

Bovine bone xenograft as orbital implants in rabbit eyes

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Received:2008-11-06 Accepted:2008-12-26

Abstract

- **AIM:** To assess the biocompatibility of bovine bone as orbital implants in rabbits.
- **METHODS:** Bovine bone graft was used as an ocular implant in rabbits to determine whether it could be successfully used in the anophthalmic socket as an alternative to the expensive synthetic alloplastics. Evisceration of eyes with and without bovine bone orbital implantation was performed in the right eyes of 12 New Zealand white rabbits. Group Y ($n = 6$) was eviscerated without implant, meanwhile Group X ($n = 6$) was eviscerated with insertion of an orbital implant using bovine bone. Observation was carried out on day 1, day 7, day 14, day 28 and day 42. Serial clinical examination was carried out based on a few fixed criteria, which included rate of infection, implant migration, evidence of wound breakdown and any restriction of intraocular movements. The implanted eyes were then enucleated on day 42. The enucleated eyes were sent for histopathological evaluation to record the type of inflammatory reaction and rate of fibrovascular ingrowth.
- **RESULTS:** Serial clinical examination showed presence of minimal infection in all eyes, both in Group X (implanted) and Group Y (control) on first postoperative day, which responded well with antibiotics. Infection occurred in the implanted group after first postoperative day, but there was no evidence of orbital migration or extrusion of implant, wound breakdown, restriction of extraocular movement, severe infection or any physical abnormality. Histopathological examination revealed good fibrovascular ingrowth in the implanted group, with minimal rejecting reaction of rabbit eye towards bovine bone implant.
- **CONCLUSION:** This study shows that bovine orbital implant has a good biocompatibility in rabbit eyes and its cost is acceptable.
- **KEYWORDS:** bovine bone; orbital implant; rabbit; fibrovascular ingrowth

Mohd Mansor S, Tan HK, Shatriah I, Wan Hazabbah WH, Hasnan J. Bovine bone xenograft as orbital implants in rabbit eyes. *Int J Ophthalmol* 2009;2(1):65-69

INTRODUCTION

Loss of an eye due to trauma, tumor or end stage ocular disease such as glaucoma can be devastating at any age^[1]. It may have a major impact on one's self-image, self-confidence and self-esteem, not to mention the adjustment required in adapting to monocular vision. Furthermore, there are several job restrictions that apply for one-eyed patients. Thus, it is important for an artificial eye patient to maintain a natural, less interactive and normal appearance of prosthetic eye. Before making the decision to remove an eye, consideration should be given to all alternatives and attempts should be made to preserve the eye that has potential for useful vision. Among patients who had their eyes removed, the damaged or diseased eyes have little or no vision remained. The search for the ideal ocular implant for the anophthalmic socket continues to evolve. An ideal ocular implant must be able to compensate the orbital volume deficits in the absence of the globe. Other important criteria include the orbital implant must be biocompatible and should have minimal rates of migration, extrusion, exposure and infection. Besides that, it should be able to accommodate ocular motility as much as possible, reproducing the normal movement of the eyeball. The hydroxyapatite crystals lie adjacent and bind tightly to collagen fibers. These bindings prevent the crystals and collagen from slipping out of place. The calcium crystals provide compressive strength, meanwhile the collagen fibers provide tensile strength. Therefore, bone is a strong structure despite its light weight^[2]. Hydroxyapatite provides a sufficient scaffold for fibrovascular ingrowth, which is important for stability of the implant.

Bovine hydroxyapatite is a safe, biocompatible and non-allergenic material. The pore size of bovine hydroxyapatite is between 300 μ m to 600 μ m. It is less dense than the coralline hydroxyapatite. M-sphere, the commercially available bovine implant is made from the cancellous bone of calf fibula. It is fully deproteinized to reduce the antigenicity. The bovine hydroxyapatite orbital implants are associated with low complication rate^[3], intraoperative handling qualities and easily be shaped with the use of scissors or a blade.

Bovine bone xenograft as orbital implants

In addition, this material offers less tissue drag than coralline hydroxyapatite and the particulate generation during manipulation easily egresses from the field with gentle irrigation^[4]. Porous coralline hydroxyapatite has been used as an ocular implant for reconstruction since 1985 and the use of this material was approved by Food and Drug Administration (FDA) in 1989^[5].

Porous polyethylene orbital implants are increasingly popular and used commonly as sheets, blocks, or spheres for volume replacement in the anophthalmic socket, orbital wall fractures and craniofacial operations in the past 40 years. Although porous polyethylene is less biocompatible than hydroxyapatite, it is widely used nowadays due to its smoother surface, inertness, less irritation to conjunctiva and it is cheaper compared to coralline hydroxyapatite^[6-8]. Medpor is the orbital implant made of high density porous polyethylene^[9]. The bioceramic orbital implant is made from aluminium oxide (Al_2O_3), which has been used in orthopaedic and dental surgery for 30 years. It was reported as good as other porous orbital implants after a few clinical studies in human beings. Synthetic hydroxyapatite, FCI₃ is a man-made hydroxyapatite. It is less costly, but has almost similar complication rate as compared to natural coralline hydroxyapatite^[10,11].

This study was carried out to assess the biocompatibility of bovine bone as orbital implant in rabbits. Besides that, the clinical and histopathological reactions towards bovine bone orbital implant in rabbits were also determined.

MATERIALS AND METHODS

This was an experimental and observational study on the consequences of bovine bone orbital implants and histopathological changes that occur in experimental eviscerated rabbits. This study involved 12 New Zealand white rabbits obtained from Animal House Unit, Health Campus, Kubang Kerian, Kelantan and was approved by the Research and Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia (USM). Twelve New Zealand white rabbits of either sex were used. All rabbits were obtained from the same animal holding facilities and were free from any eye disease. They were kept, fed and cared for in the animal house. All rabbits were treated according to the provisions of ACIOMS Ethical Code for Animal.

Primary orbital implantation was carried out using spherically shaped bone xenograft measured 10-12 mm (based on calculation applying $4/3\pi r^3$) from National Tissue Bank of Hospital Universiti Sains Malaysia (HUSM). The selection of xenograft was distributed equally to the eyes operated. All animals were selected equally for each procedure and were divided equally with or without primary orbital implant. Each group comprised six rabbits. The procedure was performed on one eye only. The study was conducted at the animal house. All rabbits which have

successfully completed the procedure of evisceration, followed by orbital implantation were selected. The rabbits would be excluded if there was any evidence of severe systemic infection, high risk of mortality, orbital extrusion of implants or restricted extraocular movement and complication that occurred during evisceration or anesthetization of the rabbits.

The sizes of the orbital implants were determined according to the ultrasonographic measurement of the eyeball before surgery. The bovine hydroxyapatite orbital implant was prepared by dissecting and cleaning femoral head from fat and soft tissue. After that, the femoral head was cut by band saw to form eye globe with size of 14, 16, 18, 20 and 22mm. The orbital implant was cleaned and was then dried in hot air oven for one to two hours. It was burned in furnace by gradually increasing the temperature up to 1 100°C for seven hours. The material was left overnight for slow furnace cooling. The prepared orbital implant was packed and sent for gamma irradiation. Gamma irradiated sintered orbital implant was ready for intraocular implant.

During the procedure, all rabbits were anesthetized with intramuscular injection of ketamine in combination with xylazine at a dose of 35 mg/kg and 5 mg/kg of body weight respectively in titrating dose. Evisceration with and without primary orbital implantation was carried out on the right eye. The left eye without primary orbital implantation acted as control group. The rabbits were randomly assigned to two groups with first group treated by evisceration with primary orbital implantation, while the other group acted as control group and underwent evisceration without primary orbital implantation. The rabbits were examined and monitored for 24 hours after every procedure for both groups. The presence of eye infection, implant extrusion or migration, ocular motility and any evidence of wound breakdown were examined on day 1, 7, 14, 28 and 42. The rabbits were also monitored for general health, which included the presence of eye discharge, feeding habits and physical activity.

The rabbits were sacrificed by a lethal dose of pentobarbitone at the end of six weeks. After that, enucleation with histopathological assessment was done to determine the presence of fibrovascular ingrowth and the rate of inflammatory reaction after with and without primary ocular implantation. All surgical procedures for evisceration and orbital implantation were conducted by a single surgeon and must follow standard ophthalmic surgical procedures. On the 42nd day after evisceration, the rabbits underwent euthanasia by an overdose of sodium pentobarbitone. The eye with orbital implants was enucleated. The technique of enucleation was based on Hersh, 1988. The affected eye was cleansed and wire eyelid speculum was applied. 360° fornix-based conjunctival peritomy was performed at the limbus through conjunctival scissors. Rectus muscle was

bluntly spread and isolated with muscle hooks. The muscle was clamped at insertion for ten seconds. The muscle was severed at its insertion and 5mm stump was left at medial rectus insertion. Oblique muscle was isolated and severed. Optic nerve was identified and clamped for five minutes. The optic nerve was then cut with enucleation scissors. Hemostasis was secure. The globe was inspected to ensure complete removal.

The enucleated eyes were fixed in 40g/L formalin for at least 24 hours before the gross sectioning was performed. The globe was examined before sectioning. Horizontal section was performed through a sharp surgical blade in sawing motion from back to front. The interior of the globe was examined. After that, the horizontal section of the enucleated eyes were fixed in 40g/L formalin and embedded in paraffin. Representative 5µm sections were stained with hematoxylin and eosin. The sections were examined under light microscope and were evaluated for the rate of inflammation and presence of fibrovascular ingrowth within the orbital implants. Rate of inflammation and fibrovascular ingrowth were graded by a standardized histopathological grading.

Rate or distribution of inflammatory cell reaction:

Grade 0 = No inflammation

Grade 1=Slight inflammation with few macrophages and giant cells.

Grade 2=Well defined inflammation with many macrophages and giant cells but no polymorphonuclear (PMN) leucocytes.

Grade 3=Moderate inflammation as grade 2 but with few PMN leucocytes.

Grade 4=Severe inflammation, abundant macrophages, giant cells and PMN leucocytes.

On the other hand, rate of fibrovascular ingrowth was graded as:

a. Grade 0=No fibrovascular tissue.

b. Grade 1=Slight fibrovascular tissue.

c. Grade 2=Marked fibrovascular tissue.

All the data collected via a standard data sheet was documented in SPSS programme and was analyzed with the same software. Student's *t* test for two samples was employed to compare characteristics of the two study groups.

RESULTS

Serial examination was carried out on all subjects on the first day after evisceration, followed by day 7, day 14, day 28 and day 42 after the procedure. All of the subjects did not show any cross infection to the other eye. In this study, all subjects with and without orbital implants showed normal body temperature throughout the entire period of clinical examination.

All of the rabbits (with or without implants) had minimal eye discharge on the first day after evisceration, as shown in Table 1. However, all subjects demonstrated improvement in



Figure 1 A gross specimen of the orbital soft tissue content without the implant

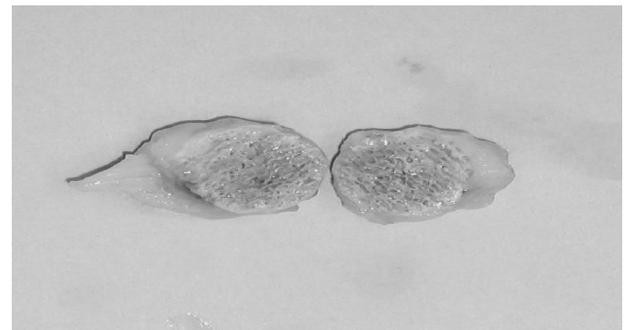


Figure 2 A gross specimen of the orbital implant sectioned into halves

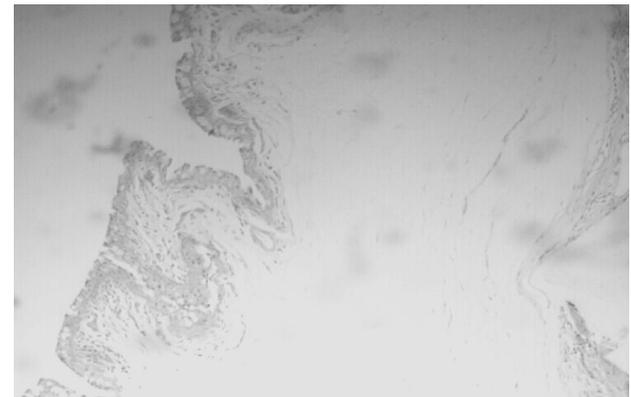


Figure 3 The histopathological examination showed fibrovascular ingrowth towards orbital implant

Table 1 Progression of discharge in evisceration of eyes with and without implants

Subject	Day 1		Day 7		Day 14		Day 28		Day 42	
	X	Y	X	Y	X	Y	X	Y	X	Y
1	+	-	-	-	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-
4	+	-	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-	-	-
7		+	-	-	-	-	-	-	-	-
8		+	-	-	-	-	-	-	-	-
9		+	-	-	-	-	-	-	-	-
10		+	-	-	-	-	-	-	-	-
11		+	-	-	-	-	-	-	-	-
12		+	-	-	-	-	-	-	-	-

(+)= present (-)= absent X= with implants (subject 1-6) Y= without implant (subject 7-12)

both evisceration with and without implants groups on day 7 and onwards. Besides that, all subjects had normal physical and feeding activities throughout the duration of study.

There was no significant difference in eye discharge, physical activity and feeding activity between the two groups.

All subjects with orbital implants (six rabbits) showed neither extrusion nor partial extrusion of implant throughout the observational period. The six rabbits with bovine bone orbital implant also had very good ocular motility. They showed full extraocular movement. None of the subjects showed total restriction or limited extraocular muscle movement throughout the observational period of six weeks. Besides that, all subjects with orbital implants did not suffer from any wound breakdown or any wound infection throughout the observational period.

The right globes in both groups of rabbits were enucleated with the rabbits being under general anesthesia. The gross specimens of the enucleated globes without and with implants are shown in Figure 1 and Figure 2 respectively. Four rabbits (67%) from Group X with orbital implants had minimal inflammatory reaction, while 2 rabbits (33%) from Group X does not show any inflammatory reaction towards the orbital implants. There was no sign of severe inflammation in all rabbits. Besides that, all rabbits ($n=6$) with bovine bone implants had fibrovascular ingrowth at six weeks post evisceration, as shown in Figure 3.

DISCUSSION

Hydroxyapatite is inert, biocompatible, non-toxic, non-allergenic and bioceramic, making it an ideal choice for ocular implant. The hydroxyapatite integrated implant used in this procedure is composed of calcium phosphate obtained from a femoral head of bovine bone. At the core of this implant, there are interconnecting pores about $500\mu\text{m}$ in diameter. These interconnecting pores allow for vascular tissue ingrowth and anchor to the ocular socket^[7]. Several animal studies have been carried out to determine the rate of tissue ingrowth in hydroxyapatite orbital implants. Rubin *et al*^[12] compared the degree of fibrovascularization in unwrapped and scleral wrapped 14mm hydroxyapatite spheres in a rabbit study. At six weeks after implantation, some of the hydroxyapatite implants had achieved complete vascularization and by 12 weeks, all of the implants were completely vascularized^[12].

Histopathological study evaluated the 14mm scleral wrapped hydroxyapatite implants which were removed and six weeks after implantation in the enucleated sockets of New Zealand white rabbits. All, except the central 2mm, of the hydroxyapatite spheres were vascularized at six weeks. The authors estimated the rate of fibrovascular tissue ingrowth to be approximately 1mm per week^[13]. They also demonstrated histopathological evidence of complete central vascularization of 12mm hydroxyapatite implants in a rabbit model as early as four weeks after implantation.

The methods used in clinical practice to estimate the extent of fibrovascular ingrowth of an orbital implant are bone scan using radioisotope, color Doppler, computed tomography

(CT) and magnetic resonance imaging (MRI) with gadolinium contrast. These methods allow better evaluation of fibrovascular ingrowth due to its ability to view vascularization in three dimensional view.

In this study, we found that bovine bone implant material has successfully shown fibrovascular ingrowth between and within the micropores of bovine bone architecture during the period of study. The large volume of microporosity that amounts to about 70 % of the total volume of the bovine bone implant and the numerous interconnections towards the core of the implant allow the invasion of capillaries, stem cells and growth factor into the implant. This explains that no clinical complication occurred in this study even during first day after evisceration. The implants show minimal infection that is resolved with antibiotic treatment. The material is not soft enough to allow a suture needle to pass through, but it is believed that surface roughness would allow a more stable fixation when fibrovascular ingrowth has occurred.

Shields *et al*^[14] have stated that MRI is the best method for distinguishing hydroxyapatite orbital implants and presence of fibrovascular ingrowth due to its high resolution and capacity to attain three-dimension. He also used MRI to find out the extent of fibrovascular ingrowth of hydroxyapatite instead of bone scan due to its limited three-dimensional imaging and distinction. In this study, the light microscopy was used to evaluate the extent of fibrovascular ingrowth within the implant and the rate of inflammatory reaction. The only pitfall of these methods is the inability to estimate the depth of fibrovascular ingrowth in three-dimensional view. During serial clinical examinations, minimal infections occurred in early phase of this study in both groups, which have responded very well to antibiotics. Late phase of this study did not show any sign of infection that can lead to failure of implant. During light microscopy evaluation after enucleation, fibrovascular ingrowth was visualized impeded between pores of the bone that indicates the biocompatibility of the material.

It is interesting to note that other clinical observations did not show any sign of clinical rejection, which was supported by the evidence that none of the subjects experienced any implant exposure, implant extrusion or any extraocular muscle restrictions. Throughout this study, none of the clinical parameter suggested clinical rejection towards this material. This phenomenon proves that there is a biocompatible environment at the host-implant interface and the favorable host tissue response has ankylosed the material with the soft tissue and has allowed it to move physiologically, thus mimicking a normal globe.

Another factor to consider is the risk of BSE (bovine spongiosa encephalopathy), which has been associated with the use of bovine product. The presence of prion in the

animal cells has been blamed for this condition. Fortunately in Malaysia and other southeast Asian countries, there is neither cases of BSE nor mad cow disease. Therefore, these bovine ocular implants which are produced from the local calves are free from such diseases^[15].

This study shows that bovine orbital implant has good biocompatibility in rabbit eyes. There was good fibrovascular ingrowth and minimal to moderate inflammatory reaction observed, as well as histological evidence of fibrovascularization within the implants as early as six weeks. This indicates a low risk of any clinical rejection. Bovine bone graft is very cost effective in regard to achieving the same objective compared with other materials. Therefore, this study demonstrates the feasibility and possibility of using bovine bone for orbital implant in human clinical trial. The success of this ocular implant material will promise a high quality healthcare that is economical and affordable to majority patients who need it.

Acknowledgement: This research was fully funded by University Sains Malaysia. The authors would like to thank Professor Dr. Abdul Rani Samsuddin, Associate Professor Dr. Afifi Sheikh Abu Bakar and animal house unit staff for their guidance and cooperation.

REFERENCES

- 1 Heimann H, Bechrakis NE, Zepeda LC, Coupland SE, Hellmich M, Foerster MH. Exposure of orbital implants wrapped with polyester-urethane after enucleation for advanced retinoblastoma. *Ophthalm Plast Reconstr Surg* 2005; 21 (2):123-128
- 2 Guyton A, Hall JE. Textbook of Medical Physiology. 11th ed. Philadelphia: WB Saunders; 2006
- 3 Suter AJ, Molteno ACB, Bevin TH, Fulton JD, Herbison P. Long term follow up of bone derived hydroxyapatite orbital implants. *Br J Ophthalmol* 2002;86(11): 1287-1292
- 4 Perry JD, Goldberg RA, McCann JD, Shorr N, Engstrom R, Tong J. Bovine hydroxyapatite orbital implant: a preliminary report. *Ophthalm Plast Reconstr Surg* 2002;18(4):268-274
- 5 Jordan DR, Gilberg S, Bawazeer A. Coralline hydroxyapatite orbital implant (bio-eye): experience with 158 patients. *Ophthalm Plast Reconstr Surg* 2004;20(1): 69-74
- 6 Jordan DR, Brownstein S, Dorey M, Yuen VH, Gilberg S. Fibrovascularization of porous polyethylene (Medpor) orbital implant in a rabbit model. *Ophthalm Plast Reconstr Surg* 2004;20(2):136-143
- 7 Klapper SR, Jordan DR, Eells A, Grahovac S. Hydroxyapatite orbital implant vascularization assessed by magnetic resonance imaging. *Ophthalm Plast Reconstr Surg* 2003;19(1):46-52
- 8 Su GW, Yen MT. Current trends in managing the anophthalmic socket after primary enucleation and evisceration. *Ophthalm Plast Reconstr Surg* 2004;20(4): 274-280
- 9 Blyden SM, Shepler TR, Neuhaus RW, White WL, Shore JW. The porous polyethylene (medpor) spherical orbital implant: a retrospective study of 136 cases. *Ophthalm Plast Reconstr Surg* 2003;19(5):364-371
- 10 Jordan DR, Bawazeer A. Experience with 120 synthetic hydroxyapatite implants (FCI3). *Ophthalm Plast Reconstr Surg* 2001;17(3):184-190
- 11 Schellini SA, Marques ME, Padovani CR, Taga EM, Rossa R. Comparison of synthetic hydroxyapatite and porous polyethylene implants in eviscerated rabbit eyes. *Ophthalm Plast Reconstr Surg* 2003;19(2):136-139
- 12 Rubin PA, Popham J, Rumelt S, Remulla H, Bilyk JR, Holds J, Mannor G, Maus M, Patrinely JR. Enhancement of the cosmetic and functional outcome of enucleation with the conical orbital implant. *Ophthalmology* 1998;105 (5): 919-925
- 13 Oria AP, Neto FA, Laus JL, Dos Santos LA, Piza ET, Brunelli AT, Nishimori CT, de Souza AL. Evaluation of a double-setting alpha-tricalcium phosphate cement in eviscerated rabbit eyes. *Ophthalm Plast Reconstr Surg* 2006; 22 (2): 126-130
- 14 Shields CL, Shields JA, Eagle RC Jr, De Potter P. Histopathologic evidence of fibrovascular ingrowth four weeks after placement of the hydroxyapatite orbital implant. *Am J Ophthalmol* 1991;111(3):363-366
- 15 Aguzzi A. Prion diseases of humans and farm animals: epidemiology, genetics, and pathogenesis. *J Neurochem* 2006;97:1726-1739