at the site of contact between the cone and the host tissue.

Conclusion

The findings show that bioglass and bioglass-ceramic FI and FII cones, when implanted in rabbit eviscerated cavities, produce no signs of systemic or local toxicity. Morphological analysis pointed to the superiority of bioglass and bioglass-ceramic FI cones, which caused a milder inflammatory reaction and less pseudocapsule formation than bioglass-ceramic FII cones. Thus, we conclude that bioglass and bioglass-ceramic FI and FII cones can be useful to repair anophthalmic sockets, with bioglass and bioglass-ceramic FI cones leading to better results.

References

1. Migliori ME. Enucleation versus evisceration. Curr Opin Ophthalmol. 2002;13(5):298-302.
2. Perry JD, Lewis CD, Levine M. Evisceration after complete evaluation an acceptable option. Arch Ophthalmol. 2009;127(9):1227-8; author reply 1229. Comment on Arch Ophthalmol. 2009;127(2):141-5.
3. Su GW, Yen MT. Current trends in managing the anophthalmic socket after primary enucleation and evisceration. Ophthal Plast Reconstr Surg. 2004;20(4):274-80.
4. den Tonkelaar I, Henkes HE, van Leersum GK. Herman Snellen (1834-1908) and Müller's 'reform-auge'. A short history of the artificial eye. Doc Ophthalmol. 1991;77(4):349-54.
5. Perry AC. Integrated orbital implants. Adv Ophthalmic Plast Reconstr Surg. 1990;8:75-81. Review.
6. Blaydon SM, Shepler TR, Neuhaus RW, White WL, Shore JW. The porous polyethylene (Medpor) spherical orbital implant: a retrospective study of 136 cases. Ophthal Plast Reconstr Surg. 2003;19(5):364-71.
7. Perry JD, Goldberg RA, McCann JD, Shorr N, Engstrom R, Tong J. Bovine hydroxyapatite orbital implant: a preliminary report. Ophthal Plast Reconstr Surg. 2002;18(4):268-74.
8. Rodrigues AC, Schellini SA, Moraes-Silva MRB, Padovani CR. Fatores relacionados a extrusão do implante de cavidade. Rev Bras Oftlamol. 1997; 56:259-64.
9. Brito MM. Análise da biocompatibilidade da esfera de quitosana porosa em cavidade eviscerada de coelho. Estudo comparativo com polietileno poroso [Dissertation]. Botucatu: Faculdade de Medicina de Botucatu da Universidade Estadual Paulista; 2008.p.94.
10. França VP, Figueiredo ARP, Vasconcelos AC, Oréfice RL. Estudo comparativo experimental de compósito bioativo de matriz polimérica para aplicação em cirurgia plástica ocular na substituição tecidual. Arq Bras Oftalmol. 2005;68(4):425-31.
11. Karesh JW. Biomaterials in ophthalmic plastic and reconstructive surgery. Curr Opin Ophthalmol. 1998;9(5):66-74
12. Hench LL. The story of Bioglass. J Mater Sci Mater Med. 2006;17(11):967-78.
13. Amato MM, Blaydon SM, Scribbick FW Jr, Belden CJ, Shore JW, Neuhaus RW, et al. Use of bioglass for orbital volume augmentation in enophthalmos: a rabbit model (oryctolagus cuniculus). Ophthal Plast Reconstr Surg. 2003;19(6):455-65.
14. Greenlee TK Jr, Beckham CA, Crebo AR, Malmorg JC. Glass ceramic bone implants. J Biomed Mater Res. 1972;6(3):235-44.
15. Roriz VM. Avaliação clínica, histológica e histomorfométrica de alvéolos dentários de cães preenchidos com biovidro ou biosilicato®, que posteriormente receberam implantes ossointegráveis [Thesis]. Ribeirão Preto: Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo; 2006. p.104.
16. Tirapelli C. Avaliação da eficácia de um biomaterial e conhecidos agentes dessensibilizantes no tratamento da hipersensibilidade dentinária: estudo *in vitro* e *in vivo* [Thesis]. Ribeirão Preto: Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo; 2007.p. 96.
17. Massuda ET. Avaliação do biosilicato como prótese de orelha média [Thesis]. Ribeirão Preto: Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo; 2007. p. 62.
18. Turrer CL, Figueiredo ARP, Oréfice RL, Maciel PE, Silveira MES, Gonçalves SP, Barbi JSF. O uso de implantes de compósito bioativo de biocerâmica em matriz polimérica na reconstrução do complexo zigomático orbitário: novas perspectivas em biomateriais. Arq Bras Oftalmol. 2008;71(2):153-61.
19. Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc. 1991;74(7):1487-510.
20. Hench LL, West JK. Biological applications of bioactive glasses. Life Chem Rep. 1996; 13:187-241.
21. Turner PV, Baar M, Olfert ED. Laboratory animal medicine - needs and opportunities for Canadian veterinarians. Can Vet J. 2009;50(3):257-60.
22. Yamada S, Ito T, Tamura T, Shiomi M. Age-related changes in serum/plasma biochemical parameters of WHHLMI rabbits. Exp Anim. 2004;53(2):159-63.
23. Cotran RS, Kumar V, Robbins SL, Shoen FJ. Robbins pathologic basis of disease. 5th ed. Philadelphia: Saunders; c1994. p. 45-83.
24. Anderson RL, Yen MT, Lucci LM, Caruso RT. The quasi-integrated porous polyethylene orbital implant. Ophthal Plast Reconstr Surg. 2002;18(1):50-5.
25. Ferraz LC, Schellini SA, Wludarski SL, Padovani CR. **Implantes de polietileno gel e poroso em cavidade anoftálmica de coelhos**. Arq Bras Oftalmol. 2006;69(3):305-8.

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