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The Inflammatory and Foreign Body Reaction of Polymethyl Methacrylate Glaucoma Drainage Device in the Rabbit Eye

Virna D. Oktariana Asrory[1,2], Ratna Sitompul[1,2], Widya Artini[1,2], Sri Estuningsih[3], Deni Noviana[3], and William H. Morgan[4]

[1] Ophthalmology Department, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
[2] Cipto Mangunkusumo Hospital, Jakarta, Indonesia
[3] Faculty of Veterinary, Institute Pertanian Bogor, Bogor, West Java, Indonesia
[4] Ophthalmology Department, University of Western Australia, Nedlands, WA, Australia

Correspondence: Virna D. Oktariana Asrory, Cipto Mangunkusumo Hospital/Faculty of Medicine Universitas Indonesia, jl Kimia no 8 Menteng, Jakarta Pusat, DKI Jakarta, 10320, Indonesia. e-mail: virnadwi_dr@yahoo.com

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Purpose: To assess the safety and tissue response of a polymethyl methacrylate (PMMA) glaucoma drainage device (GDD) in the rabbit eye.

Methods: Specially constructed PMMA GDD devices were implanted into rabbit eyes and evaluated histopathologically following euthanasia on days 5, 30, and 60 after implantation surgery. Hematoxylin-eosin, Masson's trichrome, and periodic acid-Schiff were used to stain tissue specimens dissected from the surgical site. Inflammatory cell count and capsule thickness measurements were performed.

Results: Three rabbits were sacrificed on day 5, 3 on day 30, and 4 on day 60. Macrophage and lymphocyte counts increased from day 5 to day 30 then decreased ($P = 0.0000$) with greater counts seen in the superior regions. At day 30, a fibrous capsule had formed around the plate area. Fibroblast counts increased significantly between day 5 to day 30 and again to day 60 ($P = 0.001$) with greatest numbers anteriorly. The inferior capsule thickness at day 60 was 243 µm (standard deviation, 120; 95% confidence interval: 53-433). The superior capsule thickness was 388 µm (standard deviation, 136; 95% confidence interval: 172-604). No adverse reactions were seen.

Conclusions: Histopathologically, the inflammatory response toward this PMMA glaucoma drainage device was comparable to other reported GDDs. No accentuated response or adverse event was seen suggesting that PMMA may be useful as a GDD material.

Translational Relevance: An affordable, locally built GDD is needed in Indonesia because of the high prevalence of severe glaucoma. This rabbit study is a significant step toward justifying the use of PMMA as a GDD material. PMMA is cheap and easily manufactured and sterilized in developing economies.

Introduction

Glaucoma is the second most common cause of blindness in Indonesia, after cataract.[1] The aim of glaucoma treatment is to reduce intraoccular pressure, through medical therapy, laser, or surgery, depending upon the type and severity of glaucoma. In general, when medical and laser therapy have failed, filtration surgery is performed.[2–4] The most common filtration surgery is trabeculectomy. Unfortunately, it frequently fails in high-risk patients such as in congenital, uveitic, traumatic, and neovascular glaucoma. We find that the general rates of drainage bleb fibrosis leading to failure appears to be relatively high in Indonesian patients undergoing trabeculectomy. In these circumstances, the only viable treatment to maintain aqueous outflow is glaucoma drainage device (GDD) surgery.[3,4]

In Indonesia, the cost of commercially available GDD such as Baerveldt and Ahmed GDD (US$1,000) are too high for the general population. Our aim is to create a safe GDD that can be easily

fabricated in Indonesia. All GDDs use silicone tubing connected to large plates made of various materials such as polypropylene (Molteno and Ahmed), silicone (Baerveldt), and acrysof acrylic.[5] Silicone tubing is well tolerated in the eye, with many studies demonstrating acceptable tolerability. Polymethyl methacrylate (PMMA) is known to be inert in the eye.[6] It can be molded to form various shapes that can be easily machined for suture holes and to allow connection of silicone tubing. It has a long history of use as an ocular prosthesis and intraoccular lens material. There are no reported adverse reactions of PMMA in the eye.[6–8]

This study was designed to evaluate the safety and chronic inflammatory response to our novel PMMA GDD within the rabbit eye. We chose the rabbit because of its rapid inflammatory and fibrotic response to foreign bodies. Also, rabbits have been the most common animal model used in previous assessments of GDD design and materials.[9,10] This allows us to make some comparison of the inflammatory reaction between our PMMA GDD and GDD types made with other materials. A report on the chemical and topographic properties of the PMMA material after the implantation and the comparison with other materials will be reported in another paper.

Material and Methods

This study was conducted with University of Indonesia ethics approval at the Veterinary Faculty, Institute Pertanian Bogor in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. New Zealand white male rabbits weighing 2.5 to 3 kg were used. The surgery was performed under general anesthesia by administration of ketamine (35 mg/kg) and xylazine (5 mg/kg). The implants were sutured to the superotemporal sclera using 10.0 nylon under the conjunctiva in one eye of each animal. The conjunctiva was closed with polyglactin 8.0. During surgery, the tube was ligated with 6.0 chromic catgut and inserted into anterior chamber through a trans-scleral track formed with a 23 G needle. Because the rabbit's eyes are smaller than human eyes, the size of the implant used in this study was also smaller than a planned human version. The GDD implants were manufactured by Rohto (Rohto Corp., Jakarta, Indonesia) using silicone tubing glued to PMMA plates. The dimensions of the superior surface of the implant was 14 mm in length and 11 mm in width. The total superior surface area was 146.08 mm^2. The prototype can be seen in Figure 1.

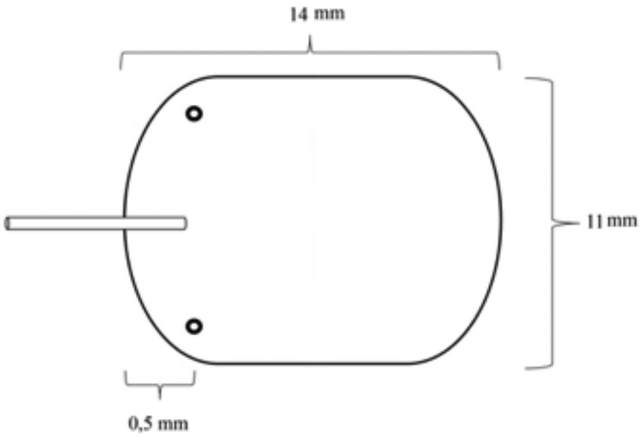

Figure 1. Schematic design of PMMA with dimensions. The tubing is silicone, glued into the PMMA plate.

After the surgery, the rabbits were administered prednisolone acetate 1% eye drops (Cendo Labs, Jakarta, Indonesia) and levofloxacin 0.5% eye drops (Cendo Labs) 6 times per day for 1 week and then 4 times per day for another week. The animals were divided into 3 groups and euthanased using ketamine (35 mg/kg) intramuscularly and xylazine (5 mg/kg) intravenously at days 5, 30, and 60. The orbits of both eyes were exenterated and prepared for histological examination. The eyelids and eyelid conjunctiva were removed in all animals leaving bulbar conjunctiva and rectus muscles attached to the globe in both eyes. The fibrous capsule around the plate was incised temporally and the GDD excised. All eyes were immersion fixed in buffered neutral formaldehyde 10% for 48 hours. The tissue was sectioned radially (longitudinally) and stained with hematoxylin-eosin (HE), Masson's trichrome, and periodic acid-Schiff.

All eyes had sections stained with HE. The region surrounding the GDD was divided into five regions; superior, inferior, anterior, middle, and posterior, as seen in Figure 3. Ten high-power fields (×20 magnification) from each of the five regions were examined histologically. Chronic inflammatory cell markers: lymphocytes, monocytes, macrophages, foreign body giant cells (FBGC), fibroblasts and plasma cells were counted per high-power field.

Sections from the day 30 and day 60 eyes were stained with Masson trichrome and the fibrous capsule thickness was measured. Microscopic images were digitized, then imported into Image J (National Institutes of Health, Bethesda, MD) for the measurement of the capsule thickness.

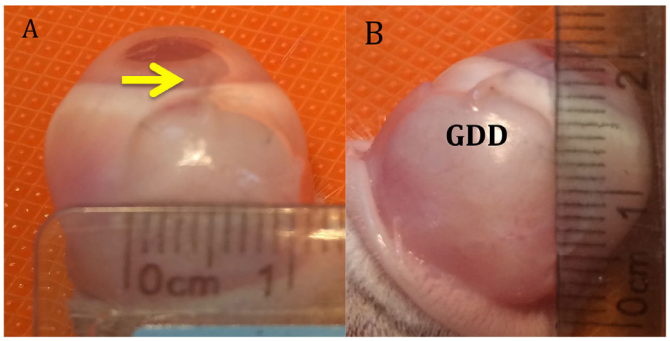

Figure 2. (A, B) PMMA GDD dimension and rabbit's eyeball at 60th day postimplantation. The bleb formed around the implant was 11 mm × 16 mm. The yellow arrow shows the tube position in the anterior chamber.

Statistical Analysis

Means and standard deviations (SD) are presented. With the capsule thickness measurements, 95% confidence interval (95% CI) were also calculated. For comparison of cell counts, we used a linear mixed model with eye as a random factor to account for the fact that 10 measurements were taken from each region. Cell count per high-power field was the response variable with region and day of sacrifice being the explanatory variables. All analysis was performed using R.[11]

Results

Ten rabbits received the GDD and none experienced GDD extrusion. Rabbits were divided into three groups: 3 rabbits were sacrificed on day 5 postoperatively, 4 rabbits on day 30, and another 4 on day 60. Several observations were consistent across all review time points macroscopically following sacrifice. The GDD remained in the implanted position throughout with no conjunctival dehiscence or breakdown visible. The anterior chamber remained deep with a visible tube tip without macroscopic signs of inflammation. A fluid-filled bleb was visible over the plate. The histological results of the implant group are summarized in Figure 4.

Macrophage counts were highest at day 5 and then tended to decrease, with a significant reduction from day 30 to day 60 ($P = 0.0004$). The superior regions contained more macrophages than inferior regions ($P = 0.0005$). There was no significant difference found between the anterior and middle regions ($P = 0.3430$); however, the anterior regions contained lower counts than posterior ($P = 0.0058$), whereas middle and posterior regions were found to have no significant differences ($P = 0.0399$).

Lymphocyte counts increased from day 5 to day 30 ($P = 0.0001$) and then decreased from day 30 to day 60 ($P = 0.0000$). Superior regions had higher lymphocyte counts than inferior regions ($P = 0.0425$). Additionally, anterior regions had higher lymphocyte counts than middle ($P = 0.0125$) and posterior regions ($P = 0.0847$), with no difference between middle and posterior regions ($P = 0.9469$).

Monocyte counts tended to increase from day 5 to day 30 ($P = 0.0466$) and then decreased significantly to day 60 ($P = 0.0109$). There was no significant difference in monocyte counts between the superior and inferior regions ($P = 0.7591$). The anterior regions had higher counts than the middle regions ($P = 0.0432$) but no significant difference found in comparison to posterior regions ($P = 0.3947$). No difference was found between the middle and posterior regions ($P = 0.2054$).

There were relatively few foreign body giant cells (mean 0.04/phpf; SD, 0.10) present in any sections. No significant variation in foreign body giant cell counts were detected between days of sacrifice or between any region.

Relatively few plasma cells (mean 0.1/phpf; SD, 0.3) were seen. There was no significant variation in plasma cell count with day of sacrifice or between the superior and inferior regions ($P = 0.0961$), although the anterior capsular region had significantly higher counts than both the middle ($P = 0.0005$) and posterior regions ($P = 0.0002$). There was no significant difference between the middle and posterior regions ($P = 0.0832$).

Fibroblast cell count increased significantly from day 5 to day 30 ($P = 0.0000$) with a subsequent increase to day 60 ($P = 0.0010$). Yet, the anterior regions contained more fibroblasts than posterior regions although it is statistically insignificant ($P = 0.4949$). No significant difference was noted in fibroblast counts between other regions.

The capsule thickness measurements were averaged for day 30 and day 60 resulting in a mean capsule thickness of 264 µm (SD, 187) superiorly and 130µm (SD 49) inferiorly at day 30, increasing to 388µm (SD 136µm, 95% CI 172 to 604µm) superiorly and 243µm (SD, 120 µm; 95% CI, 53-433) inferiorly at day 60.

Discussion

In these experiments, we found that the PMMA GDD plate was well tolerated by the rabbits, with no macroscopic inflammatory signs or extrusion.

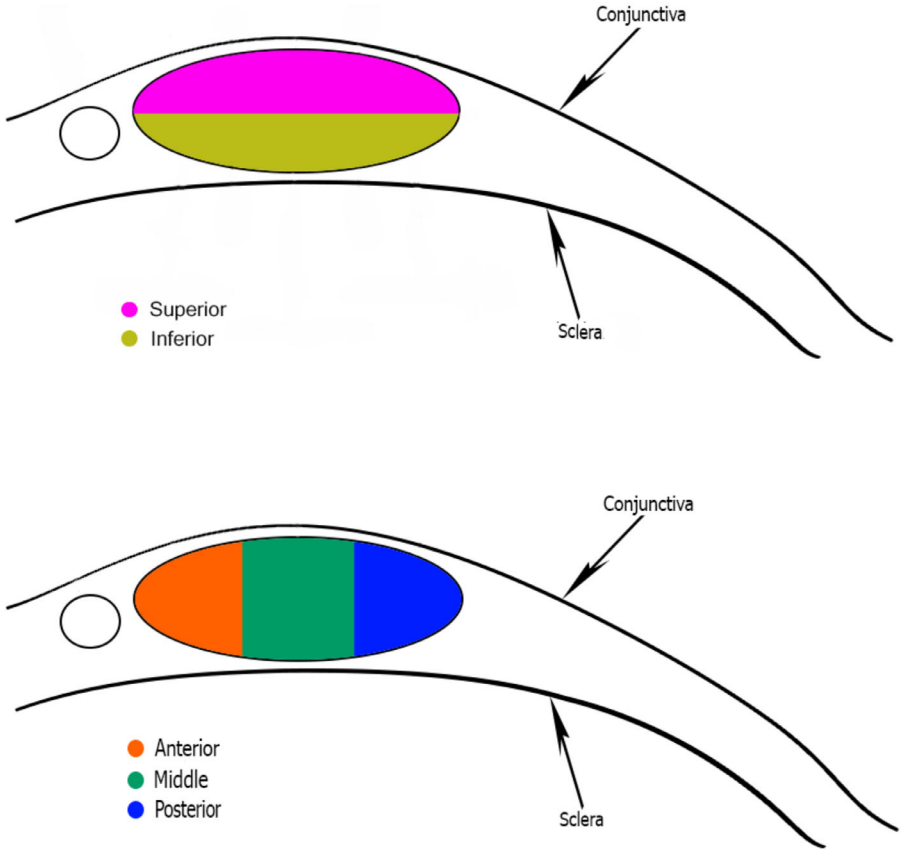

Figure 3. Schematic diagram of the implant area.

Clinically, no significant inflammatory response was seen in the eyes, similar to that published by Ayyala[5] who used polypropylene, silicone, and acrysof acrylic.

Our sampling at days 5, 30, and 60 after surgery allows us to measure the inflammatory response over time. Macrophage counts decreased suggesting that their appearance was a more acute phase response to be replaced by chronic inflammatory cells. Plasma cell counts were initially higher anteriorly along with monocytes, with the latter being maximal at 30 days and then decreasing. Perhaps the anterior region of the GDD is most prone to physical trauma with eyelid movement possibly tending to shear conjunctiva against the GDD. Lymphocytes also tended to increase by 30 days then decrease suggesting that they and monocytes are most active in the medium term. Fibroblast cell counts steadily rose consistent with the increase in fibrous capsule deposition. Our results are limited to the more chronic inflammatory cell types and do not allow examination of acute inflammatory cell subtypes.

On day 5 after surgery, mononuclear cells such as monocytes and lymphocytes that mark a chronic inflammatory process were seen. Macrophages are activated in the presence of foreign material and can lead to FBGC formation.[12–14] On histopathology examination on days 30 and 60, macrophage and FBGC were found. A study by Jung et al.[15] showed that the number of FBGCs was suppressed on the implant that was receiving aqueous humor flow compared to the implant that was not received aqueous humor flow. It was proposed that aqueous humor may inhibit FBGC. We had expected more FBGC accumulation in the capsule close to the PMMA plate given that it is rigid compared to silicone plates others have used.[16–19] However, we found that the number of foreign body giant cells near the GDD implant area (0.6 ± 0.21 phpf) was similar to that found by Jung et al.[15] (0.58 ± 1.00 phpf) at day 30.

Initial research by Molteno[20] using rabbits implanted with an implant made from polypropylene gave histological features similar to this work. An infiltrate around the implant consisting of plasma cells, lymphocytes, and occasional polymorphonuclear leucocytes were found in Molteno's research, although these cell types were not counted.

The size, form, and topography of the surface of implants may also determine the type and number of

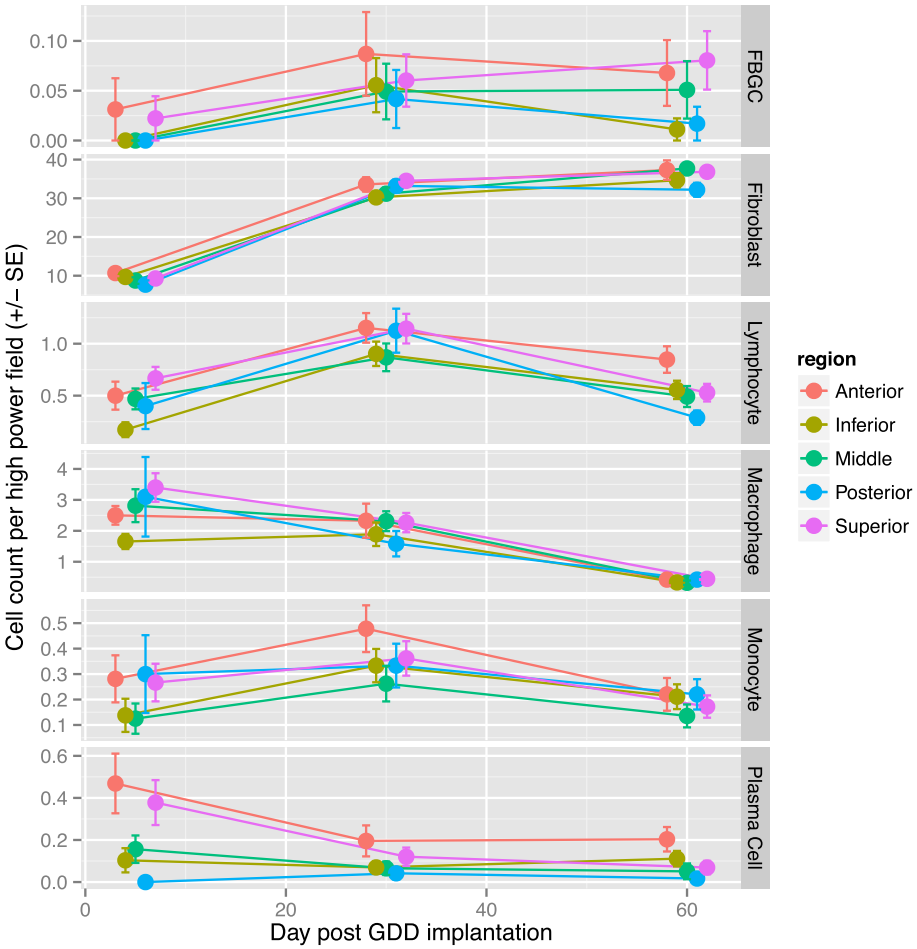

Figure 4. Mean cell count for each cell type in the GDD group with standard error bars.

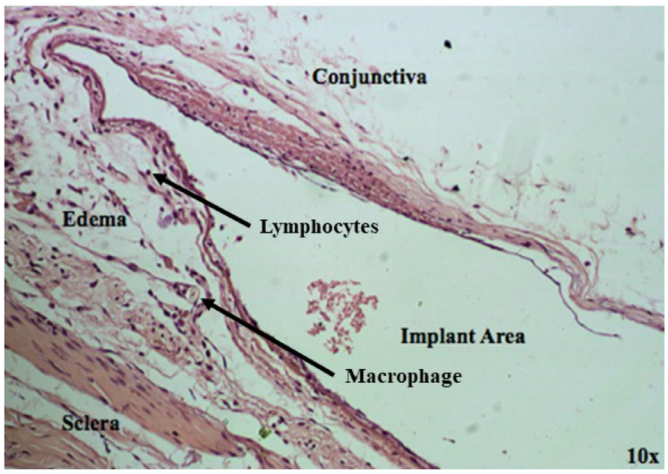

Figure 5. Anterior tissue response around PMMA GDD on day 5 with HE staining. Acute inflammatory response was seen, which was marked by infiltration of acute inflammatory cells (macrophages and lymphocytes). A fibrous capsule around the implant is starting to form. The region occupied by the GDD plate is denoted as "implant area."

inflammatory cells adhering to the material and subsequent fibrosis, according to a study by Veiseh et al. in rodents.[21] In that study, the degree of fibrosis was lower in the 0.5-mm sphere implant in comparison to the 1.5 mm, whereas in our study, the implant we used was greater in size. The previous study by Oria et al.[22] found that the rabbits receiving orbital implants with PMMA material had a less intense fibrosis compared to polyethylene and hydroxyapatite porous material. This was possibly from a lack of surface porosity in PMMA.

At day 60, our rabbit capsule thickness was 388µm (SD, 136 µm; 95% CI, 172-604) superiorly and 243 µm (SD, 120 µm; 95% CI, 53-433) inferiorly. This is similar to data published by Lee et al.,[23] who inserted polypropylene Ahmed valves into rabbits and assessed them at 60 days. Their mean superior capsule thickness was 458 µm (SD, 60; 95% CI, 413-501) and inferior capsule thickness was 233 µm (SD, 43; 95% CI, 202-264). Our capsule thickness measurements at day 60 appear to be insignificantly different to those using a

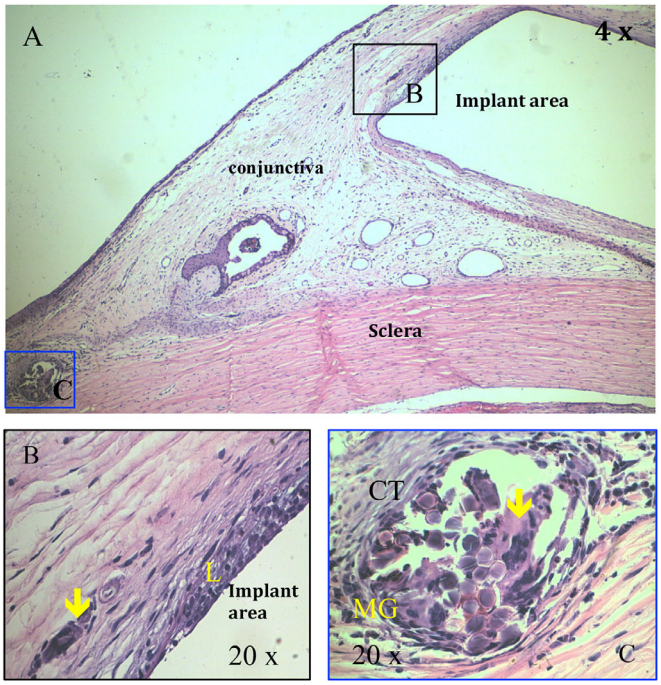

Figure 6. (A) Microscopic features of anterior tissue surrounding PMMA GDD on day 30. HE stain. Magnification ×4. Encapsulation around the implant area was seen. (B) Chronic inflammatory reaction with foreign body giant cell (*yellow arrow*), and lymphocytes (*L*) was seen. (C) At a suture site, a chronic inflammatory response toward polyglactin 8.0, there was foreign body giant cell (*arrow*), macrophage (*M*), and the fibroblasts that formed collagen (*CT*).

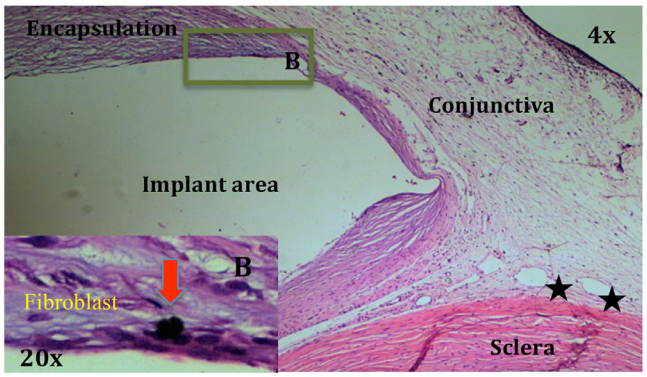

Figure 7. Posterior tissue response around PMMA implant on day 60, HE staining ×4. Implant area was well defined by encapsulation. There were foreign giant body cells (*arrow*) in magnification ×20. Presumed lymphatic vessels or venules were seen (*star*).

polypropylene Ahmed valve. We cannot predict how the capsule thickness may change beyond 60 days because all rabbits were sacrificed at 60 days, limiting our data scope over time. The histopathology finding of this GDD were seen in figure 5 (day 5), 6 (day 30) and 7 (day 60).

The rabbit's inflammatory and foreign body reaction toward this PMMA GDD was comparable to other published GDD materials such as polypropylene. This PMMA GDD was well tolerated in rabbits up to 60 days with no adverse events seen. A GDD manufactured from PMMA appears to be safe in rabbits and might be safe in human use.

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