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Original Article

ß-tricalcium Phosphate Granules as an Alternative Material for Ocular Implantation

M. Eghtedari, E. Sh. Kazemzadeh[1]

Abstract

Background: Some types of implants used for orbital implantation have side effects and complications. In this study we have evaluated beta-tricalcium phosphate (ß-TCP: ChronOS) granules as an orbital implant.

Methods: Eighteen rabbits randomly allocated to four groups. Right eye enucleation performed in groups I to III followed by filling with autologous bone particles (n=5), a mixture of autologous bone particles and pure ß-TCP granules (n=5), a mixture of autologous bone marrow and pure ß-TCP granules (n=5). Right eye evisceration was done in group IV (n=3), and then filled with one of the aforementioned materials used in groups I, II and III. After 12 weeks, exenterated contents were compared by determining the volumes of the globes, area densitometry using dual-energy x-ray absorptiometry.

Results: There was no significant difference between the volumes of enucleated globes before (groups I: 2.6±0.39, II: 2.6±0.12 and III: 2.5±0.3 (ml) and after surgery; (groups I: 2.5±0.4, II: 2.50±0.2 and III: 2.4±0.3). There was also no significant differences among the densities of exenterated contents of all groups; (I: 175±3, II: 165±1 and III: 174±3 mg/cm^2). In all enucleated groups, histopathologic evaluations showed remarkable vascularization and fibrous ingrowth which were remarkable in eviscerated group. No significant complication was observed.

Conclusion: ß-TCP granules offer good cosmetic results with low risk of infection and extrusion. However, further studies are required before they can be used in human as a new orbital implant.
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Keywords ● ß-tricalcium Phosphate ● Enucleation ● Ocular implants

Departments of Ophthalmology, Khalili Hospital and [1]Neurosurgery, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence:
Ehsan Sherafat Kazemzadeh MD, Department of Neurosurgery, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel/Fax: +98 711 6279373
Email: sherafate@sums.ac.ir

Introduction

Evisceration and enucleation are usually recommended for a variety of diseases such as severe traumatized eyes, ocular malignancies, blind painful or disfigured eyes, or end-stage diseased eyes.[1] Several types of implants are being used as a substitute of enucleated and eviscerate globes. Orbital implants have evolved three general phases including the buried, the exposed integrated, and now the buried integrated.[2]

Porous implants such as porous hydroxyapatite, aluminum oxide, or polyethylene implants allow vascularization and the placement of pegs,[3] leading to a better ocular motility. These properties of porous materials decrease the chances for infection, and decrease implant migration.[4-8] However, since almost all porous materials are solid spheres, their proper placements in the eviscerated globes require wide scleral opening.[1,9] Moreover, their rough surface may erode the overlying conjunctiva and result in implant exposure.[5]

ChronOS consists of pure ß-tricalcium phosphate (ß-TCP; $Ca_3 (PO_4)_2$). A sinter process of amorphous calcium phosphate materials synthesizes polycrystalline ceramics at over 1200°C. Beta (ß) refers to the particular alignment of the TCP crystals. These ceramics are porous with a pore size of 160 to 320 µm.[10] However, they are not osteoinductive and only serve as a scaffold for osteogenous cells.[10,11]

Unlike hydroxyapatite ceramics, which do not degrade in vivo, ß-TCP ceramics degrade slowly.[10-12] ß-TCP has been used as a bone substitute in maxillofacial, orthopedic and neurosurgical operations for several years. As far as the literature is concerned, it has not been used for orbital implantation. Therefore, the present study was designed to investigate the potentials of polycrystalline porous ß-TCP granules as a new orbital implant.

Materials and Methods

The study was performed according to the Shiraz University of Medical Sciences guidelines for animal research. Eighteen adult male New Zealand white rabbits, Laboratory Animals Breeding Center, Shiraz University of Medical Sciences, weighing from 2.5 to 3 kg were randomly assigned to four groups.

Group I was control group (n=5). We performed right eye enucleation as well as placement of two g autologous cancellous bone as a substitute implant.

Group II (n=5): Right eye enucleation was performed followed by placement of a mixture of one g autologous cancellous bone and one g β-TCP granules.

Group III (n=5): After right eye enucleation, a mixture of 0.5g autologous bone marrow and 1.5g β-TCP granules was placed.

Group IV (n=3): Right eye evisceration followed by filling with the same materials as groups I, II and III, respectively.

Animals were anesthetized with intramuscular injections of 45mg/kg ketamine hydro chloride (Rotexmedica, Trittau; Germany) and 7.5 mg/kg Xylazine (Bayer; Japan). Under aseptic conditions, a three cm skin incision was made on the right iliac crest, the muscles stripped off and the cancellous bone was removed. In group III through a five mm skin incision bone marrow was aspirated using Jamshidi needle from the same site. After irrigation, iliac wounds were closed with Nylon 3-0 sutures threads.

Enucleation and evisceration were performed using a modified version of recently published techniques.[1,13] In brief the lids were retracted, and a 360° peritomy was performed. For enucleation, all muscles were located and detached, and the optic nerve was cut. For evisceration, 270°limbus incision, and total stripping of Descemet's membrane and corneal endothelium as well as refreshment of epithelium were performed. All enucleated globes were floated in water, and their volumes were obtained through measuring the volumes of replaced waters (mL). In both procedures, all ocular tissues were removed with an evisceration spoon. The volumes of eviscerated eyes were measured by volumes of waters filling the empty eviscerated globes.

The scleral shells in enucleation groups and hollow globes in evisceration group were filled with appropriate mixtures as listed in table 1. In the enucleation groups (Groups I-III) muscles were reattached to the normal anatomic position with Vicryl 6-0 sutures. Tenon capsule and conjunctiva were closed with chromic catgut 6-0 sutures, and the lids were approximated with nylon 3-0 single suture. Tetracycline ointment (1 mL) was applied to the operated eye and the donor site periodically for one week. Moreover, systemic antibiotics therapy including 10,000 Unit/kg penicillin G (Jaberebne-Hyan; Iran) and 12.5 mg/kg cephazolin (Zakaria, Tabriz, Iran) was performed intramuscularly twice a day for five consecutive days.

After the operations, daily check was performed for complications such as infection, wound dehiscence, extrusion and other probable problems. Exenteration was performed when a rabbit died, or at the end of the 12th postoperative week.

The orbital contents were examined using three methods. First, the volumes of globes were determined by measuring the volumes of waters they replaced as described before. Then, their densities were determined using Osteocore two system (version 1.4, Medi Link; France). Finally, they were fixed in 10% formalin, cross-sectioned, dehydrated in absolute alcohol, embedded in paraffin, cut in five µm thick sections and stained using H/E tech

nique. The samples blindly evaluated for ossification and fibrovascular ingrowths.

Statistical analyses

Data are presented as mean±SD. intergroup comparisons of the volumes or densities of ocular contents of all groups before and after the operation were performed using Kruskal–Wallis test. Intragroup comparisons of preoperative and postoperative data were performed using Wilcoxon Signed Ranks test and $P<0.05$ is considered statistically significant.

Results

Three out of 18 animals were lost during the study due to unrelated causes to the intervention. One rabbit in groups I and II died due anesthesia at the beginning of the study and one rabbit of group III died due to severe gastroenteritis at the sixth post operative week. None of the animals experienced implant exposure or socket infection during follow-up. However, one of the animals in group I had wound dehiscence in donor site, which healed after irrigation, refreshing and re-suturing. Furthermore, rabbits of groups I and II had obvious limping for one weak post operation, while those in group III were symptom free.

There was no statistically significant difference between the volumes of the globes before and after implantation. Similarly the differences between preoperative and postoperative globe volumes were not statistically significant (Table 1). Moreover, there was no statistically significant differences among area of mineral densities of groups I (175±3 mg/cm^2), II (165±1 mg/cm^2) and III (174±3 mg/cm^2).

Table 1: Mean±SD globe volumes of enucleated groups of I, II, and III before and after implantation.

	Volumes (ml)		
Groups	**I**	**II**	**III**
Before	2.0 ± 0.39	2.6 ± 0.12	2.5 ± 0.3
After	2.5 ± 0.37	2.50 ± 0.2	2.4 ± 0.3
Pre&Post OPD	0.12 ± 0.02 CI=0.08,0.15	0.11± 0.10 CI=-0.04,0.27	0.1±0.1 CI=-0.02,02

Pre & Post OPD = Pre and post operative differences

Pathologic examination of the exenterated tissues revealed acceptable consistency in gross cut sections and some of them required acid preparation before processing. In microscopic examination, exenterated tissues from group I comprised of viable mixed implanted cancellous and small compact bone particles surrounded by chronic inflammatory cells and a shell of fibrovascular connective tissue (Fig 1).

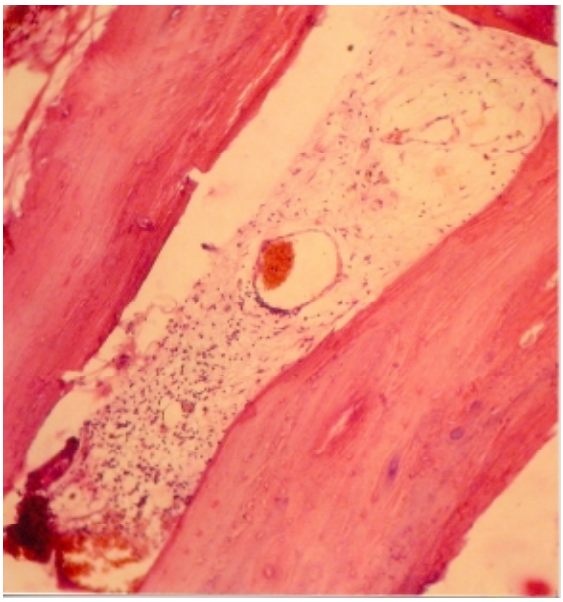

Fig 1: Cross-section of a sample obtained from a rabbit in group I shows viable bone particles and inflammatory cells with fibrovascular ingrowth (H&E stain×400).

The exenterated tissues of group II were similar to that of group I except for the presence of resorbing β-TCP granules mixed with viable bone (Fig 2). However, in one rabbit evidences of devitalized necrotic cancellous bone was existed with minimal vascularization surrounded by inflammatory cells.

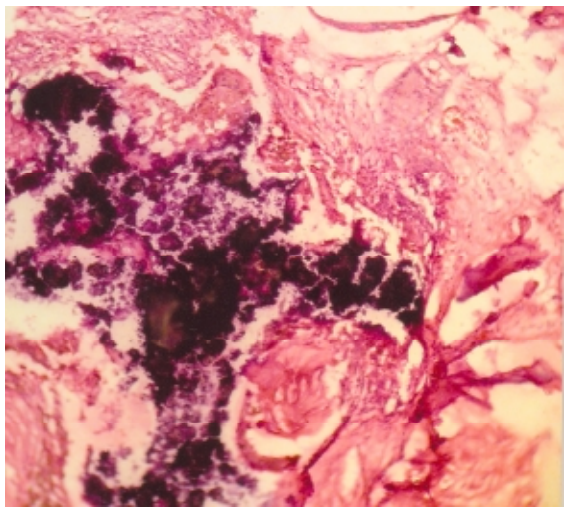

Fig 2: Micrograph of a sample of the rabbits in group II. Mixed bone particles, ß -TCP granules and fibrovascular ingrowth are seen (H&E stain ×100).

Exenterated tissues of group III did have fibrovascular ingrowth around the implanted resorbing granules. They also showed early evidences of new cancellous bone formation surrounded by fibrous connective tissues with mainly nongranulomatous inflammation (Fig 3). Exenterated tissues form group IV did show

the paucity of vascularity and fibrous ingrowths around the implanted particles.

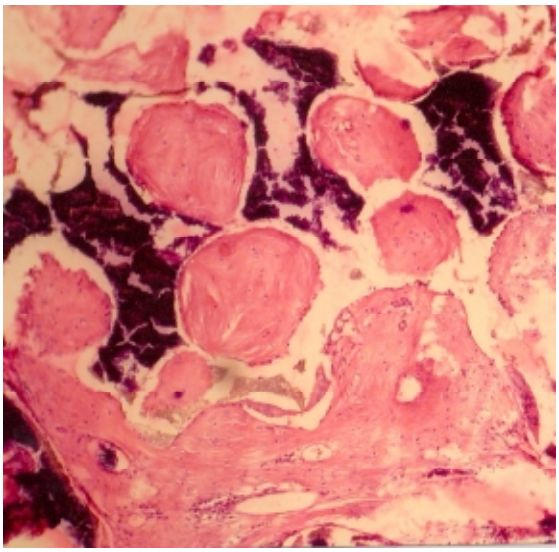

Fig 3: Fibrovascular ingrowth and new cancellous bone formation admixed with ß -TCP granules are observed in a micrograph of a sample of the rabbits in group III (H&E stain ×400).

Discussion

The use of ß-TCP as orbital implant was not associated with migration, wound dehiscence, conjunctival thinning, pyogenic granuloma formation and extrusion of the implant. These are some of the complications that may occur by using current implant materials.[7,14-17] Moreover, implant exposure with a rate ranging from 0 to 22% has been reported on hydroxyapatite implants[16],mainly in the first and12th postoperative week.[18] Only one rabbit in group I developed donor site wound dehiscence, which was due to scratching and movement of the animal. The wound healed without any significant problem.

The decrease in mean globe volumes before and after the implantation did not reach statistical significance. Such an insignificant decrease was probably due to absorption of air bobbles and fluids among particles, or remodeling of bone pieces and granules. Thus, the cosmetic results of ß-tricalcium phosphate implantation are comparable to that of the best integrated implants from decades ago, and they may even show fewer complications.[2]

Mineral density reflects the level of consistency quantitatively and correlates well with bone histology.[15] The densitometry that we used was not calibrated well and so the results were not comparable with those published in the literature.[15] However, the comparison between groups revealed relatively equal mean densities in groups I and III which was slightly more than that of group II. These findings may be influenced by resorbing β-TCP granules, as discussed in histopathologic findings. More experiments are necessary to evaluate the density appropriately.

Rabbits that received bone marrow materials (group III) showed better post operation movements compare to those undergoing removal of iliac bone particles in (groups I & II). This can be explained by the fact that bone marrow aspiration is less painful than bone removal.

There was significant vascularization and fibrous ingrowths in groups II and III. This might suggest the bioactivity of ß–TCP granules in the orbit in spite of not having direct contact with the bone. The delayed vascularization and ossification in the eviscerated group may be due to dense scleral shell. If scleral windows were created in advanced, or if the time of our study was lengthened, vascularization and bone formation might have occurred more efficiently. As the implant is vascularized, it becomes integrated into the orbital tissues, allowing it to be coupled directly to prosthesis, reducing the chance of migration, and providing excellent motility because of extraocular muscle attachments to the sclera.[2]

Minimal inflammation was found in the groups II & III, which showed excellent biologic compatibility and good tolerance of ß-TCP granules. A significant inflammation was reported on coralline hydroxyapatite implants or around pegs.[19] No rejection caused by ß -TCP has been reported in many years of cell and organ cultures as well as in the clinical application. Also, no toxic effects were established in the cell cultures.[10,12]

Finally, the cost of ß -TCP is about half of the routinely used hydroxyapatite implants.
Overall, collected data shows that the mixture of ß-TCP granules and bone marrow could maintain a density similar to bone particles. This may be explained by better osteogenic activity of bone marrow components. Although the number of animals used was low, we observed an acceptable consistency in gross cut sections, which was adequate to support the orbital structures in a stable way, in all groups. Also, this product is of low cost, and can reduce the cost of orbital implantation.

Conclusion

ß-TCP is biocompatible and osteoconductive as an orbital implant. It seems to be well tolerated and can maintain the ocular volume in the rabbit. Further studies are required to evaluate the changes in the density of mineral density changes over time after implantation.

Acknowledgements

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