



BIOCOMPATIBILITY ANALYSIS OF BIOGLASS® 45S5 AND BIOSILICATE® IMPLANTS IN THE RABBIT EVISCERATED SOCKET

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Abstract

PURPOSE Bioactive glass and bioactive glass-ceramic cone implants were placed in the rabbit eviscerated socket to assess the biocompatibility.

METHODS Fifty-one Norfolk albino rabbits underwent evisceration of the right eye followed by implantation of cones made from Bioglass® 45S5 (control group) and two types of bioactive glass-ceramic (Biosilicate®), a single- and a two-phase bioactive glass-ceramic implants into the scleral cavity. Postoperative reactions, animal behavior and socket conditions were monitored daily. Clinical exam, biochemical evaluations and orbit CT scan were done at 7, 90, and 180 days post-procedure. After that, the animals were euthanized and the orbital content was removed and prepared to light microscopy with morphometric evaluation and scanning electron microscopy examination. Statistical analysis was done by parametric and non-parametric analysis of variance, complemented by Dunn's and Tukey's tests ($p < 0.05$).

RESULTS All animals remained healthy throughout the experimental period, without orbit infection, implant migration nor extrusion. Morphological analysis demonstrated pseudocapsule around all implants, and suggested superiority of Bioglass® and single-phase Biosilicate® implants, which induced less inflammation and pseudocapsule formation than two-phase Biosilicate® cones. The inflammatory reaction was most intense 7 days post-procedure, gradually decreased throughout the experiment, and was least intense in animals receiving Bioglass® implants. No instances of implant migration or changes in the orbital cavity were detected.

CONCLUSIONS Bioglass® and single- and two-phase Biosilicate® (bioactive glass-ceramic) implants may prove useful in management of the anophthalmic socket. We observe discrete

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3 differences between the studied materials point out best responses obtained with use of
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5 Bioglass® 45S5 and single-phase Biosilicate®.
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8 **Key words** Anophthalmic socket, bioactive glass, glass-ceramic, biocompatibility, rabbit,
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10 experimental study.
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15 **Introduction** The volume loss that follows enucleation and evisceration of the eye must be
16 repaired.^{1,2} This is initially accomplished with placement of implants into the orbital cavity,
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18 allowing later placement of external prostheses.³
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23 The first implants were introduced in 1885, and were non- integrated glass spheres,
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25 initially used after evisceration and then after enucleation.⁴ Glass was the most common implant
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27 material until the 1940s,⁵ when newer materials, such as poly(methyl methacrylate) (PMMA) and
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29 silicone (also known as non-integrated implants) started becoming available. At the time, a novel
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31 spherical implant was introduced, with a sieve-like metallic structure that was exposed in the
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33 anterior portion and allowed gradual integration with the host, as orbital tissue was able to grow
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35 within the implant.⁴ Interest in this concept was rekindled in the 1980s, leading to the
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37 introduction of integrated sphere implants, first made from natural hydroxyapatite, and then from
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39 synthetic hydroxyapatite and porous polyethylene.^{6,7}
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44 The risk of complications associated with choice of material and operative technique,⁸
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46 such as implant extrusion, suture dehiscence, mechanical trauma, and exacerbated inflammation,
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48 has prompted investigation of novel materials.^{3,9-11}
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51 Bioactive glass was discovered in 1969, but was only approved for medical use in 1985,
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53 with introduction of the third generation of this material, which was sold under the trade name
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55 Bioglass® and has since gained use in several biomedical fields.^{12,13} Thermal treatment can lead
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3 to the crystallization¹⁴ of a special glass based on Bioglass 45S5, forming a bioactive glass-
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5 ceramic sold as *Biosilicate*[®], which has shown encouraging results in animal and human studies
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8 and has already been used in ossicular replacement prostheses (in humans), as a filling material
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10 for tooth sockets in canine animal models,¹⁵⁻¹⁷ and as a polymer matrix for orbitozygomatic
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12 complex reconstruction in humans.¹⁸
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15 The ceramic promotes tissue reaction and integration to the host tissue which is not
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17 observed with the inert materials like the glass. However it is necessary to evaluate if the implant
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19 induces inflammation or other complications to show if they would be a good option to be used
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21 in anophthalmic cavity repair.
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24 The purpose of this study was to ascertain, using an animal model, whether cones
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26 made from Bioglass 45S5, *Biosilicate*[®] glass-ceramic containing a single crystalline phase
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28 (henceforth, “*Biosilicate* 1P”), and *Biosilicate*[®] containing two crystalline phases (“*Biosilicate*
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30 2P”) can be useful materials for management of the anophthalmic socket.
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36 **Material and Methods** An experimental study was conducted in accordance with the
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38 ethical principles for animal experimentation adopted by the Faculdade de Medicina de Botucatu
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40 (UNESP - São Paulo) Research Ethics Committee. The study was designed as a blinded,
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42 (UNESP - São Paulo) Research Ethics Committee. The study was designed as a blinded,
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44 randomized, phase II trial, and used 51 male albino Norfolk rabbits (*Oryctolagus cuniculus*) aged
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46 three to six months. Animals were divided into three groups, each of which would receive cone
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48 implants made from a different material: Bioglass 45S5, *Biosilicate*[®] 1F, and *Biosilicate*[®] 2F. The
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50 investigator was unaware of the type of material used until unblinding at the end of the study.
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53 **DESCRIPTION OF IMPLANTS USED IN STUDY** Cones with an anterior diameter of 10
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55 mm, posterior diameter of 3 mm, and 12 mm in length were manufactured from precision
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3 graphite molds in the Universidade Federal de São Carlos Glass Materials Laboratory
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5 (Laboratório de Materiais Vítreos, LAMAV, São Carlos, Brazil). Cones were manufactured from
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7 original Bioglass 45S5, as developed by Hench,¹² and Biosilicate 1P and 2P, both having the
8
9 same chemical composition (Na_2O , CaO , SiO_2 , P_2O_5) and differing only in thermal treatment.
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11 Biosilicate 1P cones were treated so as to contain only one crystalline phase, 1 Na_2O 2 CaO 3
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13 SiO_2 , with P_2O_5 remaining in solid solution. In addition to 1 Na_2O 2 CaO 3 SiO_2 , the thermal
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15 treatment cycle chosen for Biosilicate 2P cones allowed phosphate ions to form a crystalline
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17 phase with calcium, creating a calcium phosphate—that is, an apatite (Figure 1). All cones were
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19 individually sterilized in ethylene oxide prior to use.
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25 **EXPERIMENT STAGES** The experiment was divided into three stages of observation: the
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27 baseline stage or stage (S0), in which biochemical testing and the surgical procedure were
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29 performed; S1, in which 15 animals were euthanized 7 days post S0; S2, in which 15 other
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31 animals were euthanized 90 days post S0; and S3, in which 21 animals were euthanized 180 days
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33 post S0. Five animals from each group were thus euthanized at each experimental stage of the
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35 study, except for S3, when each group comprised seven animals.
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40 **STUDY VARIABLES** The variables studied include daily clinical assessment of all animals;
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42 assessment of potential systemic toxicity by monitoring of biochemical parameters, including
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44 liver function tests (AST, ALT, LDH, FA), cardiac enzymes (CPK), and kidney function (BUN,
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46 creatinine); CT assessment of the cone implanted and its association with the socket at 45 days
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48 post S0; morphological and histological examination (H&E stain) at stages S1, S2, and S3;
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50 morphometric examination of the implant pseudocapsule and inflammatory reaction on H&E
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52 sections at four standard positions (anterior, posterior, 3 o'clock, 9 o'clock) and quantitation of
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54 pseudocapsule collagen content on Picrosirius red-stained sections; and ultrastructural
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56 examination by scanning electron microscopy (in two animals from each group, at stage S3).
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3 EXPERIMENT PROCEDURE After animals had adapted to the laboratory environment, blood
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5 was collected from the auricular vein for biochemical testing. Animals were then anesthetized
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7 with tiletamine/zolazepam (Zoletil[®] 50, IM: Virbac, France), 15 mg/kg, and underwent
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9 evisceration of the right eye with immediate volume replacement with one type of cone. The
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11 animals were daily observed. After the scheduled number of days, animals were anesthetized
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13 once more, blood was collected for biochemical testing, CT scan was done and euthanasia was
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15 immediately induced with an overdose of ketamine hydrochloride IM (Dopalen- Vetbrands- São
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17 Paulo/ Brasil). Orbital contents were then removed and prepared for morphological evaluation,
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19 followed by fixation in 10% formalin, dehydration in ascending alcohol solutions, inclusion in
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21 paraffin, and hematoxylin–eosin (H&E) and Picrosirius red staining in preparation of light
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23 microscopy. Samples chosen for scanning electron microscopy were fixated in 2.5%
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25 glutaraldehyde and coated in gold. After fixation, cones were separated from the scleral shell for
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27 sectioning. Analysis was performed on the outer aspect of the cone, the site of direct interaction
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29 between the host sclera and the cone implant.
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36 STATISTICAL ANALYSIS Results of biochemical testing and morphometric analysis were
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38 entered into Microsoft Excel[®] spreadsheets and subjected to statistical analysis by parametric
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40 and non-parametric analysis of variance (ANOVA), with calculation of medians, maximum and
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42 minimum values, means and standard deviations, complemented by post-hoc testing (Dunn's and
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44 Tukey's tests) depending on the variable. The level of significance was set at $P < 0.05$.
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Results

CLINICAL ASSESSMENT All animals had an uneventful clinical course, with normal feeding and weight gain throughout the study. Daily examination of the orbital socket showed no conjunctival hyperemia, dehiscence, or implant extrusion.

ASSESSMENT OF SYSTEMIC TOXICITY No animals showed signs of systemic toxicity as assessed by monitoring of biochemical parameters, which were within normal limits throughout the study. Statistically significant increases in ALT, alkaline phosphatase, LDH, and CPK were detected early in the experiment as compared to S3 measurements with no biological effects.

CT ASSESSMENT CT slices of the orbital cavity showed that symmetry between the eviscerated and contralateral orbit was preserved in all animals. No bone or soft tissue changes were detected. Implants were present in all animals, and no fluid collections, edema, signs of inflammation, or other demonstrable changes were present (Figure 2). Also there were no volumetric changes or migration of the orbital implants on CT assessment in all examined animals.

MORPHOLOGICAL ASSESSMENT The tissue response pattern in all three groups are described together because they had similarities and the differences between them will be pointed out.

1. **Light microscopy:** Seven days post-procedure (S1), the interface between the outer surface of the implant material and the inner scleral surface showed necrotic tissue and some regeneration

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3 underway, with spindle-shaped cells bearing rounded nuclei (morphologically consistent with
4 young fibroblasts), as well as erythrocytes, inflammatory cells (particularly neutrophils)
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6 enmeshed within fibrin, and edema—all features consistent with acute inflammatory reaction.
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8 Minute fragments of the biomaterial and some neovascularization were found amid the tissue
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10 reaction. The remained sclera tissue was edematous, particularly in the anterior portion, of the
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12 cavity. At this point, formation of a pseudocapsule was already detectable, as shown by a buildup
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14 of fibroblasts and inflammatory cells, arranged in a circular pattern, enveloping most of the outer
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16 portion of the cone implant (Figure 3). After 90 days (S2), inflammatory cells had decreased in
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18 number. The pseudocapsule was denser, and its fibroblasts had smaller nuclei than those
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20 observed at S1; it also appeared thinner, and had fewer erythrocytes and less tissue edema.
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22 Neovessels containing erythrocyte were observed. At the 180-day mark (S3), there was no
23
24 demonstrable edema and little inflammatory reaction. Bioglass or glass-ceramic fragments, seen
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26 as birefringent areas, were enveloped by connective tissue; no inflammatory reaction was present
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28 (Figure 4). Regarding fibrovascular ingrowth, all the studied cones (Bioglass and Biosilicate) did
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30 not developed it, since pores where present just in the implants surface.
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38 **2. Morphometric assessment:** although no statistically significant differences between the
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40 Bioglass, 1P and 2P Biosilicate groups for most parameters, pseudocapsule thickness values were
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42 lower in animals that received Bioglass and Biosilicate 1P at all measuring sites, and at the anterior
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44 sector in particular. Quantitation of collagen in the pseudocapsule also yielded similar results for all
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46 three groups, both at S1 and at S3. Between-group comparison of parameters at S1 and S3 showed a
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48 statistically significant difference in the Biosilicate 1P group alone: in this group, collagen was more
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50 abundant in the first stage of the experiment (S1). Comparison of cell reaction by stage within each
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52 group showed a significant reduction in the number of inflammatory cells from stage S1 to stages S2
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3 and S3. Furthermore, inflammation was most intense in the Biosilicate 2P group at stage S1 as
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5 compared with other groups (Table 1,2).
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8 **3. Ultrastructural examination:** irregular, geometric-like “cracks” were found to have
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10 developed on the biomaterial mainly in the Biosilicate cones. Implant surfaces were rough, and a
11
12 clear fracture was visible separating a narrow, rougher, superficial layer from a deeper, smoother
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14 layer. The amount of fibrosis and inflammatory cells overlying the implant was minor,
15
16 particularly so in Bioglass samples, some of which showed areas with no overlying inflammatory
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18 reaction. In other words, the tissue reaction was stronger in the Biosilicate implants and
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20 comparing both, the Biosilicate 2P had more cells covering the cone surface than the Biosilicate
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22 1P (Figure 5).
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29 **Discussion** Uneventful clinical progression and weight gain after implantation show that the
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31 study materials did not interfere with homeostasis in the study animals, which gained weight and
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33 developed normally throughout the experiment. For biochemical testing purposes, stage zero (S0)
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35 was used as a baseline for later comparison of biochemical parameters measured at the time of
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37 euthanasia. The results of most biochemical tests performed were consistent with those previously
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39 reported by other investigators,^{9,19,20} with minor physiological variations of no clinical or biological
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41 significance, as terminal test values were decreased in comparison with baseline measurements.
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46 Morphological assessment showed that tissue repair after cone placement into the
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48 anophthalmic socket was of the connective tissue (scarring) type, with inflow of fibroblasts and
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50 inflammatory cells, neovascularization, and formation of a pseudocapsule around the implant,
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52 beginning as an acute inflammatory process and progressing to chronic healing, with attendant
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3 reduction in edema and inflammatory cell counts. These changes are indicative of wound
4 healing²¹ with replacement of injured intraocular tissues by connective tissue.^{9,22,23}
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8 Pseudocapsule thickness gradually decreased on morphometric assessment, probably due
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10 to resolution of edema and reduction in inflammatory cell counts over the course of the
11 experiment. Comparison of pseudocapsule thickness according to implant material showed
12 increased thickening in the Biosilicate 2P group at 7 and 180 days post-implantation, indicative of an
13 increased inflammatory response to Biosilicate 2P throughout the experiment. Increased stage 1
14 pseudocapsule thickness in the Biosilicate 1P and 2P groups was probably due to bioreactivity, as
15 implants that underwent crystallization are more bioreactive than Bioglass cones and produce an
16 earlier inflammatory response.^{24,25} Higher values were thus found in stage S1, decreasing
17 gradually towards S3, at which point pseudocapsule thickness was statistically identical in the
18 Bioglass and Biosilicate 1P groups.
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31 Varying pseudocapsule thickness was readily visible to the naked observer eye. The
32 decision was therefore made to assess pseudocapsule thickness at four different regions: anterior,
33 posterior, 3 o'clock, and 9 o'clock. Pseudocapsule thickness varied significantly in the anterior
34 region, possibly due to more intense inflammatory response at the surgical site and suture points.
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36 Furthermore, the anterior region is most exposed to environmental factors and is therefore at a
37 higher risk of contamination.
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45 Collagen quantitation showed a significant difference in the Biosilicate 1P group between
46 S1 and S2, but overall values were not very high and not much different from those detected in
47 the Bioglass and Biosilicate 2P groups. These findings show that, regardless of material,
48 implanted cones did not produce an exacerbated inflammatory response, nor were they associated
49 with abnormal scar tissue formation or major fibrosis at the implant–host tissue interface.
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3 Phenomena observed at the ultrastructural level may be explained by the physical and
4 chemical changes in biomaterial surface brought about by contact with living tissue, with
5 formation of a double layer composed of silica (SiO₂)-containing gel and hydroxy-carbonate
6 apatite (HCA).²⁶ Glutaraldehyde fixation highlighted this bilayer due to cracking of the
7 specimens, which rendered it detectable on visual analysis. Furthermore, due to repolymerization
8 and formation of pores on the surface, implant–host integration was readily apparent. Less
9 intense formation of an inflammatory response layer on Bioglass implants than on Biosilicate 1P
10 and 2P cones was also presumed, as the implant surface was visible on Bioglass cones but not on
11 Biosilicate 1P and 2P implants, likely due to a greater buildup of inflammatory cells over the
12 implant in the latter two groups and more intense in the Biosilicate 2P cones . These findings
13 confirm light microscopy evidence and suggest that Bioglass induces less inflammatory reaction
14 than the other two materials tested and might be considered superior to them in this particular
15 point.
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34 It bears stressing that, thus far, bonding of soft tissue to synthetic materials has only been
35 reported with Bioglass 45S5 in the literature. This Bioglass formulation is therefore categorized
36 as a Class A bioactive glass, as it is able to bind with bone and soft tissues alike. All other known
37 bioactive ceramic materials are Class B, that is, capable of binding with bony tissue alone. The
38 bioactive glass-ceramic employed in the present study, however, showed the same ability to bind
39 with soft tissue as Bioglass. This is the first study to report such performance for a bioactive
40 glass-ceramic material. The Class A/Class B disparity may account for prior unsuccessful
41 attempts at using hydroxyapatite implants in the anophthalmic socket, which are associated with
42 high extrusion rates.^{24,25}
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3 CT scanning showed proper implant positioning within the orbit, absence of fluid
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5 collections or inflammatory processes, no implant migration or extrusion and symmetry between
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7 the eviscerated orbit and its contralateral counterpart.
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10 Our finding show that Bioglass and Biosilicate 1P and 2P cones produce no signs of
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12 systemic or local inflammation after implantation into the rabbit anophthalmic socket.
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14 Morphological analysis suggests discrete superiority of Bioglass and Biosilicate 1P implants,
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16 both of which were associated with less inflammation and decreased pseudocapsule formation as
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18 compared with Biosilicate 2P implants. Bioactive glass-ceramic implants proved capable of
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20 bonding to soft tissues, a well-established property of Bioglass. CT examination showed no
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22 implant migration, fluid collections, or inflammation surrounding the implant in any of the three
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24 experimental groups.
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29 We therefore conclude that bioactive glass and single- and double-phase bioactive glass-
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31 ceramic implants can be useful materials for management of the anophthalmic socket. Best
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33 responses were obtained with the bioactive glass and single-phase bioactive glass-ceramic
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35 implants, which may be considered equivalent.
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44
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Table 1. Median, maximum, and minimum thickness of the pseudocapsule formed between the sclera and cone implants according to group, stage of observation, and region (site) examined.

Group	Region	Stage of observation		
		S1	S2	S3
A	Anterior	21,9 (12,4; 37,6)	10,8 (4,7; 25,7)	18,8 (11,2; 35,1)
	Posterior	20,2 (10,3; 23,3)	12,7 (7,0; 19,5)	15,6 (8,5; 19,0)
	3 o'clock	18,3 (13,4; 23,8)	10,2 (6,4; 20,0)	12,4 (10,7; 32,3)
	9 o'clock	25,2 (20,7; 51,6)*	14,5 (5,4; 19,5)	13,8 (10,5; 30,1)
B	Anterior	93,8 (23,6; 108,9)* ^{#α}	12,7 (5,5; 30,9)	14,9 (10,4; 21,9)
	Posterior	49,8 (18,1; 59,6)*	12,8 (7,0; 60,5)	17,5 (10,0; 44,1)
	3 o'clock	15,1 (12,0; 65,3) ^α	22,1 (10,3; 28,6)	19,4 (12,9; 73,1)
	9 o'clock	30,9 (18,1; 47,2)*	12,2 (9,1; 27,0)	16,2 (14,2; 31,3)
C	Anterior	47,1 (24,3; 128,8)	20,3 (10,1; 67,8)	73,8 (20,1; 126,2) ^{#Δ}
	Posterior	52,0 (34,4; 137,2)* ^Δ	19,4 (6,8; 47,9)	17,1 (12,2; 48,8)
	3 o'clock	35,6 (13,9; 51,5)	16,4 (5,1; 43,4)	23,5 (17,1; 37,5)
	9 o'clock	37,5 (13,9; 235,2)	17,3 (5,6; 40,9)	33,3 (10,1; 40,8) ^Δ

*($P < 0.05$) M1 x (M2, M3); #($P < 0.05$) Anterior x (Posterior, 3 o'clock, 9 o'clock);

α ($P < 0.05$) C x (A, B); Δ ($P < 0.05$) B x A

Table 2. Median, maximum, and minimum cell reaction found in the regeneration tissue formed between the sclera and cone implants according to group and stage of observation.

Group	Stage		
	S1	S2	S3
A	2+ (1; 2)	1+ (1; 3)	1+ (1; 2)
B	2+ (2; 2)	2+ (1; 3)	1,5+ (1; 2)
C	3+ (3;3) ^{a b}	2+ (1; 2)	2+ (1; 2)

*(P<0.05) C x (A,B); #(P<0.05) M1 x (M2, M3)

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Fig. 1.Implants used in the study. A) Bioactive glass (Bioglass); B) Single-phase bioactive glass ceramic (Biosilicate 1P); C) Double-phase bioactive glass ceramic (Biosilicate 2P). 84x67mm (300 x 300 DPI)

View Only

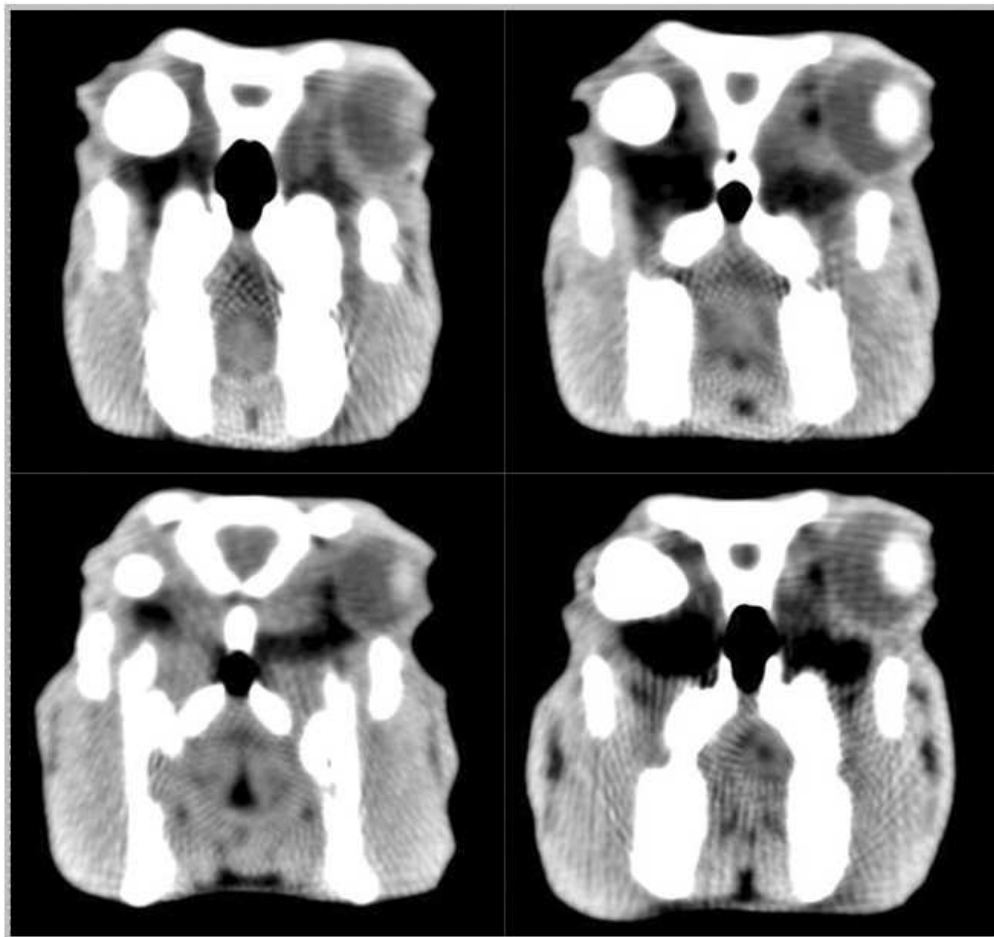


Fig. 2. Rabbit head CT slices showing Bioglass implant in the right orbit and eye in the left orbit.
55x52mm (300 x 300 DPI)

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Fig. 3. Light microscopy sections showing tissue repair reaction at the outer implant interface (arrow), stage 1 (S1) . A) Bioglass. B) Biosilicate 1P. C) Biosilicate 2P. A pseudocapsule made up of fibroblasts, erythrocytes, and inflammatory cells is visible in all sections (H&E, X40).
145x44mm (300 x 300 DPI)

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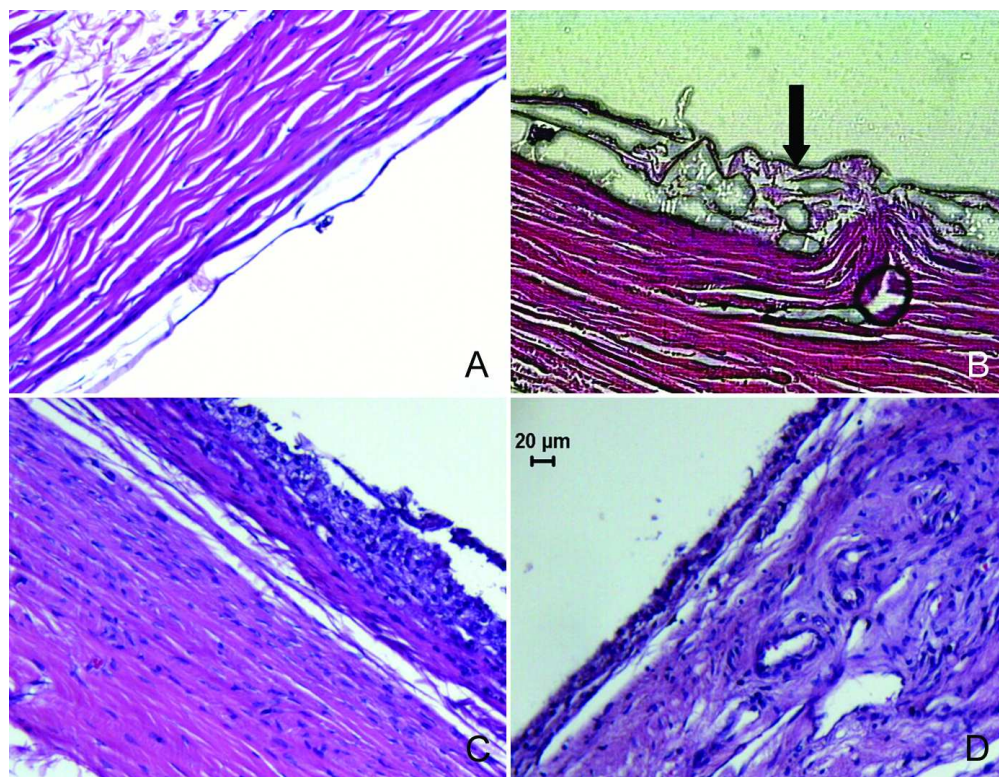


Fig. 4. Light microscopy sections showing tissue repair reaction at the sclera-implant interface, stage 3 (S3). A) Bioglass. B) Biomaterial (arrow) surrounded by host repair tissue. C) Biosilicate 1P. D) Biosilicate 2P. Pseudocapsule is visible; edema is absent and inflammatory reaction is mild (H&E, X40).

117x90mm (300 x 300 DPI)

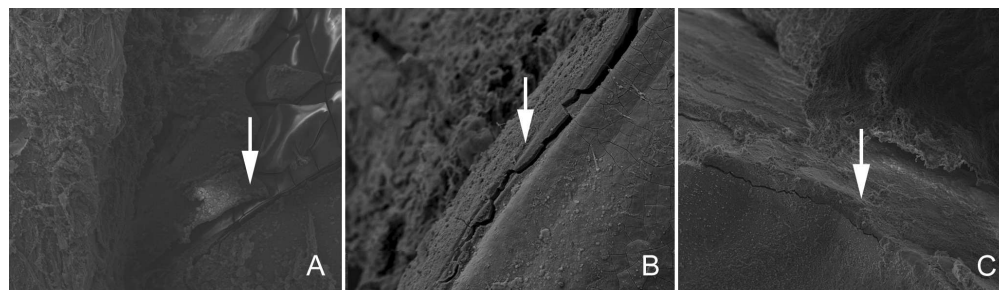


Fig. 5. Scanning ultrastructure image showing implants in the following groups: A) Bioglass. B) Biosilicate 1P. C) Biosilicate 2P. Superficial and deep layers are visible, with several geometric-shaped "cracks" (arrow) (SEM, X450).

173x50mm (300 x 300 DPI)

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