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Potential of Migration and Tissue Reactions to Intraperitoneally Implanted Polymethyl Methacrylate in Wistar Rats

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Abstract

Objective: This study aimed to evaluate the performance of polymethyl methacrylate (PMMA) when implanted into the peritoneal cavities of Wistar rats, specifically its potential for migration to the examined organs and possible tissue reactions at the implantation site.

Methods: Adult male Wistar rats (290-400 g) were distributed randomly into 2 groups: P4 (n=20), to which 0.4 mL of PMMA was administered; and P2 (n= 0), to which was 2 mL of PMMA were administered. These groups were subsequently divided into 4 subgroups according to the day on which euthanasia occurred (day 1, 7, 14, or 28).

Results: The changes in animal weight prior to PMMA implantation and after euthanasia were compared. No nodules or signs of PMMA implantation or any other macroscopic alterations were observed in the spleen, kidneys, lungs, or liver. However, mild-to-moderate inflammatory infiltration in which macrophages, lymphocytes, and neutrophils predominated was observed in at least 1 of the 4 organs examined from each animal during the experimental time points. Mild inflammatory responses were observed that were rich in histiocytes and giant cells and featured increased numbers of blood vessels and fibroblasts in the pericapsular regions of the studied organs. In 29 cases, a greyish material compatible with PMMA was identified. There was no migration of PMMA into the parenchyma of the examined organs. The changes in the tissues were the same regardless of the volume of implanted PMMA. Following staining with HE, the PMMA particles in the tissues were a grayish color, measuring approximately 30-40 μm in size, and were found to have stimulated histiocytic reactions in 29 examined organs.

Conclusions: The results showed that the most affected tissues were the visceral peritoneum of the spleen and the kidneys from group P2. Our study may contribute to add a new proof of testing implants.

Keywords: Polymethyl methacrylate; Grooves; Wrinkles; Scar; Tissue; Particles; Tissue repair

Introduction

Various types of particles have been used in body tissues with the aim of filling and restoring tissue grooves, wrinkles and scars or increasing the volumes of certain body regions for reconstructive or aesthetic purposes [1-10]. For these purposes, the ideal material should be biocompatible, safe, and stable at the implantation site; additionally, this material should not cause protrusion through the skin or mucosa, as well as, should induce only minimal reactions against the foreign body, as phagocytosis-resistant and lose the potential to migrate to distant body sites. Moreover, this material should be inert in body fluids and easily manipulated during surgery; above all, it should not induce rejection or late complications [7-17].

Some studies have shown that polymethylmethacrylate (PMMA) is biocompatible, does not generate an inflammatory response when implanted under the skin, and remains at the implantation site for long periods [11,18,19]. Because of its small number of side effects and its lasting results, PMMA is believed to be a good solution for dermal filling. However, with the popularization of its use reports of complications following PMMA implantation have increased significantly [20-29]. Therefore, understanding the tissue alterations caused by PMMA implantation is essential for discover the causes of complications inherent to this filling procedure.

The present study aimed to evaluate the performance of PMMA when implanted into the peritoneal cavities of Wistar rats, specifically its potential for migration to the examined organs and possible tissue reactions at the implantation site.

Materials and Methods

Animal model

This study was conducted on 40 adult male Wistar rats (Rattus norvegicus albinus and Rodentia: mammalia), weighing between 290 g and 400 g. The animals were reared in the Animal Facility of the Laboratory of Surgical Techniques and Experimental Surgery, Department of Surgery, at the Federal University of Uberlandia

(FAMED/UFU) and treated according to the criteria established by the Brazilian College of Animal Experimentation. All animal experimentation was conducted under a protocol in agreement with ethical principles in animal research. The experimental protocol were submitted and approved by Ethics Committee on Animal Experimentation of the Federal University of Uberlandia, protocol number: declaration of February 3/2006.

Therapeutic protocol

After weighing, the animals were anesthetized via subcutaneous injection of a solution of 2-(2,6-xylidine)-5,6-dihydro-4H-1,3-thiazine hydrochloride and ketamine hydrochloride (both at 0.4 mg/kg) on the posterior side of the left thigh. The animals were randomly divided into 2 groups (Figure 1). Either 0.4 mL (P4) or 2.0 mL (P2) of polymethylmethacrylate (PMMA), commercially known as Newplastic® (ANVISA - Brazilian Regulatory Agency - Register number 80256510001, Le Bon, RS, Brazil), in 30% carboxymethyl cellulose (vehicle), was inoculated into the peritoneal cavities of rats.

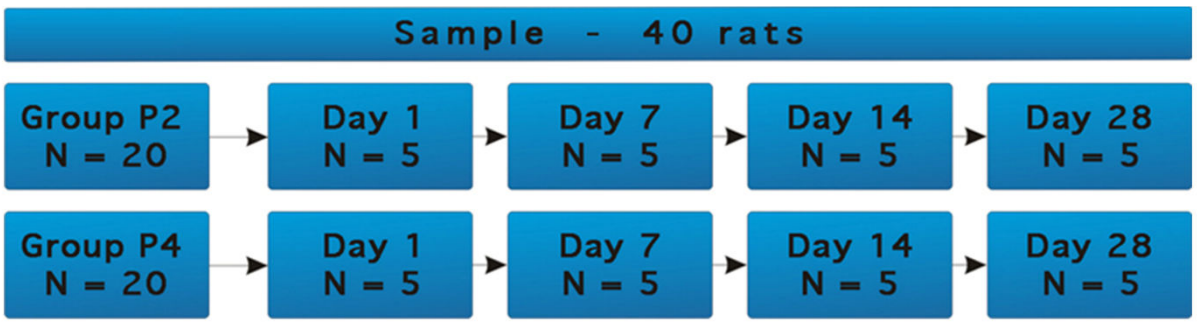

Figure 1. Illustration of the experimental design. The animals were randomly divided into 2 groups. Either 0.4 mL (P4) or 2.0 mL (P2) of polymethylmethacrylate (PMMA), was inoculated into the peritoneal cavities of rats. The 2 groups were further divided into 4 subgroups according to the day of euthanasia (days 1, 7, 14, and 28).

The 2 groups were further divided into 4 subgroups according to the day of euthanasia (days 1, 7, 14, and 28; Figure 1).

Histological analysis

After the euthanasia the organs were collected (spleen, kidneys, lungs, and liver). Tissue specimens were fixed in 10% buffered formalin and routinely processed for paraffin embedding. Sections of 5 µm were obtained and stained with: Hematoxylin-eosin to assess tissue integrity under an optical microscope (Zeiss®; Carl Zeiss AG, Oberkochen, Germany).

Assessment of inflammatory response

The inflammatory infiltrates were rated according to intensity using a grading scale that ranged from 0-3, as: Grade 0 (absent); Grade 1(mild); Grade 2 (moderate) and Grade (intense). Fibrogenesis, neovascularization, necrotic areas, and the presence of PMMA micro particles was graded as: Grade 0 (absent) and Grade 1 (present). The organs were assessed for the presence of the following cell types: neutrophils, mast cells, lymphocytes, plasmocytes, histiocytes, eosinophils, lymphohistiocytes, and lymphoplasmocytes.

Statistical analysis

All the values were expressed as means ± S.D. Qualitative variables were expressed as frequencies and percentages. The analysis of variance test was used for comparisons of the means. Pearson's chi-squared test or Fisher's exact test was used to evaluate the correlations between the intensity of inflammation, cell-type predominance, neovascularization, fibrogenesis, necrosis, presence of PMMA particles, and granulomatous foreign-body reactions in the P2 and P4 groups. A significance level of 5% was adopted. The analysis were obtained by SPSS statistics, version 21 (SPSS Inc., Chicago, IL, USA) for statistical analysis.

Results

Body weight changes

The changes in animal weight prior to PMMA implantation and after euthanasia were compared. After the experimental period, it was possible to observe an increase in the weight of the animals, compared to the weight initial. The animals of P4 group had an increase in body weight, in all the subgroups, with values of 342 ± 32.1 g (day 1); 322 ± 16.4 g (day 7); 383 ± 19.5 g (day 14) and 396 ± 68.8 g (day 28) (Table 1).

P4	P2

Euthanasia	Weight initial (g)	Weight final (g)	Variation (g)	Weight initial (g)	Weight final (g)	Variation (g)
	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
Day 1	316.00 (24.08)	342.00 (32.11)	26.00 (15.17)	308.00 (24.90)	352.00 (26.83)	44.00 (23.02)
Day 7	312.00 (17.89)	322.00 (16:43)	10.00 (18.71)	310.00 (29.15)	316.00 (48.27)	6.00 (21.91)
Day 14	326.00 (36.47)	384.00 (19.49)	58.00 (29.00)	310.00 (25.50)	330.00 (39.37)	20.00 (14.14)
Day 28	350.00 (47.96)	396.00 (68.78)	46.00 (15.07)	380.00 (23.45)	350.00 (14.14)	-30.10 (33.17)

Table 1: Mean initial weights, mean final weights, and mean weight variations (in grams). Weights of Wistar rats implanted with 0.4 mL or 2.0 mL of PMMA, according to the day of euthanasia.

The P2 group also had an increase in body weight, with the exception of the subgroup "day 28" that showed a decrease in body weight. The values to this group were 352 ± 26.8 g (day 1); 316.0 ± 48.3 g (day 7); 330.0 ± 39.3 g (day 14) and 350.0 ± 14.1 g (day 28). The subgroup "day 28" presented a difference of - 30.10 ± 33.2 g, compared to the weight initial (Table 1).

Anatomopathological assessment-macroscopic examination of the organs

The macroscopic examinations of the peritoneal cavities in all study groups indicated no signs of extrinsic intestinal obstruction, abscesses, adherence between the intestinal loops and the parietal peritoneum, or the presence of peritoneal fluid. No nodules or signs of PMMA implantation or any other macroscopic alterations were observed in the spleen, kidneys, lungs, or liver (Figure 2).

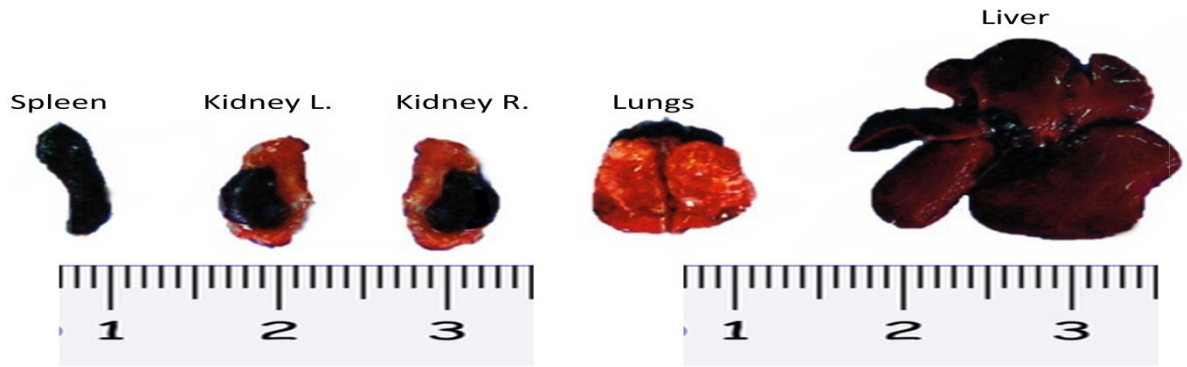

Figure 2: Macroscopic examination of the organs. After the euthanasia the organs were collected and analyzed. No nodules or signs of PMMA implantation or any other macroscopic alterations were observed in the spleen, kidneys, lungs, or liver in all study groups.

Anatomopathological assessment-microscopic examination of the organs

The analysis parameters, including the inflammation intensity, predominant cell type, neovascularization, fibrogenesis, necrosis, presence of PMMA particles, and granulomatous foreign-body reactions, were compared at different periods after euthanasia, as shown in Tables 2-5.

Group/Animal	Organ	Infiltrate	PC	Neo	Fib	Necrosis	PMMA	FBR
P4/1	Spleen	2	H/N	1	1	0	0	0
P4/2	Kidney	1	H/N	1	1	0	0	0
P4/3	Kidney	1	H/N	1	1	0	0	0
P4/4	Kidney	1	H/N	1	0	0	0	0

P4/5	Spleen	1	H/N	0.1	0	0	0	0
P2/1	Spleen	1	H/N	0	1	0	0	0
	Kidney	1	H/N	1	1	0	0	0
P2/2	Kidney	1	H/N	1	1	0	0	0
P2/3	Kidney	1	H/N	0.1	1	0	0	0
P2/4	Kidney	1	H/N	1	0	0	0	0
P2/5	Kidney	1	H/N	1	1	0	1	0

Table 2: Histological examination following euthanasia at 1 day post-injection. Sub-group/Identification (Group/Animal); Organ affected (organ);Inflammation intensity (infiltrate): Mild (1), Moderate (2); Predominant cell (PC) type: Neutrophils (N), Histiocytes (H); Neovascularization (Neo): Absent (0), Present (1);Fibrogenesis (Fib): Absent (0), Present (1); Necrosis: Absent (0), Present (1); PMMA: Absent (0), Present (1); Granulomatous foreign-body reaction (FBR): Absent (0), Present (1).

Mild-to-moderate inflammatory infiltration in which macrophages, lymphocytes, and neutrophils predominated was observed in at least 1 of the 4 organs examined from each animal during the experimental time points (Tables 2-5).

Group/Animal	Organ	Infiltrate	PC	Neo	Fib	Necrosis	PMMA	FBR
P4/1	Spleen	1	H	0	1	0	0	0
	Kidney	1	H	0	1	0	0	0
P4/2	Spleen	2	LH	0	1	0	1	1
	Kidney	2	H	1	1	0	1	0
P4/3	Spleen	1	H/N	0	1	0	0	0
	Kidney	1	H/N	0	1	0	0	0
P4/4	Spleen	2	LH	0	1	0	1	0
P4/5	Kidney	1	H	0	1	0	1	0
P2/1	Liver	2	H/N	1	1	0	1	0
	Spleen	3	H/N	1	1	0	1	0
	Kidney	2	H/N	1	1	0	1	0
P2/2	Spleen	1	H/N	1	1	0	0	0
	Kidney	1	H/N	1	1	0	0	0
P2/3	Liver	2	H/N	0.1	1	0	1	1
	Kidney	2	H/N	1	1	0	1	0
P2/4	Spleen	2	H	1	1	0	1	0
	Kidney	1	H/N	1	1	0	0	0
P2/5	Spleen	2	H/N	0	1	0	1	0

Table 3: Histological examination following euthanasia at 7 days post-injection. Sub group/Identification (Group/Animal); Organ affected; Inflammation intensity (infiltrate): Mild (1), Moderate (2), Intense (3); Predominant cell (PC) type: Lymphohistiocytes (LH), Neutrophils (N), Histiocytes(H);Neovascularization (Neo): Absent (0), Present (1);Fibrogenesis (Fib): Absent (0), Present (1); Necrosis: Absent (0), Present (1); PMMA: Absent (0), Present (1); Granulomatous foreign body reaction (FBR): Absent (0), Present (1).

Histological assessment of organs

In the implantation sites where PMMA particles could be visualized, the inflammatory infiltrates were initially located peripherally to the implant and were more centrally located at 28 days post-euthanasia (Figure 3). The areas of hyperemia and neovascularization around the PMMA implantation site increased progressively during the evaluated time points (Figure 3).

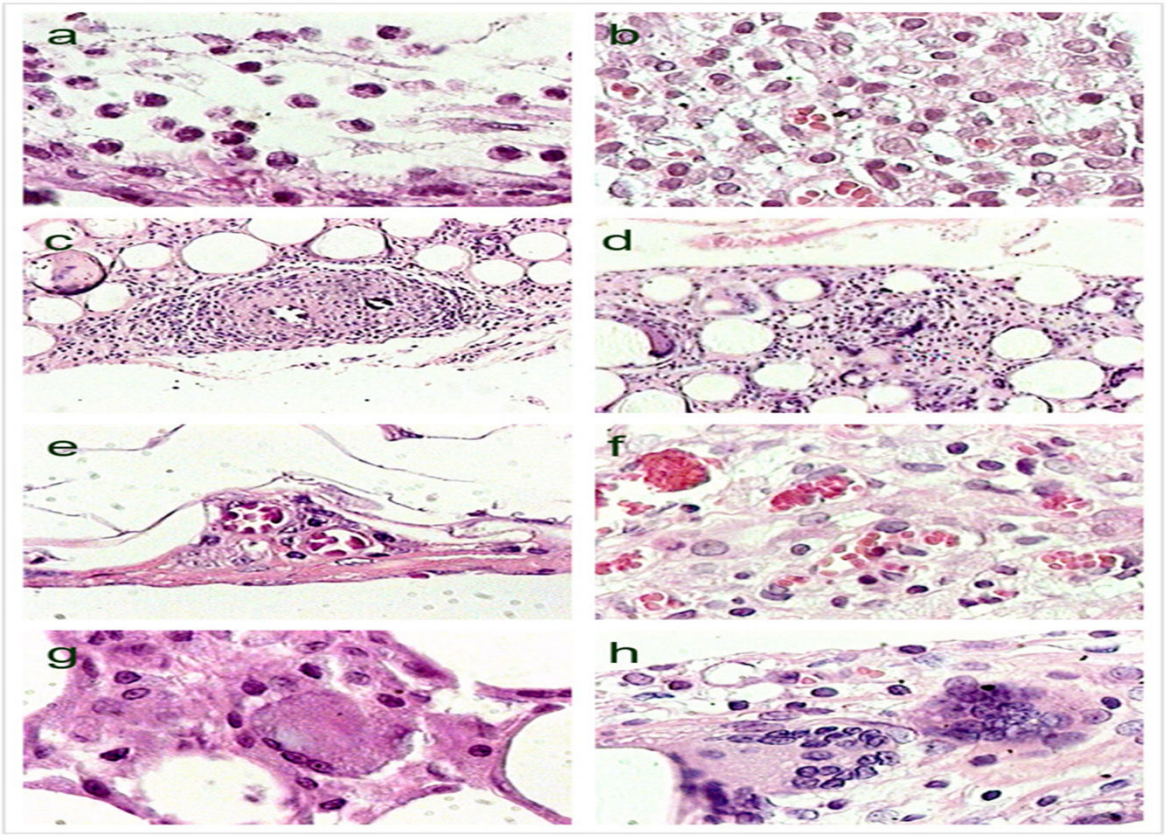

Figure 3

Figure 3: Photomicrograph of perirenal tissue from rat P2/1 at 14 days after euthanasia. The tissue was stained with HE and examined at a 10x magnification to reveal the predominant neutrophilic inflammation. (a) Perisplenic tissue from rat P2/2 at 28 days after euthanasia; predominant lymphohistiocytic inflammation. (b) Perihepatic tissues from rats P2/1 and P2/5 at 7 and 28 days after euthanasia, respectively; inflammatory infiltrates on the periphery of the implantation site and (c) increase of spaces between the microspheres in the presence of the granuloma. (d) Perisplenic tissues from rats P2/1 and P2/2 at 28 days after euthanasia. (e and f) Areas of mild hyperemia and intense neovascularization. (g and h) Perisplenic tissue from rat P4/4 at 28 days after euthanasia, thus revealing the presence of giant cells.

Group/Animal	Organ	Infiltrate	PC	Neo	Fib	Necrosis	PMMA	FBR
P4/1	Spleen	2	LH	0	1	0	1	0
	Kidney	1	H/N	1	1	0	0	0
P4/2	Spleen	2	LH	0	1	0	1	0
	Kidney	2	LH	1	1	0	1	1
P4/3	Kidney	1	H/N	0	1	0	0	0
P4/4	Kidney	1	H	0	1	0	0	0

	Spleen	3	LH	1	1	0	1	0
P4/5	Kidney	1	H/N	1	1	0	0	0
	Spleen	2	LH	1	1	0	0	0
P2/1	Kidney	1	H/N	1	1	0	1	0
P2/2	-	0	-	0	0	0	0	0
P2/3	-	0	-	0	0	0	0	0
P2/4	Liver	2	H	0	1	0	1	0
	Liver	2	H	0	1	0	1	0
	Spleen	2	LH	1	1	0	1	0
P2/5	Kidney	2	LH	1	1	0	1	0

Table 4: Histological examination following euthanasia at 14 days post-injection. Sub-group/Identification (Group/Animal); Organ affected; Inflammation intensity (infiltrate): Mild (1), Moderate (2), Intense (3); Predominant cell (PC) type: Lymphohistiocytes (LH), Neutrophils (N), Histiocytes (H);Neovascularization (Neo): Absent (0), Present (1); Fibrogenesis (Fib): Absent (0), Present (1); Necrosis: Absent (0), Present (1); PMMA: Absent (0), Present (1); Granulomatous foreign body reaction (FBR): Absent (0), Present (1).

However, necrotic areas were not observed in any case (Figure 4). The granulomatous foreign-body reaction incidence was higher at 28 days post-euthanasia (Table 5).

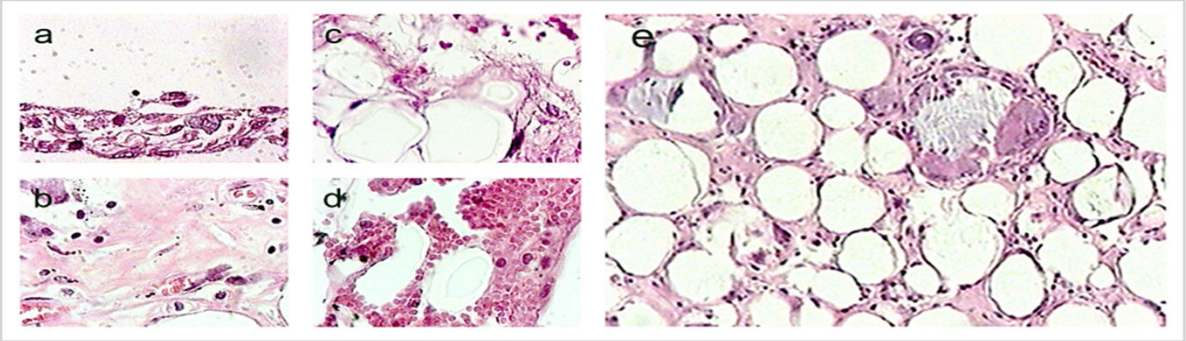

Figure 4: Photomicrographs of perihepatic tissue from rat P2/1 at 7 days and rat P2/5 at 28 days after euthanasia. The tissues were stained with HE and examined at 10x magnification to reveal young fibroblasts. (a) Mature fibroblasts; (b) surrounded by dense collagen. Perihepatic tissue from rat P2/1 at 7 days after euthanasia (c) and perisplenic tissues from rat P2/1 at 28 days after euthanasia (d) and rat P4/5 at 14 day after euthanasia (e). The images reveal the presence of differently sized PMMA microspheres surrounded by lymphohistiocytic inflammatory infiltrates.

Beginning at day 7 post-euthanasia in both animal groups, fibrous tissue deposition occurred between the microspheres and near the implant sites. This deposition exhibited a pseudo capsular aspect with varying thicknesses (Figure 4).

Group/Animal	Organ	Infiltrate	PC	Neo	Fib	Necrosis	PMMA	FBR
P4/1	Spleen	1	H	0	1	0	0	0
P4/2	Spleen	1	LH	0	1	0	1	0
P4/3	Spleen	1	H	0	1	0	0	0
P4/4	Spleen	2	LH	0	1	0	1	1

	Kidney	2	LH	0	1	0	1	1
	Spleen	2	LH	0	1	0	1	1
P4/5	Kidney	2	LH	0	1	0	0	1
	Spleen	3	LH	1	1	0	1	0
P2/1	Kidney	1	H	1	1	0	0	0
	Spleen	3	LH	1	1	0	1	0
P2/2	Kidney	1	H/N	1	1	0	0	0
	Spleen	3	LH	1	1	0	1	0
P2/3	Kidney	1	H/N	1	1	0	0	0
	Spleen	1	LH	0	1	0	0	0
P2/4	Kidney	1	H/N	1	1	0	0	0
	Liver	1	H	0	1	0	1	0
P2/5	Kidney	1	H	0	0	0	0	0

Table 5: Histological examination following euthanasia at 28 days post-injection. Sub group/Identification (Group/Animal); Organ affected; Inflammation intensity (infiltrate): Mild (1), Moderate (2), Intense (3); Predominant cell (PC) type: Lymphohistiocytes (LH), Neutrophils (N), Histiocytes (H);Neovascularization (Neo): Absent (0), Present (1); Fibrogenesis (Fib): Absent (0), Present (1); Necrosis: Absent (0), Present (1); PMMA: Absent (0), Present (1); Granulomatous foreign body reaction (FBR): Absent (0), Present (1).

Following staining with HE, the PMMA particles in the tissues were a grayish color, measuring approximately 30-40 μm in size, and were found to have stimulated histiocytic reactions in 29 examined organs. The most affected tissues were the visceral peritoneum of the spleen and the kidneys from group P2 (Figure 4).

Discussion

The PMMA microspheres used in this study exhibited ideal characteristics in terms of inertness, stability at room temperature, availability, low production cost, physical consistency at the implantation site similar to that of un implanted tissue, and durability [7-12,18,19,28,29].

The microsphere sizes followed the recommendations of previous studies that showed that the use of 30-50 μm diameter particles prevented phagocytosis and migration [30-32].

The inflammatory reactions induced by the intraperitoneal administration of PMMA to rats were mild-to-moderate in intensity and characterized by the presence of histiocytes, foreign-body giant cells, and newly formed connective tissue. These results were similar to those reported in the literature for other sites of PMMA implantation [7, 20,31,32].

PMMA microspheres stimulate local neovascularization via the induction of inflammatory reactions (granulomatous foreign-body reaction) involving monocytes, histiocytes, and fibroblasts [31,33]. Moreover, growth factors secreted by histiocytes, such as fibroblast growth factor and vascular endothelial growth factor, might be responsible for the vascular proliferation observed at the implantation site, considering that histiocytes predominated in the inflammatory infiltrates [31,33].

Giant cells were observed at 7, 14, and 28 days post-euthanasia without significant differences between the 2 groups. These results were similar to those reported in previous studies, wherein giant cells were always present at the implantation sites [7,11,12,28,29].

Impurities on the surfaces of the PMMA microspheres might be primarily responsible for stimulating immune reactions against the polymer; these reactions manifest histologically by the formation of foreign-body granulomas [34,35]. However, it is unclear whether this acute inflammatory process is directly associated with PMMA implantation, as granulomatous foreign-body reactions may occur up to 6 years after the subcutaneous implantation of PMMA [22,35].

PMMA particles associated with a vehicle containing bovine collagen are encapsulated by the collagen immediately after the procedure. In addition, the collagen is replaced by connective tissue at approximately 2-3 months after implantation. Therefore, an observation period longer than 1 month is needed to accurately assess fibroplasia onset with collagen deposition [35-38].

In this study, granulomatous foreign-body reactions were only found in 5 examined organs. In contrast to the results obtained when PMMA was associated with bovine collagen, the vehicle used in the present study was carboxymethyl cellulose, which might have reduced the formation of granulomas. Furthermore, collagen deposition around the PMMA implantation site increased progressively throughout the study period. However, individual encapsulation of the beads was observed in the groups euthanized at 28 days.

When using carboxymethyl cellulose gel as the vehicle, the time required for its reabsorption was found to be less than 2 weeks [2]. Because of its hydrophilic nature, this gel will most likely be reabsorbed more rapidly than would a colloid. This rapid reabsorption could explain the close proximity of the microspheres that persisted

until day 14, and this proximity might hinder the migration of the inflammatory infiltrate to the implantation area [2].

The peritoneum was chosen as the route of PMMA administration because of its high absorption capacity, which would facilitate particle migration to the cavity organs [39]. A comparative study of Zyderm® (collagen) and Artecoll® application in guinea pigs demonstrated that these products were subject to degradation and phagocytosis and could undergo transepithelial elimination after intradermal application [40].

In previous studies, 3 routes by which PMMA microspheres can migrate have been described [41,42].

Hematogenic route: after an inadvertent injection of particles into a large-caliber vessel, the particles are ultimately transported to the pulmonary capillaries.

Lymphatic route: after the injection of particles into large-caliber lymphatic vessels, the particles are transported to the lungs and regional lymph nodes.

Phagocytic route: upon phagocytosis by macrophages, PMMA particles are absorbed in the implantation site and subsequently migrate to the regional lymph nodes.

The peritoneum is a serious membrane that lines the walls of the abdominal cavity and its internal organs. This membrane forms a large cavity that is divided into smaller cavities via the mesofolds and corresponding organs. Although generally inactive, the peritoneum can be used as a large filtering system to replace, at least temporarily, kidneys that are not functioning efficiently [41,42].

Peritoneal absorption involves lymphatic absorption through the stomata or through orifices located on adjacent sides of the peritoneal mesothelial cells. The material absorbed through the stomata is directed to terminal lymphocytes known as lacunae that are located on the peritoneal surface of the diaphragm. From the lacunae, the particles are transported to the venous system via the main thoracic duct. Therefore, if secondary migration were detected after peritoneal PMMA implantation, the local lymph nodes and lungs would be the most probable final destinations of these particles. However, secondary migration did not appear to occur because PMMA particles were not observed in the lungs [41,42].

In this study, the presence of PMMA micro particles was only detected in the visceral peritoneum of 29 organs studied. In addition, the high viscosity of the vehicle containing PMMA may explain the ability of PMMA to accumulate at the site of implantation through adherence.

There are several possible explanations for the absence of PMMA in the parenchyma of the examined organs: PMMA particles were not absorbed because their diameters might have exceeded those of the stomata;

PMMA particles might have been absorbed via phagocytosis and enzymatic degradation;

PMMA might have migrated to body compartments that were distinct from the filtering organs;

The sites selected for histological examination were distinct from those containing the implantation sites; however, this is unlikely because the nodules formed by the injection of PMMA were easily noticeable.

Similar to our results, other study conducted experimental injections of PMMA into peritoneal cavity of female B6C3F1 mice, whose was euthanized 1, 7 and 28 days later. The histological studies found PMMA particles determinate enlarged and activated spleens with marked deposits of particles in the red pulp. These authors used PMMA particles with size 1.4 and 6.4 micro in diameter. The small size of PMMA particles in this study my reforces our negative findings into visceral organs in our mice, probably due the large PMMA particles used in our experiment (measuring approximately 30-40 μm) [42,43].

With regard to the different volumes of PMMA administered in this study, larger volumes are believed to induce stronger tissue reactions at the implantation sites and increase the probability of migration to cavity organs. However, these assumptions were confirmed, as no significant differences were observed between groups P2 and P4.

Studies concluded that this kind of study into abdomen of mice is an excellent testing situation for implants of different composition and shape before use in humans. In concordance to these authors, our study may contribute to add a new proof of testing implants. This point is relevant to dermatological practice due ethical and legal issues, which imply considers extensive tests of new products in experimental models before use in humans for therapeutic or aesthetic proposes [44]. PMMA micro particles were observed predominantly in the visceral peritoneum but not in the parenchyma of some organs, including the spleen, kidneys, lungs, and liver, after intraperitoneal implantation in rats. Tissue alterations were similar between groups P2 and P4 and thus did not depend on the volumes of injected PMMA. These alterations were also similar to those observed in other implantation sites, as reported in the literature.

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Author contributions

The authors contributed equally to the conception, design, conduct, interpretation, writing and editing of the current work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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