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ORIGINAL ARTICLE

Polydioxanone for orbital reconstruction – an ARRIVE-guided preclinical assessment of its inflammatory profile compared to titanium

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Abstract

Introduction and Objective. Polydioxanone (PDO), a biodegradable synthetic polymer, has been proposed for small orbital reconstructions due to its absorbable nature. However, past findings have raised concerns about its inflammatory response, warranting further investigation. The aim of this study is to evaluate the inflammatory profile and behaviour of periorbital tissue in rabbits using a PDO membrane.

Materials and Method. The trial was carried out in 17 male rabbits of the species Oryctolagus Cunilicus, in which one of the orbits was submitted to surgical exposure and positioning a PDO membrane (intervention). In the contralateral orbit, a titanium sheet (TS) was positioned (positive control). The orbits were evaluated to identify the inflammatory profile (neutrophils, plasma cells, lymphocytes, and blood vessels) by histometry at 7, 15, and 60 days.

Results. The PDO group had more plasma cells in the 7 days than the titanium group ($p = 0.012$). In the 15 days, the PDO group had a higher number of lymphocytes ($p = <.001$), neutrophils ($p = <.001$), and plasma cells ($p = <0.004$) compared to the titanium group. Analysis of the granulation tissue area revealed a higher mean around the PDO in the periods of 7 and 15 days ($p = 0.009$ and $p = <.001$). PDO assessment did not suffer significant changes between the periods, and no remnants of the membrane were observed at 60 days. In contrast, the titanium group showed a significant decrease between 7–15 days ($p = 0.041$), and no changes between 15–60 days.

Conclusions. Considering the limitations, it was concluded that the PDO membrane caused a greater inflammatory process than TS, however, this inflammation did not extend to adjacent periorbital tissues or result in relevant clinical changes during post-operative follow-up.

Key words

titanium, eye injuries, orbital fractures, polydioxanone, orbital implants

INTRODUCTION

Despite regional characteristics, orbital trauma accounts for approximately 10% of facial traumas requiring surgical intervention [1]. New materials and improved indications for their use are the subject of various studies in the field. Currently, orbital reconstructions are performed using implants categorized into one of five groups: biological grafts, ceramics, metals, polymers, and composites. Despite the variety of available biomaterials and formats, with the exception of the biocompatibility of Titanium, there is no robust evidence proving the superiority of any implant regarding improvement in enophthalmos, elimination of diplopia, orbital volume correction, lower rates of post-operative infections, improvement/reduction of ocular motility, or elimination of muscle entrapment [2].

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Although titanium meshes are more commonly used, they present aspects that require attention, especially in the face of a second traumatic event. Their strength, significantly higher than that of the orbital floor bone [3], raises concerns, particularly regarding the possibility of new traumas in the region. A case report showed extensive deformation of the titanium mesh towards the orbital cone [4], along with finite element analyses revealing hazardous behaviour of the mesh in such cases [5]. Therefore, it is essential for surgeons to have absorbable options available for use when the structural support of the orbit is not compromised, such as in Jaquiéry type I fractures [6]. In this context, polydioxanone (PDO) was the subject of this laboratory trial.

PDO is a fully absorbable polyester polymer by hydrolysis within one year, with a 50% reduction in tensile strength after the third week [7–9]. In its 0.15mm version, it exhibited an initial tensile strength of 2.57N/mm^2, whereas the orbital floor bone showed 0.8N/mm^2 [3]. In the past, PDO has shown some concerning outcomes, such as increased inflammation, enophthalmos, and diplopia [10]. As understanding of its physicochemical properties has improved and its range of

uses has been better defined, subsequent studies have yielded more favourable results. Currently, PDO is also used in absorbable sutures, aesthetic suspension threads, and alveolar reconstructions. However, the results presented by Kontio et al. [10] raises concerns regarding inflammation that have not yet been fully answered: Is the inflammation generated by PDO during its absorption process clinically significant enough to harm the repair process compared to other implants?

To address this question, the PICOT (Population, Intervention, Comparison, Outcome, and Time) objective of this laboratory trial was to assess the inflammatory profile (O) by counting and characterizing inflammatory cells and measuring the area of granulation tissue (O) around PDO membranes (I) and titanium sheets (C) in rabbit orbits (P), at 7, 15, and 60 day (T) intervals. It was also assessed whether the granulation/inflammation tissue progressed to the surrounding areas.

MATERIALS AND METHOD

Ethical approval. The laboratory trial was approved by the Ethics Committee on Animal Use of São Paulo State University (Unesp), School of Dentistry in Araçatuba (Approval No. 0639–2021). The paper was written according to the ARRIVE 2.0 guidelines [11] which serve as a framework for ensuring the rigorous conduct and transparent reporting of animal research. The authors of the presented study will make available the complete ARRIVE checklist for this study to readers who wish to review it.

Sample. The sample size calculation was based on data previously published by Lee and Baek [12]. Clusters of analysis were separated for different groups and euthanasia times. The extent of granulation tissue was defined as the primary outcome, and quantitative results of mean and standard deviation were compared between test and control groups. The α error was set at 5%, and the study power at 80%. The minimum difference to be observed was defined as 5mm² with a mean standard deviation of 1.8. Sample calculation indicated the need for 5 samples per group at each time point, totaling 30 orbits (15 rabbits).

Considering an average loss of 20%, 18 animals were included in this study. The rabbits were quarantined for 2 weeks. During this period one animal died. The final number was 17 animals (Fig. 1). No samples were excluded from the analysis.

Seventeen male rabbits of the Oryctolagus Cunilicus lineage (New Zealand) without genetic alterations were used, with an average body weight between 3–4 kg and approximately 5 months old. Each animal served as its own control, with one orbit receiving a PDO membrane (intervention group) and the other a Titanium Sheet (TS) (positive control group). The animals were randomly taken to the operating room, one at a time. Surgeries were performed sequentially, and animals were assigned to different euthanasia periods by systematic allocation according to the order in which they were taken. The first 6 animals were allocated for euthanasia at 7 days, the next 6 for euthanasia at 15 days, and the last 5 for euthanasia at 60 days (Fig. 1). Blinding was not performed, as the evaluation of histological slides allows direct visualization of the implant. The animals were kept separate, with standardized feeding and *ad libitum* water,

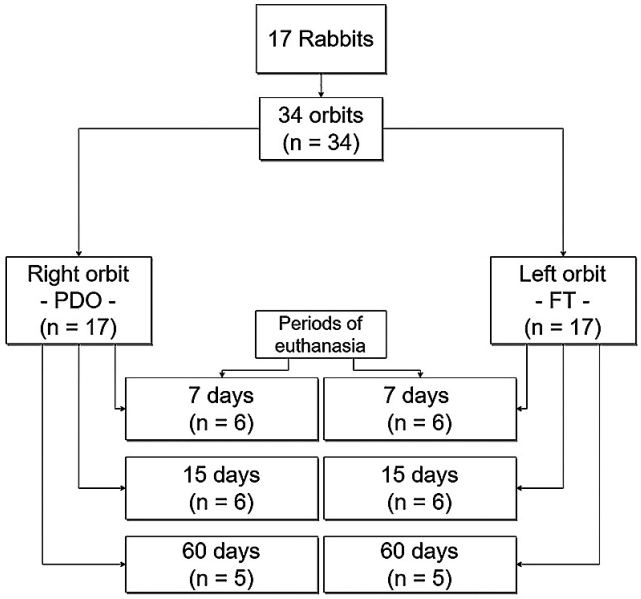

Figure 1 – Distribution of animal samples by groups. Source: Own elaboration

controlled temperature around ≅22ºC, and a daylight cycle of approximately 12 hours. Animal handling was performed by the same animal care technician to minimize stress.

Surgical procedure. After pre-operative fasting, the animals were sedated intramuscularly with a combination of 50 mg/kg Ketamine (Fracontar – Vibrac do Brasil Ltda., São Paulo, Brazil) and 5 mg/kg Xylazine hydrochloride (Rompum – Bayer AS – Animal Health, São Paulo, Brazil). Sedation was confirmed by the absence of pupillary light reflex. The eyes were protected with an ophthalmic ointment containing Retinol Acetate, Amino Acids, Methionine, and Chloramphenicol (Regencel – Latinofarma, Cotia, São Paulo, Brazil). Antisepsis of the surgical area was performed using 2% chlorhexidine digluconate with surfactants (Riohex 2% – Rioquímica Pharmaceutical Industry, São José do Rio Preto, São Paulo, Brazil). Tarsorrhaphy with 5–0 nylon suture (Ethicon, Johnson Prod., São José dos Campos, São Paulo, Brazil) was performed to keep the eyes occluded and protected during the procedure.

Local anaesthesia was achieved by infiltrating approximately 0.9 ml (0.3 ml/kg) of 2% mepivacaine hydrochloride with 1:100,000 epinephrine (Mepiadre – Nova DFL, Rio de Janeiro, Brazil) subcutaneously in the bilateral zygomatic arch region, and deep into the inferomedial region of the orbit.

All surgical procedures were performed by the same surgeon (LHGM). The surgical accesses were performed in the infraorbital region, which was ≅1.5cm in length. The dissection was performed in layers: skin and subcutaneous tissue, orbicularis oculi muscle, and periosteum until reaching the anterior portion of the infraorbital rim bone, and then progressing medially in a subperiosteal manner to reach the maxillary tuberosity (MT) – a bony projection that covers the tooth roots within the orbital cavity.

After exposing the MT, a 1 mm thick PDO membrane (Plenum Guide, M3 Health Indústria e Comércio de Produtos Médicos, Odontológicos e Correlatos SA – Jundiaí, São Paulo, Brazil) with a diameter of 8 mm was installed, fully supported by bone tissue (Fig. 4). The contralateral orbit received an 8 mm diameter and 0.04 mm thick titanium sheet (TS) (Surgitime

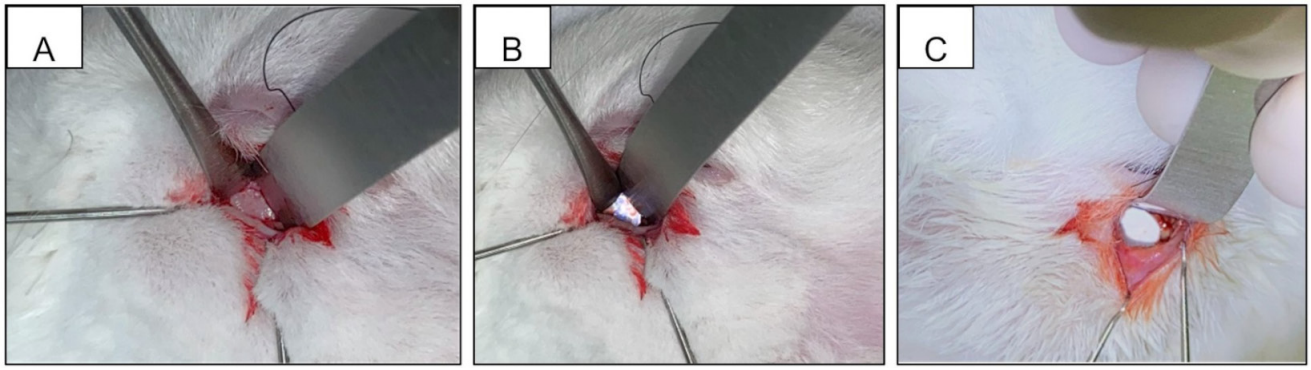

Figure 2 – Implant Installation Source: Author's personal archive. A: Exposure of TM; B: Titanium sheet positioned; C: PDO membrane positioned

Titanium Seal, Bionnovation Produtos Biomédicos LTDA. – Bauru, São Paulo, Brazil) (Fig. 2). The orbicularis oculi muscle was sutured with 4–0 polyglactin 910 (Vicryl, Ethicon, Johnson Prod., São José dos Campos, São Paulo, Brazil), and the dermis with 5–0 nylon suture (Ethicon, Johnson Prod.).

The animals were treated with Enrofloxacin (Venco Saúde Animal, Londrina, Paraná, Brazil) at a dose of 5mg/kg once daily for 5 days and Morphine (União Química, São Paulo, Brazil) at a dose of 2.5 mg/kg for analgesia for 2 days. Additional doses of analgesic were not necessary as the animals did not show signs of pain. Anti-inflammatories were not used to avoid altering the inflammatory response, which was the focus of this study.

Euthanasia. Euthanasia was performed in a separate room from other animals. Initially, the animal was sedated intramuscularly with 50mg/kg of Ketamine (Fracontar – Vibrac do Brasil Ltda.) and 5 mg/kg of Xylazine hydrochloride (Rompum – Bayer AS – Animal Health). After confirming sedation, 150mg/kg of Thiopental (Thiopentax – Cristália, Itapira, São Paulo, Brazil) and 10mg/kg of Lidocaine (Anestt – Sintec, Santana de Parnaíba, São Paulo, Brazil) were administered intravenously. Death was confirmed by asystole, pale mucous membranes, and loss of corneal reflex.

Histological processing. A cranial block containing the intact orbit was removed, identified, and fixed in 4% formaldehyde buffered with 0.1M sodium phosphate, pH 7, for 24 hours. Subsequently, it was decalcified in 4% EDTA buffered with 0.1M sodium phosphate, pH 7.2. During decalcification, sequential reductions in the size of the specimens were performed to facilitate EDTA contact with deeper portions of the orbit. After decalcification, the TS was gently removed, and the specimens underwent dehydration in increasing concentrations of ethanol before being embedded in paraffin wax. Samples were cut into 6µm thickness axially using a microtome (Microm HM 325, Thermo Fisher Scientific, Waltham, MA, USA) [13]. Following this stage, one slide was designated for histology and the next discarded, alternating in this manner until 3 slides were obtained for histometry at different depths of the inflammatory process (Fig. 3B). The slides were then stained with haematoxylin and eosin and observed under an optical microscope (Diastar – Leica Reichert Jung Products, Wetzlar, Germany) at 2.5× and 100× magnification. Images were captured in TIFF format using a digital camera CAMEDIA C50/60 Wide Zoom (Olympus Corporation's, Ishikawa-machi, Hachioji-shi, Tokyo, Japan) attached to the microscope.

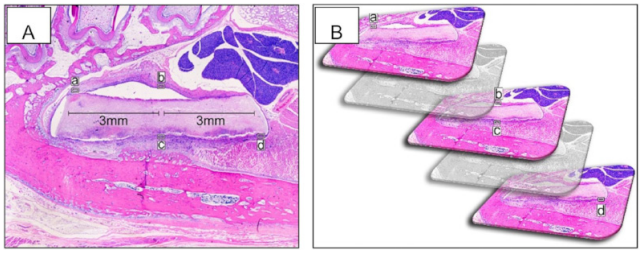

Figure 3 – Schematic representations of the areas photographed at 100x magnification and the use of histological slides. Source: Own elaboration with images from the author's personal files. A: Four photographs were taken from each slide, spaced 3 mm apart between each field; B: Scheme used for cell counting in different slides and fields

Images at 2.5× magnification were digitally stitched together using AutoStitch software (Department of Computer Science, University of British Columbia, Vancouver, Canada) [14] to generate a 1:1 scale panoramic image (confirmed by comparative physical measurements) used for measuring granulation area. Each of the 3 histological slides was measured 3 times at different intervals, and the average of these measurements was used. At 100× magnification, photographs were taken in specific fields (Fig. 3A). The purpose was to identify and count inflammatory cells (neutrophils, plasma cells, lymphocytes) and blood vessels within a grid of 130 points. Both analyses were conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis. Tests were conducted using Jamovi software (Sydney, Australia), version 2.5. A significance level (α) of 95% was considered. Samples receiving the same implant were treated as paired.

Granulation area (mm²). The Shapiro-Wilk test indicated, except for the PDO and TS groups at 15 days – p=0.028 and p=0.015, respectively, normal distribution of data (p=0.179; p=0.772; p=0.489). Thus, a normal distribution for this variable was assumed. The T-test was used to compare means between PDO vs. TS implants and to compare means within the PDO group. Intragroup TS comparison was performed using ANOVA and Tukey's *post hoc* test. Given the assumption of normality and the reduced sample size, effect size calculation was possible with Hedges' g test, where a result of >0.8 represents a large effect size.

Inflammatory profile (count of neutrophils, plasma cells, lymphocytes, and blood vessels). The data did not show normal distribution, according to the Shapiro-Wilk test.

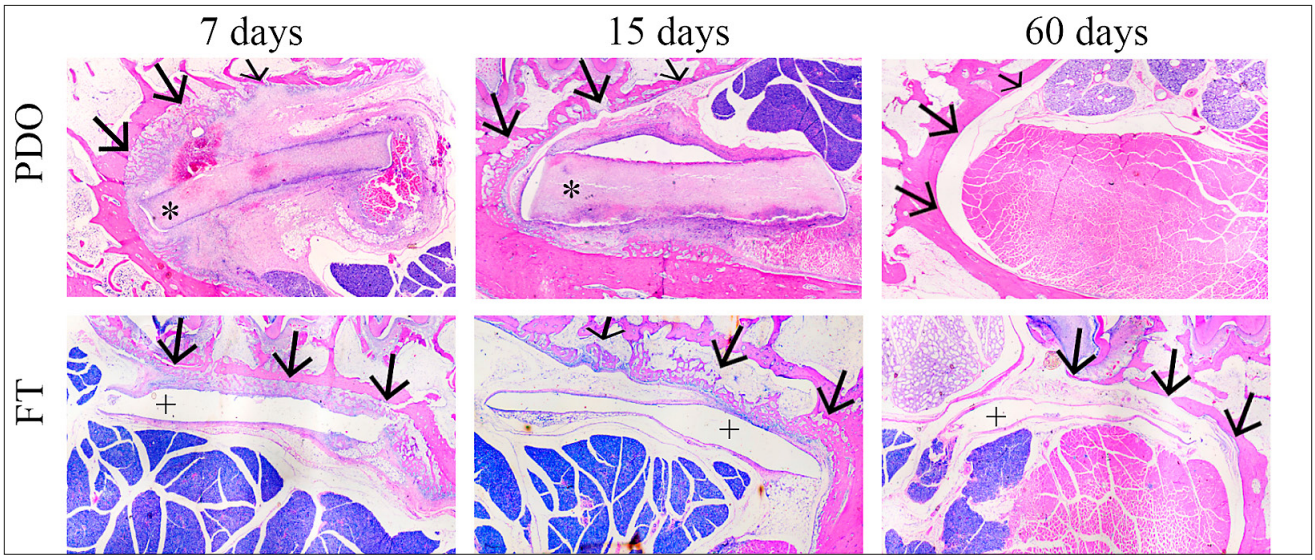

Figure 4 – General histological slide Source: Author's personal archive. Panoramic reconstruction of photomicrographs taken at 2.5x magnification, from the histological region where the biomaterial was in contact with the MT. *: indicates the PDO membrane. +: represents the cavity where the TS was located. Arrows indicate the bone cortex where the TS or PDO membrane was positioned

The Mann-Whitney U test was used to compare the medians between the PDO and TS groups. The Friedman test with the Durbin-Conover *post hoc* test was used to compare the medians within the TS group at different euthanasia times, while the Wilcoxon signed-rank test was employed for the PDO group.

RESULTS

No clinical complications, such as incompatible inflammation, oedema, bleeding, abscess, or signs of animal stress, were observed in any of the samples during daily clinical evaluations. At the 60-day mark, none of the orbits implanted with PDO showed remnants of the material, preventing membrane analysis during this period. Due to the lack of fixation of the implants, both the PDO membrane and the TS implants frequently shifted from their initial positions. Figure 4 shows a histological slide of the different groups across the 3 periods.

Granulation area. Comparison between implants showed a larger average granulation tissue area around PDO at 7 and 15 days (p=0.009 and p=<.001, respectively), with a large effect size in both. Intragroup evaluation of PDO showed no significant difference between the 7 and 15-day periods (p=0.387). The TS group showed differences between periods (p=0.003), and post-tests revealed significance between the 7 vs. 15 days (p=0.041) and 7 vs. 60 days (p=0.033), while the comparison between 15 vs. 60 days showed no statistical difference (p=0.516) (Tab. 1, Fig. 5).

Inflammatory profile. Comparison between inflammatory cells in PDO vs. TS groups at 7 days revealed a higher number of plasma cells in the PDO group (p=0.012). At 15 days, the PDO group showed a higher number of neutrophils (p=<0.001), plasma cells (p=0.004), and lymphocytes (p=<0.001). Intragroup evaluation of PDO at 7 vs. 15 days showed no differences in cell counts. In the TS group, neutrophils were more abundant at 7 days compared to 15

Table 1. Area of granulation tissue measured in 1:1 panoramic reconstructions of photographs at 2.5 × magnification (mean in $mm^2 \pm SD$)

	PDO	TS	Effect size
7 days (n=6)	$13.6 \pm 7.9^{A, a}$	$3.2 \pm 0.9^{B, b}$	1.84
15 days (n=6)	$10.1 \pm 4.2^{A, a}$	$1.6 \pm 0.5^{B, c}$	2.84
60 days (n=5)	Ø	1.4 ± 0.4^{c}	

- Uppercase letters compare results in the same row (PDO_7 vs. TIT_7; PDO_15 vs. TIT_15).
- Lowercase letters compare results in the same column (PDO_7 vs. PDO_15; TIT_7 vs. TIT_15 vs. TIT_60).
- Identical letters indicate no statistical difference in the test, e.g., $^{A=A}$, while different letters indicate significant difference, e.g., $^{a \neq b}$.

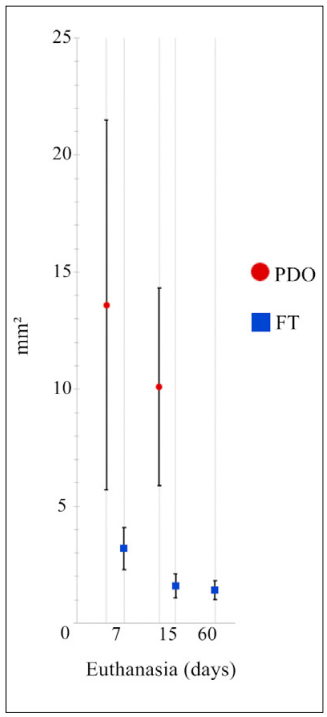

Figure 5 – Confidence interval of the means and SD of the granulation area Source:

(p=0.003) and 60 days (p=0.009). The cumulative count of inflammatory cells and their analysis in different post-operative periods showed that PDO had a higher count (thus, higher density) of these cells at 7 (p=0.01) and 15 days (p=<0.001). Blood vessels did not show statistical differences among the different groups (Tab. 2).

Table 2. Count of inflammatory cells and blood vessels in photographs at 100 × magnification (units found)

	PDO		TS		
	7 days	15 days	7 days	15 days	60 days
Neutrophils	30 $^{A, a}$	24 $^{A, a}$	21 $^{A, a}$	4 $^{B, b}$	5 ^{b}
Plasmocytes	32 $^{A, a}$	32 $^{A, a}$	14 $^{B, a}$	10 $^{B, a}$	14 ^{a}
Lymphocytes	24 $^{A, a}$	38 $^{A, a}$	19 $^{A, a}$	11 $^{B, a}$	9 ^{a}
Blood vessels	11 $^{A, a}$	5 $^{A, a}$	6 $^{A, a}$	4 $^{A, a}$	6 ^{a}

- Uppercase letters compare the results of the variable (row) between different implants within the same time frame (PDO_7 vs. TIT_7; PDO_15 vs. TIT_15).
- Lowercase letters compare the results of the variable (row) within the same implant across different time frames (PDO_7 vs. PDO_15; TIT_7 vs. TIT_15 vs. TIT_60).
- Identical letters indicate no statistical difference in the test, e.g., $^{A=A}$ or $^{a=a}$, while different letters indicate statistical significance, e.g., $^{A\neq B}$ or $^{a\neq b}$.

DISCUSSION

Although it is quite an underexplored area, previous studies have used New Zealand White Rabbits for histological evaluation following orbital reconstructions [12,15–20]. Some of these studies chose to create bone defects in the orbital margins [16–20], medial wall [15], and floor [12] to also assess the mechanical support provided by the materials to the orbital contents. To isolate the inflammatory response solely to the presence of the implant (which is a variable highly sensitive to surgical technique), in the current study, the least invasive surgical intervention possible was selected. The PDO and TS membranes were simply placed over the MT, which reduced exposure to intervening variables. This approach ensured that the inflammatory response observed was a controlled result of the peri-orbital contact with the implant.

The chosen methodology for quantitative histometric measurement of inflammation varies significantly among studies. Görgülü et al. [17] assessed the peri-orbital inflammatory response to contact with human cadaver nails using a scoring scale specifically designed to evaluate various aspects of inflammation. Notably, although this scale was initially developed to characterize wound healing in rabbits, particularly for cutaneous and mucosal tissues, its applicability to assessing the periorbital inflammatory response remains uncertain. Lee and Baek [12] used cell counting to assess the inflammatory response to an anti-adhesive solution added to Medpor®. Gu et al. [18] quantified the expression of VEGF (Vascular endothelial growth factor) in response to polyetheretherketone implants. Zheng et al. [16] evaluated compressive load-to-failure and bone ingrowth in reconstructions performed with a composite of calcium phosphate cement and recombinant human bone morphogenetic protein-2 through histomorphometry. Aral et al. [15], in their comparison of Poly L-Lactide/Glycolic Acid and Porous Polyethylene, employed scoring scales to evaluate orbital content protrusion as visualized on postoperative tomography. They also used these scales to assess histological findings.

Due to descriptive heterogeneity, it was not possible to correlate the results obtained in the current study with other studies. In the study, inflammatory cell counting and granulation area measurement were chose as quantitative measures that reflect the extent and intensity of tissue repair. This approach reduces the subjectivity associated with score-based assessments and allows for direct comparisons between different studies using implants of the same diameter.

The inflammatory density and granulation area of PDO were greater and took longer to regress than TS implants. The inflammatory profile at 7 days was similar between groups, except for plasmocytes, which were found in greater quantities in the PDO group. Plasma cells are responsible, among other functions, for producing immunoglobulins [21]. From the results of the current study it was not possible to assert that the difference in plasma cell count would lead to a heightened immune response upon a second exposure, as this response involves several other factors that were not assessed in the study. Moreover, the authors are not aware of any studies that have exposed experimental units to PDO more than once to compare reactions between the first and second exposures.

Because PDO is fully absorbable, it is expected that it will induce more inflammation than titanium, which is considered the gold standard in biocompatibility. Despite this difference, the biological safety of PDO has already been established [22]. Although PDO for orbital reconstruction has not yet been approved by the FDA for use in the United States, it has been approved in Europe and is utilized in orbital reconstruction procedures. It has been studied in randomized clinical trials [23,24], retrospective cohorts [25], prospective case series [10], and retrospective observational studies [8,26].

The authors believe that the presented study has partially addressed the knowledge gap regarding the histometric evaluation of periorbital inflammatory processes in contact with PDO. Further studies comparing the periorbital inflammatory response to PDO with other absorbable materials – such as hyaluronate/carboxymethylcellulose (HA/CMC), polylactic acid/polyglycolic acid (PLA/PGA), polyglactin 910/PDS, among others – may be beneficial in providing greater insight into this field.

Limitations of the study. Although the 60-day period to evaluate the final stages of PDO membrane degradation was excessively long, it demonstrated that the tissue in contact showed no signs of fibrosis or permanent alterations. Deep decalcification of the orbit was only achieved after serial reductions of the histological specimens, which led to some detachment between tissue planes within the blocks.

CONCLUSIONS

As this is a laboratory study of animal samples, direct extrapolation of the results to humans should not be performed. Considering the limitations of the methodology employed, it was concluded that the PDO membrane caused a greater inflammatory process compared to TS, as expected. However, this inflammation did not extend to adjacent periorbital tissues and did not result in relevant clinical changes during the post-operative follow-up. Therefore, PDO may be considered for controlled clinical studies in humans,

aiming at the reconstruction of small orbital defects that do not require mechanical support.

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