Aesth Plast Surg
https://doi.org/10.1007/s00266-022-03243-5

ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Polymethylmethacrylate Microspheres are Immunologically Inert in Mouse Tissues

Anália Cirqueira Milhomem[1] · Rodrigo Saar Gomes[1] · Fernanda Dias Tomé[1] · Felipe dos Santos Arruda[1] · Pablo Igor Ribeiro Franco[1] · Eduardo Luiz da Costa[2] · Jonathas Xavier Pereira[1] · Marina Clare Vinaud[1] · Ruy de Souza Lino Júnior[1]

Received: 22 November 2022 / Accepted: 18 December 2022

Abstract
Nowadays, aesthetic concerns have gained attention, especially by patients looking for a less invasive alternative to minor facial corrections. Polymethylmethacrylate (PMMA) is widely used as a soft tissue filler; the demand for this polymer has increased, and along with it, there are some reports of adverse reactions. Such adverse reactions stem from consequences of immune and inflammatory reactions to PMMA. Some animal models have been used to unravel the causes of these reactions, among other factors involving the management of PMMA. The aim of this study was to determine the immunogenic profile of PMMA implantation in different anatomical planes of mice, over up to 360 experimental days. In this study, BALB/c mice were divided into 30 groups for immune evaluation of the interaction between the organism and the polymer; 2% PMMA was implanted subcutaneously, 10% intramuscularly and 30% in periosteal juxtaposition and followed during five experimental days (7, 30, 90, 180 and 360 days after implantation-DAI). Pro- and anti-inflammatory cytokines (IL-2, IL-4, IL-6, IFN-gamma, TNF, IL-17A, IL-10 and TGF-beta) were quantified in all experimental days. There was no statistical difference between the groups analyzed considering the evaluated parameters. Therefore, at all implanted depths, PMMA behaved inertly in a murine model.
No Level Assigned This journal requires that authors assign a level of evidence to each submission to which Evidence-Based Medicine rankings are applicable. This excludes Review Articles, Book Reviews, and manuscripts that concern Basic Science, Animal Studies, Cadaver Studies, and Experimental Studies. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Polymethylmethacrylate · Anti-inflammatory agents · Esthetics · Bioplasty · Cytokines

✉ Ruy de Souza Lino Júnior
ruy@ufg.br

Anália Cirqueira Milhomem
analiabmdc@gmail.com

Rodrigo Saar Gomes
rodrigosaar@ufg.br

Fernanda Dias Tomé
fernandadiast@gmail.com

Felipe dos Santos Arruda
arrudaf@gmail.com

Pablo Igor Ribeiro Franco
pablo_igor@hotmail.com

Eduardo Luiz da Costa
dreduardo@bioplastiabrasil.com

Jonathas Xavier Pereira
jonathasxp@gmail.com

Marina Clare Vinaud
mvinaud@ufg.br

[1] Department of Biosciences and Technology , Institute of Tropical Pathology and Public Health (IPTSP), Federal University of Goias (UFG), Rua 235, s/n.° - Setor Leste Universitário, Goiânia, Goiás, Brazil

[2] Bioplastia Brasil, Rua T-55, n° 938, sala 1009; Edifício EBM Walk Bueno – Setor Bueno, Goiânia, Goiás, Brazil

Published online: 06 January 2023

Introduction

Bioplasty is the term that defines the application of polymethylmethacrylate (PMMA) to correct depressions in soft tissues or bone volumes. It is a non-invasive and non-surgical approach. Currently, this technique is widespread around the world, and along with the increase in its application, reports of adverse reactions to this polymer have also grown [1, 2]. It is believed that these reactions are the result of incompatibilities related to the biomaterial, the patient or even the implantation technique [2, 3]. One of the important factors in the post-implantation outcome is the immunological reaction generated in the patient by the presence of the polymer [3, 4].

Mostly, immunity to PMMA is activated from the moment of its implantation, through the adsorption of blood proteins on its surface, adhesion of macrophages (MO) and activation of the complement system. Simultaneously, the coagulation cascade is activated and provisional extracellular matrix (ECM) is formed around the biomaterial. The damage-associated molecular patterns (DAMPs) released at the time of implantation signal the migration of mast cells and neutrophils, which degranulate, activate MO, and acute inflammation sets in [5–9].

Between two and five weeks after implantation, the MO are polarized in classically activated (M1) or alternatively activated (M2) from the interaction with the ECM; CD4+ T lymphocytes potentiate MO differentiation and foreign body giant cell (FBGC) formation. In parallel, MO activate fibroblasts that phagocyte damaged tissue and stimulate the formation of a fibrous capsule to isolate PMMA, characterizing chronic inflammation [5–9]. Alarmins released by tissue damage from polymer implantation also activate inflammasomes, which, depending on how they are modulated, can exacerbate the pro-inflammatory reaction or signal tissue repair [7].

FBGC, M1 and neutrophils are responsible for secreting nitric oxide (NO) and other proteolytic enzymes. These corrode the surface of the biomaterial and release molecular patterns associated with biomaterials, the BAMPs, which are considered by the body as alarmins, intensifying the inflammatory reaction to the implant [10].

The formation of FBGC occurs due to the inability of MO to phagocyte PMMA; thus, they fuse due to the stimulation mainly from interleukin 4 (IL-4) and interleukin 3 (IL-3) cytokines. The FBGC is more effective against the polymer and protects against the apoptosis mechanism. In up to 30 days, these cells secrete cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor (TNF), highly pro-inflammatory components, in an attempt to degrade and destroy the polymer. Subsequently, the FBGC begins to secrete regulatory cytokines, such as interleukin 10 (IL-10) and the transforming growth factor beta (TGF-β); given its inability to destroy the polymer, the organism converges to its isolation. In this phase, neutrophils secrete extracellular neutrophil traps (NETs), responsible for covering the surface of the material and facilitating its recognition by other cell types, such as MO [5–8, 11, 12].

The microenvironment of chronic inflammation associated with FBGC results in the formation of a fibrous capsule. For this, M2 and fibroblasts secrete TGF-β, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Additionally, metalloproteinases (MMP) secreted by MO are also involved in ECM remodeling. Fibroblasts and endothelial cells produce collagen and other proteins; MO, fibroblasts, collagen and angiogenesis form the granulation tissue. Over time, this tissue matures into a less cellular and more collagenous capsule and type III collagen is gradually replaced by type I [5–8, 11]. However, this immunological and inflammatory cascade is not always triggered in this way, there are reports of adverse reactions, and the reasons why these events occur are not fully elucidated [4, 13, 14]. Experimental models serve as tools that make it possible to unravel these events [11, 15–17].

Understanding the immunological factors responsible for the biocompatibility and long-term stability of PMMA is important to avoid further complications and allows greater usability of this biomaterial. Therefore, the objective of this study was to describe the immunogenic profile of PMMA implantation in different anatomical planes of mice, over up to 360 experimental days.

Materials and Methods

Ethical Considerations

The Ethics Committee on the Use of Animals (CEUA) of the Federal University of Goiás (UFG) approved this study, under protocol number 027/2017.

Experimental Design

Male BALB/c mice aged between 8 and 12 weeks and weighing between 20 and 30 grams were used. Their matrices were kept in the animal's facilities of the Tropical Pathology and Public Health Institute (IPTSP) in Federal University of Goias (UFG), Brazil.

Three hundred animals were divided into 30 groups (n=10), where the implantation of 2% PMMA in subcutaneous, 10% in intramuscular and 30% in juxta-periosteal

Table 1: Experimental design considering implantation period, anatomical plane and concentration of polymethylmethacrylate (PMMA).

DAI	Anatomical plane		
___	Subcutaneous	Intramuscular	Juxta-periosteal
7	CTS 7DAI	CTI 7DAI	CTJ7DAI
	2%S 7DAI	10%I 7DAI	30%J 7DAI
30	CTS 30DAI	CTI 30DAI	CTJ 30DAI
	2%S 30DAI	10%I 30DAI	30%J 30DAI
90	CTS 90DAI	CTI 90DAI	CTJ 90DAI
	2%S 90DAI	10%I 90DAI	30%J 90DAI
180	CTS 180DAI	CTI 180DAI	CTJ 180DAI
	2%S 180 DAI	10%I 180DAI	30%J 180DAI
360	CTS 360DAI	CTI 360DAI	CTJ 360DAI
	2%S 360 DAI	10%I 360DAI	30%J 360DAI

DAI days after implantation, *S* subcutaneous, *I* intramuscular, *J* periosteal juxtaposition, *CTS* control group of the subcutaneous region, *CTI* control group of the intramuscular region, *CTJ* control group of the juxta-periosteal region.

was performed over 5 experimental periods (7, 30, 90, 180 and 360 days after implantation-DAI). Such groups are described in Table 1.

Implantation Procedure

Mice were randomly selected and anesthetized intraperitoneally with a solution of ketamine 100 mg/mL and xylazine 20 mg/mL in the proportion of 0.1 mL/10 g of animal weight. Then, they were shaved in the distal portion of the hind limbs for implantation according to the gold standard method of PMMA injection, tunneling [18].

1 μL of PMMA Linnea Safe® (Lots: 1608J, 1608G and 1608I) was implanted in the right hind limb of the mice, varying the implantation depth, to form the test groups, while in the contralateral paw of these same animals, sterile 0.9% NaCl (1 μL) was injected, varying the depth, to form the control groups.

Euthanasia

Upon completing 7, 30, 90, 180 or 360 DAI, euthanasia was performed with a lethal dose of anesthetic (ketamine 300mg/kg + xylazine 30mg/kg intraperitoneally). The right and left hind limbs were dissected to remove the muscle where PMMA or NaCl was implanted and bone separation. Samples were maintained in a cell lysis and protease inhibition solution containing 1% NP40 + 1% Protease Inhibitor Cocktail (SIGMA-ALDRICH P8340) + 98% PBS 1x. Then, the content was mechanically macerated for 10 seconds with the aid of a tissue macerator (Tissue Master 125 Homogenizer-Elisabeth® Pharmacon). After maceration, the samples were centrifuged for 10 minutes at 25°C and 2000rpm; the supernatant was collected and stored at – 20 °C.

Flow Cytometry Quantification of Cytokines

The cytokines interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-17A (IL-17A), interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) were measured using the Cytometric Bead Array (CBA) technique, according to the manufacturer's instructions ("Mouse,Th1/Th2/Th17, Cytokine,Kit,RUO-560485 | BD Biosciences-US") [19].

The samples were incubated with beads, at different fluorescence intensities, conjugated with a specific capture antibody for each cytokine. Then, specific antibodies for each cytokine, conjugated with phycoerythrin (PE), were added. After incubation, the beads were washed with the kit's own solution and analyzed in a flow cytometer (BD FACSCanto II Flow Cytometer). The specific beads for each cytokine were separated by emitting different fluorescence. The acquired data were analyzed using the FCAP Array 2.0 software (SoftFlow-USA), and the cytokine concentrations were calculated from the standard curve. Cytokine concentrations were normalized from the concentration of total proteins, measured by the microLowry method, according to the manufacturer's instructions.

Statistical Analysis

The data obtained were organized in spreadsheets in the Microsoft Excel 2010 software. To determine the concentration of the analyzed cytokines, the equation of the straight line was calculated. Finally, such concentrations had their normality and variance tested in the GraphPad Prism program (8.0), so that the groups could then be tested and compared using the most appropriate test. The Wilcoxon test was used to compare the test and control groups on the same experimental day and two-way ANOVA to compare the test groups over the five experimental periods; differences were considered statistically significant when $p \leq 0.05$.

Results

The implantation of 2% PMMA in the subcutaneous region (Fig. 1), at 10% in the muscle tissue (Fig. 2) and at 30% in the juxta-periosteal region (Fig. 3) did not change the kinetics of production of the cytokines analyzed throughout all the experimental periods ($p > 0.05$). The quantification of IL-4 in all experimental groups did not reach the lower limit of detection (data not shown). It is important to highlight that there were no animal losses during this study, nor infections in the experimental sites.

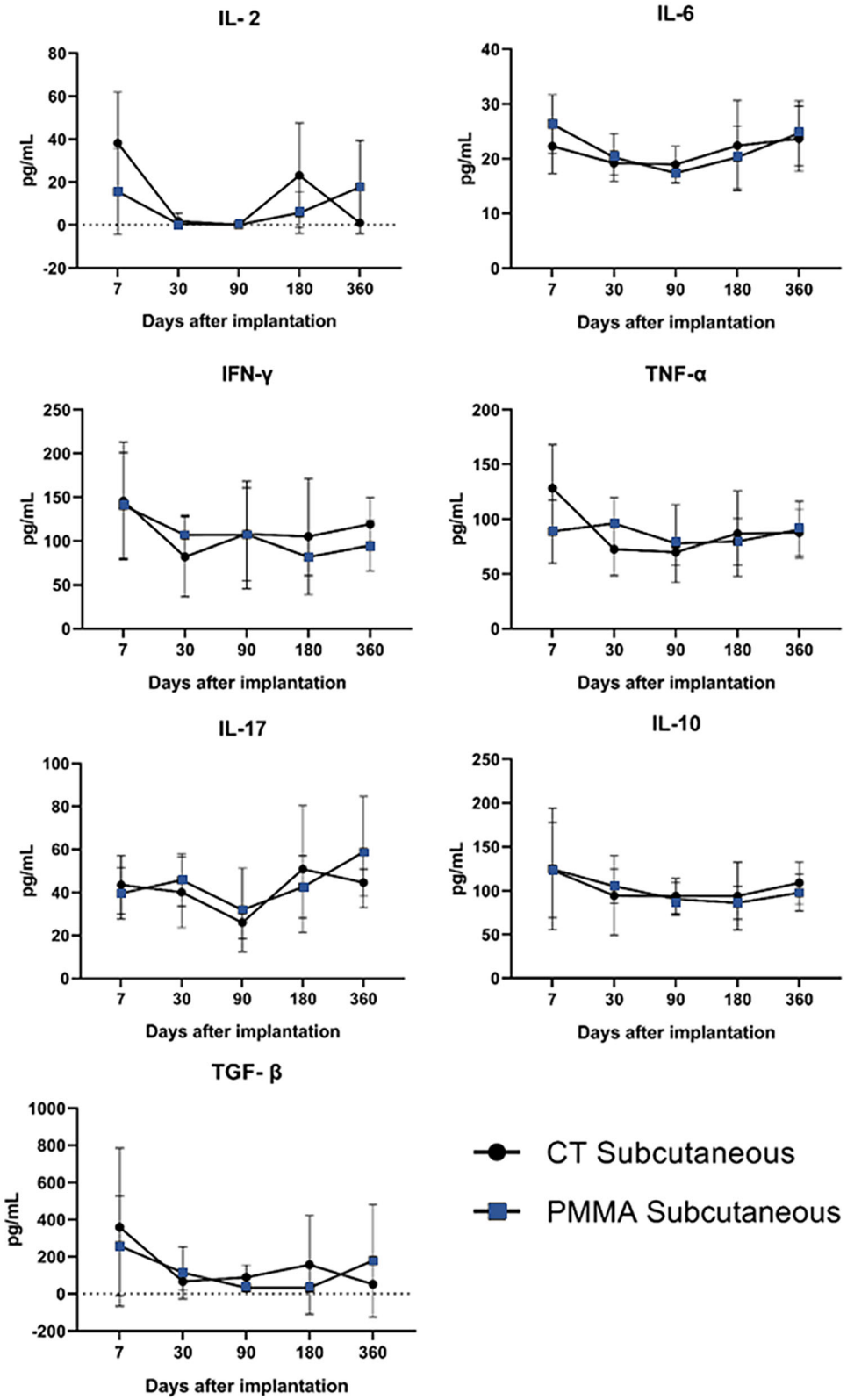

Fig. 1 Quantification of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin -17A (IL-17A), interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) in the subcutaneous region of mice after implantation or not of 2% polymethylmethacrylate (PMMA) on different experimental days. CTS7: subcutaneous control group at 7 days after implantation (DAI); CTS30: subcutaneous control group at 30 DAI; CTS90: subcutaneous control group at 90 DAI; CTS180: subcutaneous control group at 180 DAI; CTS360: subcutaneous control group at 360 DAI; 2%S7: group that received 2% PMMA implantation in the subcutaneous region, at 7 DAI; 2%S30: group that received 2% PMMA implantation in the subcutaneous region, at 30 DAI; 2%S90: group that received 2% PMMA implantation in the subcutaneous region, at 90 DAI; 2%S180: group that received 2% PMMA implantation in the subcutaneous region, at 180 DAI; 2%S360: group that received 2% PMMA implantation in the subcutaneous region, at 360 DAI. Wilcoxon and two-way ANOVA tests were applied, and differences were considered statistically significant when $p \leq 0.05$ (*)

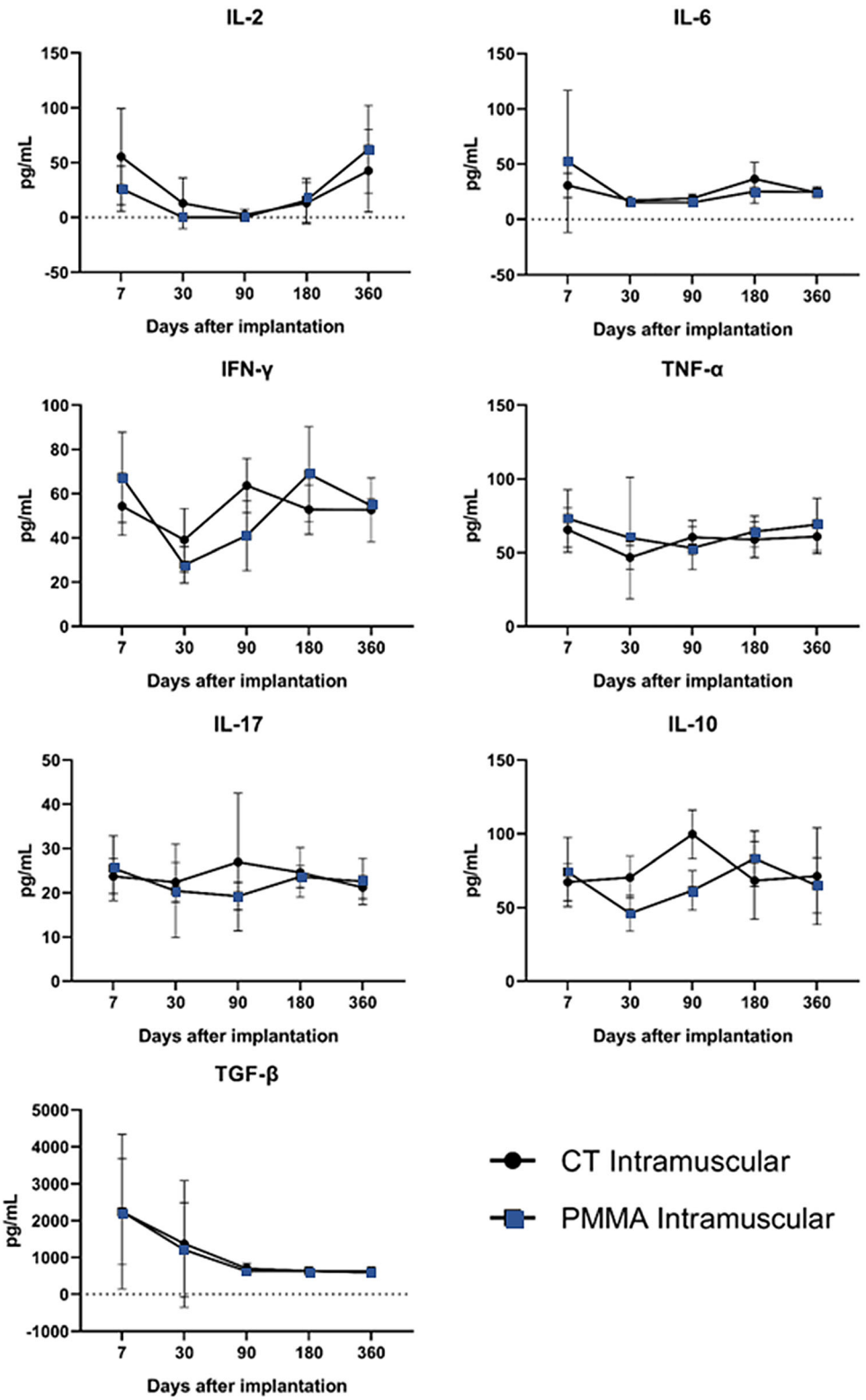

Fig. 2 Quantification of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-17A (IL-17A), interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) in the intramuscular region of mice after implantation or not of 10% polymethylmethacrylate (PMMA) on different experimental days. CTI7: intramuscular control group at 7 days after implantation (DAI); CTI90: intramuscular control group at 90 DAI; CTI180: Intramuscular control group at 180 DAI; CTI360: intramuscular control group at 350 DAI; 10%I7: group that received 10% PMMA implantation in the subcutaneous region, at 7 DAI; 10%I30: group that received 10% PMMA implantation in the subcutaneous region, at 30 DAI; 10%I90: group that received 10% PMMA implantation in the subcutaneous region, at 90 DAI; 10%I180: group that received 10% PMMA implantation in the subcutaneous region, at 180 DAI; 10%I360: group that received 10% PMMA implantation in the subcutaneous region, at 360 DAI. Wilcoxon and two-way ANOVA tests were applied, and differences were considered statistically significant when $p \leq 0.05$ (*)

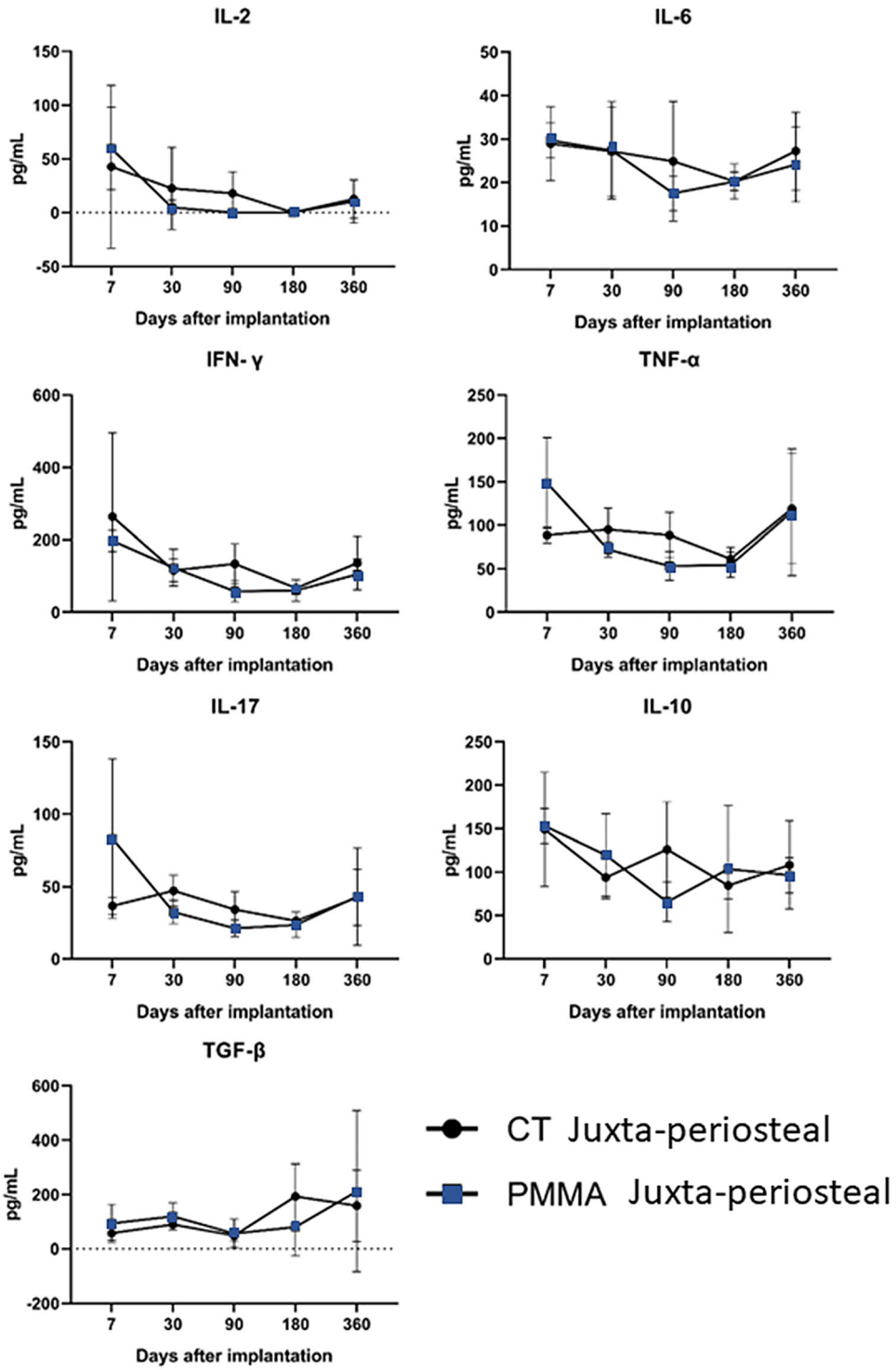

Fig. 3 Quantification of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-17A (IL-17A), interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) in the periosteal fair region of mice after implantation or not of 30% polymethylmethacrylate (PMMA) on different experimental days. CTJ7: juxta-periosteal control group at 7 DAI; CTJ30: control group juxta-periosteal at 30 DAI; CTJ90: juxta-periosteal control group at 90 DAI; CTJ180: juxta-periosteal control group at 180 DAI; CTJ360: juxta-periosteal control group at 360 DAI; 30%J7: group that received 30% PMMA implantation in the periosteal juxtaposition at 7 DAI; 30%J30: group that received 30% PMMA implantation in the periosteal juxta region, at 30 DAI; 30%J90: group that received implantation of 30% PMMA in the periosteal juxta region, at 90 DAI; 30%J180: group that received 30% PMMA implantation in the periosteal fair region, at 180 DAI and 30%J360: group that received 30% PMMA implantation in the periosteal fair region, at 360 DAI. Wilcoxon and two-way ANOVA tests were applied, and differences were considered statistically significant when $p \leq 0.05$ (*)

Discussion

The presence of PMMA in the tissue of mice stimulates an immune response, but statistically similar to that generated after injection of saline solution. These results allow us to infer that, under these conditions, PMMA is immunologically inert, and most likely biocompatible and safe.

The use of different concentrations of PMMA was chosen in order to understand the interaction between the product and the tissue. It has been described that higher concentrations of PMMA in superficial planes are more related to the development of local complications such as fibrosis, nodules and telangiectasia [20]. However, our results have shown that these complications are more

related to individual immunological response and the application technique.

Williams (1987) proposed the first widely accepted definition for biocompatibility, based on the principle that a material implanted in the organism must perform its function successfully and generate an appropriate and adequate biological response according to its application [21]. Currently, biocompatibility is defined as the ability of a material to function with an appropriate host response. A biocompatible material must interact with living systems without developing injury, toxicity or rejection by the immune system. Furthermore, it is to induce an appropriate and beneficial response in a host, without generating local or systemic undesirable effects [22].

According to Raut et al. (2020), the successful outcome after application of a biomaterial is the complete acceptability of its presence by the organism, that is, its biocompatibility. This, in turn, depends on three main factors: 1—the polymer cannot cause cytotoxicity, genotoxicity, mutagenicity, carcinogenicity and exacerbated immunogenicity; 2—the biomaterial must fulfill its function in relation to the medical therapy and purpose in which it was applied; 3—in addition to having to generate a beneficial and appropriate cellular and tissue response for its purpose [23].

It is possible to infer that the first factor that interferes with biocompatibility must already be tested and specified by the manufacturer prior to the clinical use of the polymer, while the second should be a factor of attention for the professional responsible for the use of the material, directing its best management depending on the purpose. The third interfering factor in the biocompatibility of a biomaterial is the cellular and tissue response of the host to it. In this study, we demonstrated that the cellular response, evaluated by the production of the main cytokines involved in the entire inflammatory process, was inert, that is, similar to the physiological expression. Our research group, in unpublished data, also showed that the tissue response was appropriate for the purpose, depending on the depth of PMMA implantation.

The immunological inertia of PMMA in the subcutaneous region was maintained throughout the experimental period, since there was no statistical difference regarding the quantification of cytokines between the experimental groups. Of the three anatomical regions of PMMA implantation, the subcutaneous one is the most vascularized. This feature facilitates greater adsorption of blood proteins to the surface of the biomaterial, therefore, amplification of immune and inflammatory signaling and, therefore, vast migration of immune cells to the implantation site [24]. Fortunately, these inflammatory processes did not occur significantly in the groups analyzed.

There was no difference between the test and control groups of the same experimental day regarding the production of IFN-γ after PMMA implantation in the subcutaneous region. Cassini-Vieira et al. (2018) reported after their experimental study with implantation of polymeric polyether-polyurethane sponges that the low expression of IFN-γ attenuates the foreign body reaction to this subcutaneous implant in mice, since this cytokine seems to contribute to inflammatory angiogenesis and the fibrogenesis of synthetic implants [25].

The DAMPs released in the subcutaneous region after the implantation procedure are probably the main responsible for the production of pro-inflammatory cytokines (IL-2 and IFN-γ) at 7 DAI. On the other hand, at 180 and 360 DAI, there was greater expression of IL-2 and IL-17 cytokines, which may be related to the animal's natural aging processes. The life expectancy of mice kept in the laboratory varies from 2 to 3 years, depending on the physical conditions of the place and the level of disturbance to which the animal is subjected throughout life [26]. Aging generates a picture of systemic inflammation, with increased cellular oxidative stress and increased infiltration of immune cells, especially in adipose tissue, such as neutrophils, lymphocytes and macrophages, inducing secretion of pro-inflammatory cytokines [27, 28].

Subcutaneous IL-17 and TGF-β secretion tends to increase in the late phase of the foreign body reaction (180 and 360 DAI). When associating these data, a trend of IL-2 increase in the same period is observed. This increase may be related to the involvement of aging processes, oxidative stress, in addition to the presence of immune response regulation. Additionally, it is suggested that at 360 DAI there is a pro-inflammatory stimulus of BAMP's, for the activation and proliferation of IL-17- and IL-2-producing cells, which generate a positive feedback microenvironment [26, 29].

The late increase in IL-17 may also indicate a greater propensity for the development of autoimmune diseases, since it plays a central role in the management of this class of diseases, due to its highly pro-inflammatory characteristic [30, 31]. The manufacturer of the PMMA used in this study contraindicates the use of this product in patients with a history of or evolving autoimmune diseases (ANVISA/MS Registry No. 802565100006 - Linnea Safe) [32]. On the other hand, the exacerbated presence of IL-17 in the subcutaneous tissue may be beneficial due to its relationship with epithelial protection. When there is skin injury and disruption of the epithelial barrier, IL-17 stimulates the proliferation of epithelial cells to aid in the healing process, in addition to favoring the secretion of VEGF and IL-10 [8, 31].

The profile of cytokines produced in the intramuscular region after PMMA implantation also suggests

immunological inertia of the biomaterial. There is no statistical difference between the groups analyzed regarding the secretion of cytokines of different immunological profiles. Teixeira et al. (2021) showed that PMMA is biocompatible when injected into the muscular region of Wistar rats, without evidence of antigenic activity and considerable acute inflammatory response [33].

The intramuscular implantation of PMMA is used to induce a mechanical projection of the region which is intensified due to the collagen production throughout the time and the consequent repositioning of the region [18]. Also, the juxta-periosteal implantation has been used as bone cement and bone filler with satisfactory long-term properties as described previously by Sas et al. (2021) [34].

The PMMA implanted in the juxta-periosteal region, as well as in the other anatomical planes analyzed, did not generate a significant immunological reaction. The contact of PMMA with the periosteum seems to cause mild bone remodeling by endochondral ossification, triggering resolution of the pro-inflammatory response and formation of a fibrin scaffold [35]. The blood vessels that supply the periosteum are small, but still capable of releasing proteins when injured, which will adsorb to the surface of PMMA [36].

In the juxta-periosteal region, there was a high production of IL-17, IL-2, IFN-γ and TNF-α at 7 DAI, even though it was not statistically different from their respective controls. According to the tissue characteristics, the presence of a semi-solid substance generates focal pressure, causing the release of DAMPs, which may have aided in the secretion of these pro-inflammatory cytokines. On the other hand, it is considered a mild inflammatory response, where at 30 DAI, there is a drop in the concentration of these cytokines, equaling the basal levels, without the need for high release of regulatory cytokines.

The low production of TGF-β up to 180 DAI did not impact the increase in pro-inflammatory cytokines (IL-2, IL-6, IFN-γ, TNF-α and IL-17) in the same period, which indicates a lack of need of regulation, in addition to mild inflammation and little PMMA immune stimulation. The expression of IL-10 was sufficient to regulate the levels of pro-inflammatory cytokines throughout the experimental period. The data presented here confirm the immunological inertia of PMMA. The low concentration of IL-4 at all depths and analyzed periods demonstrates that the pro-inflammatory response is subtle, with no need for a relevant anti-inflammatory response.

Reports in the literature show the biocompatibility and safety of PMMA in applications for aesthetic or repair purposes: as for the correction of bone defects of genetic origin, such as hemifacial microsomia [37]; as an alternative to a less invasive and effective method for penile enlargement [38, 39]; in post-mastectomy nipple projection with nipple–areolar reconstruction [40]; as a substitute for bone allografts in facial reconstructions after trauma [41]; and for urethral augmentation as a treatment for urinary incontinence [42]. Our results corroborate the safety of the use of PMMA, since it was shown to be immunologically inert, which reinforces its applicability.

Conclusion

The implantation of PMMA in mouse tissues stimulates a mild immune response, without detection of high concentrations of anti-inflammatory or regulatory cytokines to control it. The absence of statistically significant differences between the control and test groups on all experimental days indicates that PMMA is immunologically inert, inducing reactions similar to physiological ones by the release of DAMP's or resulting from aging. This is the first article that demonstrates the immunological inertia of PMMA implantation over 360 experimental days in a murine model.

Acknowledgments ACM, ELC and RSLJ conceived the study design. ACM, PIRF, RSG, JXP and FDT performed experiments. ACM, FSA, FDT collected data. ACM, RSG and RSLJ analyzed experimental data and designed the figures. ACM, JXP MCV and RSLJ interpreted the data. ACM carried out bibliographic research. ACM, MCV and RSLJ wrote the manuscript. All authors performed a critical review of the manuscript's intellectual content, in addition to approving the final version to be published.

Funding This work was supported by the Bioplastia Brazil, Rua T-55, n° 938, sala 1009; Edifício EBM Walk Bueno - Setor Bueno, Goiânia - Goiás - Brazil. CNPJ: 24.755.478/0002-70; the Coordination for the Improvement of Higher Education Personnel—Brazil (CAPES)—project number: 88882.461654/2019-01.

Data Availability The raw/processed data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Human and Animal Rights, or Ethical Approval Animal rights were completely respected and this study followed the regulations of the ethical committee in animal use, approval protocol number 027/2017 (CEUA/UFG).

Informed Consent For this type of study, informed consent is not required.

References

1. Ersek RA, Beisang AA (1992) Bioplastique: a new biphasic polymer for minimally invasive injection implantation. Aesthet Plast Surg 16:59–65. https://doi.org/10.1007/BF00455150

2. De Boulle K, Heydenrych I (2015) Patient factors influencing dermal filler complications: prevention, assessment, and treatment. Clin Cosmet Investig Dermatol 8:205–214. https://doi.org/10.2147/CCID.S80446
3. Funt D, Pavicic T (2013) Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. Clin Cosmet Investig Dermatol 6:295–316. https://doi.org/10.2147/CCID.S50546
4. Alijotas-Reig J, Fernández-Figueras MT, Puig L (2013) Late-onset inflammatory adverse reactions related to soft tissue filler injections. Clin Rev Allergy Immunol 45:97–108. https://doi.org/10.1007/s12016-012-8348-5
5. Carnicer-Lombarte A, Chen S-T, Malliaras GG, Barone DG (2021) Foreign body reaction to implanted biomaterials and its impact in nerve neuroprosthetics. Front Bioeng Biotechnol 0:271. https://doi.org/10.3389/FBIOE.2021.622524
6. Franz S, Rammelt S, Scharnweber D, Simon JC (2011) Immune responses to implants—A review of the implications for the design of immunomodulatory biomaterials. Biomaterials 32:6692–6709. https://doi.org/10.1016/J.BIOMATERIALS.2011.05.078
7. Vasconcelos DP, Águas AP, Barbosa MA, Pelegrín P, Barbosa JN (2019) The inflammasome in host response to biomaterials: bridging inflammation and tissue regeneration. Acta Biomater 83:1–12. https://doi.org/10.1016/j.actbio.2018.09.056
8. Sheikh Z, Brooks PJ, Barzilay O, Fine N, Glogauer M, Macrophages, (2015) Foreign body giant cells and their response to implantable biomaterials. Materials (Basel, Switzerland) 8:5671–5701. https://doi.org/10.3390/MA8095269
9. Bachour Y, Kadouch JA, Niessen FB (2021) The aetiopathogenesis of late inflammatory reactions (LIRs) after soft tissue filler use: a systematic review of the literature. Aesthet Plast Surg 45:1748–1759. https://doi.org/10.1007/S00266-021-02306-3
10. JunZhu F, LinTong Y, YongSheng Z, MingYao Y (2019) Role of dendritic cells in the host response to biomaterials and their signaling pathways. Acta Biomater 94:132–144. https://doi.org/10.1016/j.actbio.2019.05.038
11. Jhunjhunwala S, Aresta-DaSilva S, Tang K, Alvarez D, Webber MJ, Tang BC, Lavin DM, Veiseh O, Doloff JC, Bose S, Vegas A, Ma M, Sahay G, Chiu A, Bader A, Langan E, Siebert S, Li J, Greiner DL, Newburger PE, Von Andrian UH, Langer R, Anderson DG (2015) Neutrophil responses to sterile implant materials. PLoS One. https://doi.org/10.1371/journal.pone.0137550
12. Selders GS, Fetz AE, Radic MZ, Bowlin GL (2017) An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. Regen Biomater 4:55. https://doi.org/10.1093/RB/RBW041
13. Funt D, Pavicic T (2013) Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. Clin Cosmet Investig Dermatol 6:295–316. https://doi.org/10.2147/CCID.S50546
14. Park TH, Seo SW, Kim JK, Chang CH (2011) Clinical experience with polymethylmethacrylate microsphere filler complications. Aesthet Plast Surg 362(36):421–426. https://doi.org/10.1007/S00266-011-9803-Z
15. De Jesus LH, De Campos Hildebrand L, Martins MD, Da Rosa FM, Danilevicz CK, Filho MS (2015) Location of injected polymethylmethacrylate microspheres influences the onset of late adverse effects: an experimental and histopathologic study. Clin Cosmet Investig Dermatol 8:431. https://doi.org/10.2147/CCID.S81467
16. Costa EL, Milhomem AC, De Moura Filho RM, Lino RS (2016) Polymethyl methacrylate (Linnea Safe) causes local inflammatory response after intramuscular implant in BALB/c mice but it is not observed in distant organs. J Bras Patol e Med Lab 52:400–406. https://doi.org/10.5935/1676-2444.20160058
17. Saleh LS, Bryant SJ (2017) In vitro and in vivo models for assessing the host response to biomaterials. Drug Discov Today Dis Models 24:13. https://doi.org/10.1016/J.DDMOD.2018.04.002
18. Lemperle G, Knapp TR, Sadick NS, Lemperle SM (2010) ArteFill® permanent injectable for soft tissue augmentation: I. Mechanism of action and injection techniques. Aesthet Plast Surg 34:264. https://doi.org/10.1007/S00266-009-9413-1
19. Mouse Th1/Th2/Th17 CBA Kit, (n.d.). https://www.bdbiosciences.com/en-us/products/reagents/immunoassay-reagents/cba/cba-kits/mouse-th1-th2-th17-cba-kit.560485 (accessed November 9, 2021).
20. Medeiros CCG, Cherubini K, Salum FG, Figueiredo MAZ (2014) Complications after polymethylmethacrylate (PMMA) injections in the face: a literature review. Gerodontology 31:245–250. https://doi.org/10.1111/ger.12044
21. Williams DF (1987) Tissue-biomaterial interactions. J Mater Sci 22:3421–3445. https://doi.org/10.1007/BF01161439
22. Ghasemi-Mobarakeh L, Kolahreez D, Ramakrishna S, Williams D (2019) Key terminology in biomaterials and biocompatibility. Curr Opin Biomed Eng 10:45–50. https://doi.org/10.1016/J.COBME.2019.02.004
23. Raut HK, Das R, Liu Z, Liu X, Ramakrishna S (2020) Biocompatibility of biomaterials for tissue regeneration or replacement. Biotechnol J 15:2000160. https://doi.org/10.1002/BIOT.202000160
24. Kastellorizios M, Tipnis N, Burgess DJ (2015) Foreign body reaction to subcutaneous implants. Adv Exp Med Biol 865:93–108. https://doi.org/10.1007/978-3-319-18603-0_6
25. Cassini-Vieira P, de Carvalho Santuchi M, da Silva RF, Russo RC, Araújo FA, dos Santos RAS, Andrade SP, Teixeira MM, Barcelos LS (2018) Lack of interferon-gamma attenuates foreign body reaction to subcutaneous implants in mice. J Biomed Mater Res Part A 106:2243–2250. https://doi.org/10.1002/JBM.A.36416
26. Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW (1999) Growth curves and survival characteristics of the animals used in the biomarkers of aging program. J Gerontol A Biol Sci Med Sci. https://doi.org/10.1093/GERONA/54.11.B492
27. Frasca D, Blomberg BB (2020) Adipose tissue, immune aging and cellular senescence. Semin Immunopathol 42:573. https://doi.org/10.1007/S00281-020-00812-1
28. Ferrucci L, Fabbri E (2018) Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 15:505. https://doi.org/10.1038/S41569-018-0064-2
29. Ross SH, Cantrell DA (2018) Signaling and function of interleukin-2 in T lymphocytes. Annu Rev Immunol 36:411. https://doi.org/10.1146/ANNUREV-IMMUNOL-042617-053352
30. Omidian Z, Ahmed R, Giwa A, Donner T, Hamad ARA (2019) IL-17 and limits of success. Cell Immunol 339:33. https://doi.org/10.1016/J.CELLIMM.2018.09.001
31. McGeachy MJ, Cua DJ, Gaffen SL (2019) The IL-17 family of cytokines in health and disease. Immunity 50:892. https://doi.org/10.1016/J.IMMUNI.2019.03.021
32. Produtos | Linnea Safe | O PMMA mais seguro do Brasil | Laboratório Lebon | Rio Grande do Sul, (n.d.). https://www.laboratoriolebon.com.br/linneasafe Accessed Nov 9 2021
33. E. Teixeira, F. Bortolozo, H. Menezes, (2021) Biocompatibility and fibrous response of polymethylmethacrylate in skeletal muscles. Int J Dev Res (IJDR), 11:51034–51039. https://www.journalijdr.com/biocompatibility-and-fibrous-response-polymethylmethacrylate-skeletal-muscles Accessed July 29 2022
34. Sas A, Helgason B, Ferguson SJ, van Lenthe GH (2021) Mechanical and morphological characterization of PMMA/bone composites in human femoral heads. J Mech Behav Biomed Mater 115:104247. https://doi.org/10.1016/j.jmbbm.2020.104247

35. Bahney CS, Zondervan RL, Allison P, Theologis A, Ashley JW, Ahn J, Miclau T, Marcucio RS, Hankenson KD (2019) Cellular biology of fracture healing. J Orthop Res 37:35. https://doi.org/10.1002/JOR.24170
36. A. Nahian, P.R. Chauhan, (2021) Histology, periosteum and endosteum, statpearls. https://www.ncbi.nlm.nih.gov/books/NBK557584/ Accessed Nov 9 2021
37. Milhomem AC, Jorge IMDS, Costa ELD, Vinaud MC, Lino Júnior RDS (2020) Polymethyl methacrylate (PMMA) in the treatment of a case of hemifacial microsomia. Aesthetic Surg J Open Forum. https://doi.org/10.1093/ASJOF/OJAA002
38. Lemperle G, Casavantes L (2019) Penisvergrößerung durch Injektion von PMMA-Mikrosphären. Intimchirurgie. https://doi.org/10.1007/978-3-662-57392-1_7
39. Casavantes L, Lemperle G, Morales P (2016) Penile girth enhancement with polymethylmethacrylate-based soft tissue fillers. J Sex Med 13:1414–1422. https://doi.org/10.1016/J.JSXM.2016.06.008
40. McCarthy CM, VanLaeken N, Lennox P, Scott AM, Pusic AL, (2010) The efficacy of artecoll injections for the augmentation of nipple projection in breast reconstruction, Eplasty. 10 e7. /pmc/articles/PMC2848402/ Accessed July 29 2022
41. Milhomem AC, Alves LM, de SouzaJorge IM, Costa EL, Vinaud MC, de SouzaLinoJúnior R (2018) Facial trauma reconstruction with polymethyl methacrylate—A case report. J Cosmet Dermatol 17:1037–1040. https://doi.org/10.1111/JOCD.12786
42. Lemperle G, Lappin PB, Stone C, Lemperle SM (2011) Urethral bulking with polymethylmethacrylate microspheres for stress urinary incontinence: tissue persistence and safety studies in miniswine. Urology 77(1005):e1-1005.e7. https://doi.org/10.1016/J.UROLOGY.2010.12.021