Patient-specific testicular prosthesis prototype produced with polylactic acid and coated with cyanoacrylate or polymethylmethacrylate

Estudo experimental de prótese testicular paciente-específico produzido com ácido polilático e revestido por cianocrilato ou polimetilmetacrilato em ratos

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**Camila Calvi Menegassi Ferreira**
Master in Veterinary Sciences
Institution: Universidade Federal de Mato Grosso (UFMT)
Address: Av. Fernando Corrêa da Costa, 2367, Boa Esperança, Cuiabá – MT, CEP: 78060-900
E-mail: camila_menegassi@hotmail.com

**Lianna Ghisi Gomes**
PhD in Veterinary Sciences
Institution: Universidade Federal de Mato Grosso (UFMT)
Address: Av. Fernando Corrêa da Costa, 2367, Boa Esperança, Cuiabá – MT, CEP: 78060-900
E-mail: liannaghisi@gmail.com

**Sabela Godoy Menezes**
PhD in Veterinary Sciences
Institution: Universidade Federal de Mato Grosso (UFMT)
Address: Av. Fernando Corrêa da Costa, 2367, Boa Esperança, Cuiabá – MT, CEP: 78060-900
E-mail: belinhagodoy@hotmail.com

**Paloma Moraes Lobo**
Master in Veterinary Sciences
Institution: Universidade Federal de Mato Grosso (UFMT)
Address: Av. Fernando Corrêa da Costa, 2367, Boa Esperança, Cuiabá – MT, CEP: 78060-900
E-mail: pml.lobomoraes@gmail.com
In order to reduce the psychological and aesthetic consequences caused by elective or therapeutic orchiectomy, we developed two types of testicular prostheses, which were produced using a 3D printer, with polylactic acid filament, and coated with cyanoacrylate or polymethylmethacrylate (PMMA). They were tested in Wistar rats, with orchiectomy performed on the left testicle and the prosthesis implanted inside the tunica vaginalis, in group 1 we implanted the prosthesis coated with cyanoacrylate and in group 2 with PMMA. The implants showed interesting features such as low cost, reduced production time, and ease of customization for the patient. Two rats in group 1 had monocular vaginalitis in the region of the tunica vaginalis implant and 3 (3/10, 30%) had fibrous dermatitis. In group 2, 5 rats (50%) had fibrous dermatitis, and none had alterations in the
tunica vaginalis. We performed a logistic regression model, the results showed no significant association between the type of prosthesis and the presence of inflammation in the tunica vaginalis (p=0.08) or fibrous dermatitis (p=0.22). However, we suggest further studies with cyanoacrylate. PMMA proved to be an excellent option as a biomaterial for prosthesis. Fibrous dermatitis occurred due to the dermal healing process caused by the surgical incision.

Keywords: implants, 3D printer, biomaterials.

1 INTRODUCTION

The use of implants to repair or replace parts of the human body has increased in recent years, mainly because of the increase in life expectancy of the population (Bertol et al., 2017). Thus, the demand for biomaterials grows by 5–10% per year (Pereira et al., 2005).

Several conditions have led to the need for patients to undergo therapeutic orchietomy, including unilateral cryptorchidism, torsion of the spermatic cord, and prostate tumors. Although effective, orchietomy has both aesthetic and psychological drawbacks. However, in 1939, the first testicular implant procedure in human patient was
accomplished inside the tunica albuginea, thus mitigating the consequences of the procedure, as it resolved the problem from both functional and aesthetic perspectives (Palma & Rodrigues, 1987).

Silicone patches and silicone gel prostheses have been used since the 1970s (Puranick et al., 1973). Although silicone is known as an inert material, the observation of reactive cells over time, appearance of local inflammation, and migration of silicone particles have created a lot of suspicion about this inertia. When the high cost of a material is added to the aforementioned disadvantages, the search for a new material becomes necessary (Hage et al., 1999).

Cyanoacrylate is an ester of cyanoacrylic acid with an alkyl side-chain. Commercially, it is presented as a liquid and transparent synthetic adhesive that undergoes polymerization at room temperature when in contact with an adherent surface, thereby promoting low heat release (Tessarioli et al., 2014)). Its adhesion power was quickly recognized after its discovery in 1949, and in the late 1950s, cyanoacrylates began to be used in medicine as tissue adhesives in medicine (Leggat et al., 2007) including plastic surgery, digestive tract, orthopedic, and ophthalmologic surgeries. Its application has also been described in surgical procedures involving the kidneys, liver, and blood vessels of men and animals (Ko et al., 2019).

Polymethylmethacrylate (PMMA) is another material with a chemical structure similar to that of silicone and is also known to be inert as a denture material. Another positive factor is that it has a low acquisition cost and is easy to manufacture (Karademir et al., 2004).

In veterinary medicine, prostheses are used frequently in orthopedics, ophthalmology, and soft tissue surgeries such as herniorrhaphy. In order to obtain adequate population control of animals in Brazil, actions such as conscious guarding and elective castration have been promoted (Oliveira et al., 2012). The population's prejudice in relation to the aesthetic alteration in the males as a result of castration, often makes authorization of the surgical procedure difficult.

To date, there is no more effective medical treatment than orchiectomy to drastically reduce serum testosterone levels (Resnick, 1984), and this surgical procedure...
leads to the development of psychological problems in men (Chapple & McPherson, 2004). In addition, within the scope of veterinary medicine, developing an option to maintain the aesthetic standard of the animal can encourage elective orchiectomy and consequently assist in population control. Therefore, it is extremely important to work on projects that develop alternative testicular prostheses that meet the requirements considered ideal, which can be useful to minimize eventual problems caused by orchiectomy. In this context, we produced two models of testicular prostheses using a 3D printer and combined materials and tested their application in an experimental study in rats. The results obtained were significant to validate a new type of testicular prosthesis, allowing its wide acquisition by the population and use.

2 MATERIAL AND METHODS

Twenty male Wistar rats (*Rattus norvegicus*) aged between 6 months and 1 year with an average weight of 400 g (ranging from 250 g - 500 g) were used (CEUA-UFMT 23108.028184-2019-98). Group 1 consisted of 10 animals that underwent implantation of a prosthesis made of polylactic acid and coated with cyanoacrylate. Group 2 was composed of 10 animals that underwent implantation of a testicular prosthesis produced with polylactic acid and coated with polymethylmethacrylate.

The prostheses were produced in patient-specific sizes, so before surgery an ultrasound was performed for the purpose of to evaluate the dimensions.

The data obtained in the ultrasound were sent through the software to the 3D printer (GTmax3D, model coreA3. V2). The filament used was 3DX made of polylactic acid (PLA) with a diameter of 1.75 mm.

The prosthesis had a firm consistency with a lattice-like interior. They were printed with a thin rod to facilitate handling when coating the second material used. The cyanoacrylate or polymethylmethacrylate coating was made by submerging the prototype through the stem, removing the excess, and cutting the stem. The implants had the following dimensions on average: 19.2 mm × 11.14 mm × 8.1 mm. The coating layer was approximately 1 mm thick and smooth. After completion, the prostheses were submitted to a polishing process.
Before implantation, the prostheses were washed with 2% chlorhexidine, submerged in 70% alcohol, kept under ultraviolet light for 1 h, and then sent to the operating room in sterile packaging.

Rats were premedicated with intramuscular administration of ketamine (Cetamin® 40 mg/kg, Rhobifarma Indústria Farmacêutica Ltda., Brazil), midazolam (midazolam generic drug 1 mg/kg, União Química Farmacêutica Nacional S/A, Brazil), and methadone (Mytedom® 2mg/kg, Cristália Produtos Químicos Farmacêuticos Ltda., Brazil). Anesthetic induction and maintenance were performed using isoflurane with universal bubbling through a mask. During the operation, meloxicam (Maxicam® 2% 1 mg/kg, Ouro Fino Saúde Animal Ltda., Brazil) was administered subcutaneously, and enrofloxacin (Floxiclin® 10 mg/kg, Biofarm Tecnologia em Veterinária, Brazil) was administered subcutaneously. During the entire anesthetic recovery period, 1 drop of glucose was administered orally every 30 min.

Wide shaving of the scrotum region and previous antisepsis of the site were performed, and then the animals were subjected to orchiectomy of the left testis, followed by implantation of the prosthesis under the tunica vaginalis, and a simple stitch was made with absorbable multifilament thread 4-0 Polyglactin (Vicryl®, Johnson & Johnson do Brasil Indústria e Comércio de Produtos para Saúde Ltda., Brazil). A retaining stitch was made in the muscles above the prosthesis to keep it inside the scrotum, thus preventing it from passing through the inguinal canal. This is followed by a simple pattern skin suture of non-absorbable 3-0 nylon monofilament thread (Procare®, Labor Import Comércio de Importação e Exportação Ltda., Brazil). The right testis was preserved for use as a negative control for inflammation.

The scrotum was examined daily for 30 d after the surgical procedure, and the animals were assisted until the surgical wound was completely healed. All animals showed normophagia, normodipsia, normochezia, and normouria throughout the postoperative period.

Thirty days after the surgical procedure, the animals were euthanized, in strict accordance with the protocols defined in Federal Council of Veterinary Medicine (CFMV) Resolution Nº 1.000, on March 11, 2012. Necropsy was performed at the
Laboratory of Veterinary Pathology at UFMT, and the complete scrotum and tissues adjacent to the implants were evaluated and fixed in 10% formalin for 24 h. Tissues of interest were routinely sectioned and processed, embedded in paraffin blocks, cut at 5 μm and stained with hematoxylin and eosin (H&E), and analyzed under an optical microscope. Inflammatory and cellular reactions in the scrotum and tissues adjacent to the lining material used in the prototype were evaluated.

Data was analyzed using a logistic regression model. The test was performed to evaluate the dependency and the effects of each prosthesis biomaterial on the local tissues. The prosthesis type was used as predictor (independent variable) and tunica vaginalis inflammation or fibrosis dermatitis as a fixed effect (dependent variable). The response variable was evaluated considering the presence or absence of damage. Results were considered statistically significant at a p-value below 0.05. All statistical analysis were performed with the R Core Team (2021) software, Package glm – method binomial.

3 RESULTS

The implant production process is fast, simple, and effective. One day before the surgical procedure, the process began by determining the dimensions of the patient's testicles, through the ultrasound of the scrotum, followed by the production and printing of the interior of the prosthesis with polylactic acid by the 3D printer, therefore the procedure was carried out, coating and finishing with combined materials, which can be cyanoacrylate or polymethylmethacrylate. This was followed with sterilization of the prototype.

In all animals, healing of the surgical wound was completed within one week. No surgical wound infection, implant contamination, or signs suggestive of prosthesis rejection were observed.

In both groups, scrotal asymmetry or swelling was not present, and the prostheses were mobile in the region of the scrotum and without palpable reactive tissue.

In the histopathological examination of the scrotum, of the 10 rats included in group 1 (cyanoacrylate), three (30%) showed multifocal dermal fibrosis in the deep dermis, adjacent and serous musculature, proliferation of fibroblasts with slight collagen
deposition, and moderate edema. Additionally, there was a slight infiltration of lymphocytes, plasma cells, and occasional mast cells (Fig. 1). In seven rats (70%), no changes were observed (Tab. 1).

Figure 1. Photomicrograph of the scrotum of *Rattus norvegicus* after 30 days of cyanoacrylate group. The tunica vaginalis is irregularly thick, and in most of the section evaluated, it is enlarged from two to three times the normal size, often containing a mild to moderate inflammatory infiltrate composed of lymphocytes, plasma cells, macrophages and rare neutrophils. In some areas, the musculature adjacent to the serosa contains a moderate infiltrate, with a predominance of mononuclear cells and proliferation of fibroblasts with slight collagen deposition. Morphological diagnosis of mononuclear and erosive, irregular, extensive mild to moderate vaginalitis.

Table 1. Data was analyzed using a logistic regression model. The results showed no significant association between prosthesis type and the presence of tunica vaginalis inflammation (p-value = 0.08) or fibrous dermatitis (p-value = 0.22).

<table>
<thead>
<tr>
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<th>PMMA (%)</th>
<th>Cyano (%)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Tunica vaginalis inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0%)</td>
<td>2 (20%)</td>
<td>0.08</td>
</tr>
<tr>
<td>No</td>
<td>10 (100%)</td>
<td>8 (80%)</td>
<td></td>
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<tr>
<td>Fibrous dermatitis</td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5 (50%)</td>
<td>7 (70%)</td>
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</tr>
</tbody>
</table>

Source: Data from the survey itself.

After surgery, only two rats in group 2, with the PMMA prosthesis coating, presented with edema and incisional hematoma until the fifth day after surgery, after which appearance and consistency normalized in the following days.
In group 2 (PMMA), out of 10 (100%) rats, five (50%) showed dermal fibrosis similar to that observed in groups 1, and five (50%) did not present with any type of lesion.

Regarding the histopathological changes in the tunica vaginalis region (Tab. 1), in group 1 (cyanoacrylate), two (20%) had mononuclear and erosive vaginalis, noting that the tunica vaginalis was irregularly thickened and diffusely enlarged, three times the normal size, containing moderate infiltrate composed of lymphocytes, plasma cells, macrophages, and rare neutrophils and eosinophils. There was an occasional disappearance of the lining mesothelium, and mesothelial proliferation (hyperplasia) was observed in a few areas. In some areas, the musculature adjacent to the serosa had a moderate inflammatory infiltrate, with a predominance of mononuclear cells and fibroblast proliferation with mild collagen deposition (Fig. 2). Eight rats (80%) showed no change.

![Photomicrograph of the scrotum of *Rattus novergicus* after 30 days of PMMA group. Multifocal dermal fibrosis in the deep dermis, adjacent and serous musculature, proliferation of fibroblasts with slight collagen deposition, and moderate edema. Additionally, there was a slight infiltration of lymphocytes, plasma cells, and occasional mastcells.](image)

Source: Data from the survey itself.

No animal in the PMMA group showed histological changes in the tunica vaginalis region.
Logistic regression is often used to evaluate causality effect where the response variable is categorical. The results showed no significant association between prosthesis type and the presence of tunica vaginalis inflammation (p-value = 0.08) or fibrous dermatitis (p-value = 0.22).

4 DISCUSSION

The technology used to print objects using 3D printers is becoming increasingly accessible and useful. For surgeons, 3D printing has been widely used in research, surgical training, and preoperative planning (Jacobs & Lin, 2017). In this study, using the 3D printer, we were able to obtain implants quickly and efficiently, with a period of only 30 min spent to print the interior of each prosthesis. In addition, because software is used as a tool for sending data to the printer, it is possible to set up a database in which only the dimensions of the testicles are changed before printing. This accelerates production and makes the process more flexible, which allows the prostheses to be fully customized and/or patient-specific, helping to reduce the risk of inflammation since the prosthesis is individually modeled.

Three-dimensional (3D) printing is a process in which a product is built layer-by-layer based on a sketch developed in software. This technology facilitates free-form design and production of custom objects and shapes, including complex geometries. In human medicine, this technique is beginning to revolutionize medical and surgical procedures (Hoy, 2013; Chae et al., 2015). However, although 3D printing has advanced recently, mainly in the manufacture of ocular prostheses, there is still much room for research in the field of development of new techniques and the use of this material (Alam et al., 2017; Ko et al., 2019).

Numerous biomaterials have been investigated worldwide for use in implants; however, only a few have been approved and certified by health agencies. For example, in Brazil, there is only one certified product currently available for orbital implants, distributed under the trade name Medpor®8. However, as these products are not competitive, they have a high acquisition cost, which prevents their use by the broad population as it makes the presence of these materials in most public hospitals difficult.
(Kormann et al., 2019). In veterinary medicine, cost interferes even more, as it is an elective procedure. Therefore, we developed two low-cost prostheses that would facilitate the acquisition of the material for a broad population in both human and veterinary medicine.

A disadvantage of both prostheses created in this study is their extremely firm consistency, which significantly alters the sensitivity of the patient in relation to the implant. The future of testicular implants is primarily geared towards achieving complete patient satisfaction from the point of view of generating the most natural sensitivity and appearance (Hayon & Coward, 2020).

The most important parameters in the evaluation of prostheses are the degree of inflammatory response and level of formation of the surrounding fibroconnective tissue. Therefore, to be considered a good implant material, it must induce minimal inflammation and be surrounded by a thin fibrotic capsule (Ye et al., 1999).

In the present study, two rats in the group with the cyanoacrylate-coated prosthesis showed a mild degree of inflammation in the tunica vaginalis. Studies have shown that cyanoacrylate derivatives with a short carbon chain, such as the ethyl cyanoacrylate used here, are poorly biocompatible and present rapid degradation at application sites, causing intense inflammatory reactions (Leggat et al., 2007). Although no statistically significant difference was found between the two groups in this study, the tunica vaginalis analysis demonstrated a p-value near to significant (p-value = 0.08) when compared with PMMA group. Since, when the categorial data is evaluated as a response variable probability of incidence in the population should be considered to avoid false positives. The fact of the p-value be not significant reflects the small incidence in this population (PMMA group). In other words, this result can be attributed to the small number of animals in each group, and for this reason, we suggest further experiments by increasing the sample group.

Another possibility would be to change the type of cyanoacrylate used in the experiment, since cyanoacrylate derivatives with a long carbon side chain (more than four carbons) are considered more biocompatible and degrade slowly, promoting a slower release of degradation products and less toxicity, thus causing fewer local inflammatory
reactions when compared to short-chain cyanoacrylates, as used in this study (Leggat et al., 2007).

Rats with implants coated with polymethylmethacrylate did not show any inflammatory reaction in the tissues adjacent to the prosthesis. In a study that histologically analyzed the bone repair process in the frontal bone of rats in the face of the addition of PMMA, no signs of inflammation or bone necrosis were observed, and the biocompatibility of the material was observed through the formation of a fibrous capsule (Lins et al., 2008). Since 1960, PMMA has been shown to be immunologically inactive as an implant material, and reports have described its use as a hip prosthesis and dental prosthesis, and it has been widely used in the field of ophthalmology (Karademir et al., 2004), has described its use in urology, which makes our experiment unusual and motivates us to expand studies in this area.

PMMA is produced through an exothermic reaction, in which the temperature can reach more than 80ºC. Although the monomer liquid is extremely allergenic and cytotoxic (Eppley, 2002), the mixing and initial polymerization process took place outside the implantation site one day before the surgical procedure; thus, there is minimal contact between the free monomers and the tissue.

One advantage of PMMA as a coating for our implant is that it is affordable, which would facilitate its commercialization, especially for elective procedures in veterinary medicine, where the cost factor is decisive for the authorization of surgical procedures.

Both groups had rats with fibrous dermatitis, but the p-value was not significant, and considering that the dermatitis fibrosis was observed 30 days after the surgical incision, it is suggested that such observation is only a consequence of the final healing process of the dermis. Karademir et al., (2004) evaluated testicular prostheses made of polymethylmethacrylate in rats and made a sample group of 10 rats, in which only one incision was made in the tunica vaginalis, followed by suturing, to serve as a control group. After 3 months, the testes were removed along with the scrotum, and histological analysis was performed, which revealed that 100% of the animals did not show any type of inflammation, thus, corroborating that the dermatitis found in this study should be considered just the end of the skin healing process.
5 CONCLUSIONS

Biomaterials and new technologies for implants need to be intensively studied and evaluated as the demand increases every year, with an increase in life expectancy in both humans and animals.

The 3D printer is a great work tool for surgical centers, both for its efficiency and for fast production. To reduce the cost of prostheses, it is only necessary to cover them with biomaterials.

PMMA has proven to be a great option for use as a testicular implant, as it does not present any type of rejection or inflammation and is easily accessible. However, cyanoacrylate requires further studies with a larger sample size, so we can conclude its efficiency as a biomaterial.
REFERENCES


