Sultan Qaboos University Med J, May 2022, Vol. 22, Iss. 2, pp. 233–240, Epub. 26 May 22
Submitted 20 Oct 20
Revision Req. 11 Jan 21; Revision Recd. 24 Jan 21
Accepted 10 Feb 21
https://doi.org/10.18295/squmj.6.2021.082

CLINICAL & BASIC RESEARCH

Evaluation of Different Polymerisation Methods of Ocular Prosthesis Acrylic Resins on Subcutaneous Tissue Inflammatory Response in Rats

Emily V.F. da Silva,[1] *Marcelo C. Goiato,[1] Sandro B. Bitencourt,[1] Victor G.B. Brito,[2] Aline S. Takamyia,[3] Paulo A. Penitente,[1] Sandra H.P. de Oliveira,[2] Daniela M. dos Santos[1]

abstract: ***Objectives:*** This study aimed to evaluate the influence of different polymerisation methods of acrylic resin for ocular prostheses on the subcutaneous tissue inflammatory response of rats. ***Methods:*** The study was conducted at the Basic Sciences Department, Araçatuba Dental School, São Paulo State University (UNESP), Brazil, from 2016 to 2018. The samples were prepared by water bath (WB), microwave energy (MW) or autopolymerisation (AP) (n = 20 samples per group). The inflammatory response (cell count and immunohistochemical analysis of interleukin [IL]-1β, IL-6, tumour necrosis factor [TNF]-α, IL-17 and macrophage inflammatory protein-3α) was analysed by the implantation of a sample from each group in the subcutaneous tissue of 20 Wistar rats and evaluated after seven, 15, 30 and 60 days. The quantitative and qualitative data were analysed using analysis of variance and Tukey tests (P <0.05) and visual comparison, respectively. ***Results:*** There was a moderate inflammatory infiltrate for the MW and AP groups and a light infiltrate for the WB group after seven days. The inflammatory infiltrate and the immunolabeling of tested targets decreased gradually during the 60-day period. The AP group had the highest immunolabeling of TNF-α (seven days), IL-1β and IL-17 (at 15 and 30 days) and IL-6 (at 30 and 60 days). The WB and MW groups showed greater immunolabeling at 15 and 30 days, while the MW group also had high results at 60 days. ***Conclusion:*** Polymerisation by microwave energy and by chemical activation resulted in a higher inflammatory response.

Keywords: Ocular Prosthesis; Acrylic Resins; Biocompatibility Testing; Wistar Rats; Polymerization; Brazil.

Advances in Knowledge

- *Different polymerisation methods of ocular prostheses acrylic resin were analysed.*
- *The subcutaneous tissue of Wistar rats was assessed for inflammatory response analysis.*
- *Materials were polymerised according to the manufacturer's instructions.*
- *Polymerisation by microwave energy and chemical activation resulted in a higher inflammation.*

Application to Patient Care

- *The knowledge of ocular prosthesis material's biocompatibility is important to ensure safe use in patients.*
- *The polymerisation by microwave energy and chemical activation resulted in a higher inflammatory response, which is interesting information for clinicians.*
- *Patients benefit from the identification of the polymerisation method that causes fewer undesirable effects.*

The use of ocular prostheses to improve the self-esteem of anophthalmic individuals through the re-establishment of facial aesthetics and healthy maintenance of the remaining structures is a viable and safe rehabilitation procedure.[1] These prostheses, composed of a larger volume of white acrylic resin (AR) and a smaller volume of colourless resin, can be polymerised in a heated water bath, by microwave energy or chemical activation.[2,3]

Among the factors involved in the success of prosthetic rehabilitation, it is essential that the artificial material used causes no local or systemic undesirable effects in the individual who received it.[4] However, there are reports in the literature of irritation associated with the use of ocular prostheses, which may occur when there is an incomplete conversion of methyl methacrylate (MMA) monomers into polymers.[2,5] This material then releases potentially toxic substances such as formaldehyde, methacrylic acid and residual MMA monomers, among others.[4,6]

In vivo toxicity tests allow the study of the mechanisms of adverse reactions to a material through the analysis of interactions among cells and tissues, simulating a condition similar to what would occur in humans.[7,8] The implantation of a sample in the subcutaneous tissue of rats allows for the analysis of local effects of a given stimulus and enables, for example, the identification of inflammatory cells and the analysis of the local tissue response.[9,10] An excessive inflammatory response of the organism can promote irreversible damage, so it is important to evaluate the ability of ocular prostheses to induce the release of

Departments of [1]Dental Materials and Prosthodontics, [2]Basic Sciences and [3]Surgery and Integrated Clinic, School of Dentistry, São Paulo State University (UNESP), Araçatuba, Brazil

**Corresponding Author's e-mail: goiato@foa.unesp.br*

pro-inflammatory cytokines and chemokines and to understand their chemotactic activity mechanism.[11] These mediators secreted under inflammatory response conditions include two patterns: the T helper Type 1 (Th1) cytokine pattern, with pro-inflammatory action mainly caused by interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α and the Th2 pattern, with anti-inflammatory action.[12]

It is a known fact that human studies involve multiple ethical issues in the performance of certain types of intervention studies. Since the results obtained from animal models have acceptable predictability for toxicity in humans (approximately 70–80%),[8] being no more resistant than models in humans, the present study can provide useful information for patients and professionals. This study aimed to evaluate the influence of different polymerisation methods of white-coloured AR for ocular prostheses on the subcutaneous tissue inflammatory response of Wistar rats. The null hypothesis is that the different polymerisation methods of white AR tested do not produce inflammatory tissue responses in the subcutaneous tissue.

Methods

The current study was conducted at the Basic Sciences Department, Araçatuba Dental School, São Paulo State University (UNESP), Brazil, from 2016 to 2018. A total of 60 AR samples in the colour white (Artigos Odontológicos Clássico Ltda, São Paulo, Brazil) were prepared and distributed across three groups (n = 20 each), according to the polymerisation method: thermopolymerised resin in water bath (WB; Clássico, Artigos Odontológicos Clássico Ltda), thermopolymerised resin by microwave energy (MW; Onda Cryl, Artigos Odontológicos Clássico Ltda) and autopolymerisation (AP; chemically activated) resin (Jet, Artigos Odontológicos Clássico Ltda).[3]

Samples were obtained from a metallic matrix containing 10 circular compartments (10 mm in diameter and 3 mm in thickness).[1,3] The AR was handled according to the manufacturer's recommendations and inserted into the compartments.[13,14] For the WB polymerisation, a flask was immersed in water, heated for 30 min, placed at room temperature for 30 min and boiled at 100 °C for 1 h. For the microwave energy MW polymerisation, a flask was placed in a microwave for three minutes at 30% power (320 W), four minutes at 0% power (0 W) and three minutes at 60% power (720 W). For AP, an open flask with the required amount of water was placed in a device under 140 kPa for 20 minutes. The samples were then finished with an abrasive drill.[3]

Twenty male albino Wistar rats at the age of four months were used. The animals were kept in an environment with controlled temperature and received *ad libitum* water and food.[9,10] Initially, samples were washed three times in Milli-Q water (Merck Millipore, Burlington, Massachusetts, USA) and sterilised by exposure to ultraviolet light for 30 minutes per side.[15] Animals were anesthetised with an intraperitoneal injection of ketamine (25 mg/kg) and xylazine (10 mg/kg). Then, the trichotomy of the dorsal region was performed, followed by asepsis with 5% povidone-iodine solution.[9,10] An incision of 2 cm in a head-to-tail direction was performed in the dorsal region using a no. 15 Bard-Parker® blade (Aspen Surgical Corp., Caledonia, Michigan, USA). The connective tissue was laterally divulsed with a blunt-tipped scissor to form three surgical pockets. One sample of each group was inserted in each surgical pocket. It is important to attest that the pockets remained distant from each other. The skin was sutured with a 4.0 silk thread (Ethicon Inc., Raritan, New Jersey, US).[16]

After seven, 15, 30 and 60 days of implantation (n = 5), the animals were euthanised with an overdose of isoflurane, an inhalation agent (Cristália, São Paulo, Brazil). The specimens (samples and adjacent tissues) were removed and fixed in 10% buffered formalin (pH 7.0). For histological processing, the specimens were dehydrated in increasing concentrations of ethyl alcohol (70%, 80%, 90%, 100%-I, 100%-II, 100%-III). Subsequently, the AR samples were carefully removed from the interior of the tissue, which was diaphanised in a xylol bath and, finally, infiltrated and embedded in paraffin. The specimens were sectioned into 3 μM pieces and stained with haematoxylin and eosin (HE) for further microscopic study of their cell count.[9,10] The tissue response was classified as 0 (no or few inflammatory cells and no tissue reaction), 1 (less than 25 cells and a mild reaction), 2 (between 25 and 125 cells and a moderate reaction) and 3 (more than 125 cells and a severe reaction). The mean number of cells for each group was obtained from the count of mast cells, eosinophils, lymphocytes, macrophages and neutrophils counted in 10 separate areas (×100 magnification) of one slide by a single evaluator.[9,10] Representative images for each group were obtained (×40 magnification) [Figure 1].

From each group, 25 slides were randomly selected to perform the immunohistochemical (IHC) reactions, where goat primary antibodies (Santa Cruz Biotechnology Inc., Dallas, Texas, USA) were used to detect IL-1β, -6 and -17 and rabbit primary antibodies (Abcam plc., Cambridge, UK) were used to detect TNF-α and macrophage inflammatory

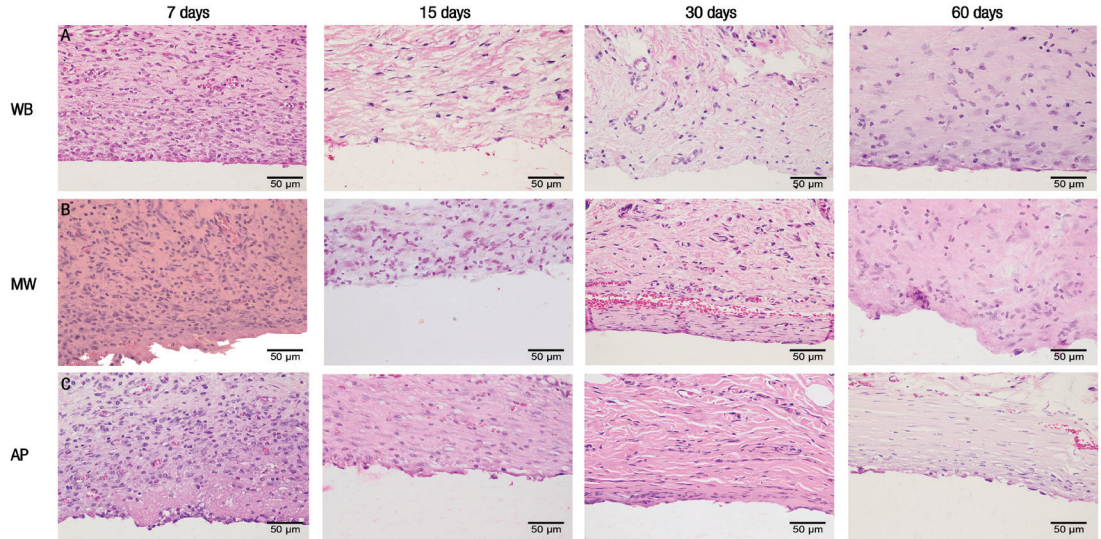

Figure 1: Hematoxylin and eosin-stained subcutaneous tissue slides at ×40 magnification of three groups of male Wistar rats prepared using **(A)** water bath (WB), **(B)** microwave energy (MW) and **(C)** autopolymerisation (AP). The slides show moderate inflammatory infiltrates at seven days for slides prepared using MW and AP (found in sub-images B and C) and light inflammatory infiltrates for all other remaining slides (A, B and C).

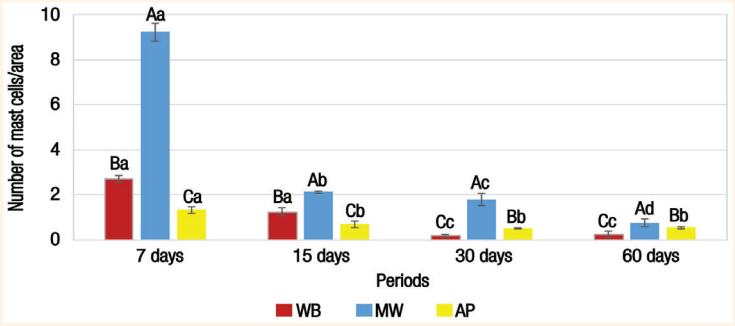

Figure 2: Number and standard deviation of mast cells per area for each group of male Wistar rats (water bath, microwave energy and autopolymerisation) at four different points of time with different uppercase letters indicating the statistical difference between groups at the same time (P <0.05) and different lowercase letters indicating statistical difference between durations within the same group (P <0.05).

protein 3α (MIP-3α/ C-C motif chemokine ligand 20 [CCL20]) (n = 5). These antibodies were selected due to their high performance in inflammatory processes. Biotinylated rabbit anti-goat IgG antibodies (Santa Cruz Biotechnology Inc.) and goat anti-rabbit IgG antibodies (Abcam plc., Cambridge, UK) were used as secondary antibodies. The reactions obtained were amplified with the avidin-biotin complex (Vectastain Elite ABC Kit, Standard) (Vector Laboratories, Inc., Burlingame, California, US) and chromogen diaminobenzidine (DAB, Dako, Glostrop, Denmark). Following this, the slides were counter-stained by Harris haematoxylin for 5 seconds and dehydrated for sealing with a coverslip using DPX mounting medium (Sigma Aldrich, St. Louis, Missouri, US). The negative control consisted of the protocol described previously but in the absence of primary antibodies.[17,18]

Photographic recording of the slides was performed at ×20 magnification; except for MIP-3α, where a ×10 magnification was used) with an Olympus XC50 camera (Olympus Corp., Tokyo, Japan) coupled with the Olympus BX53 microscope (Olympus Corp.) Further, IL-1β, IL-6, IL-17 and TNF-α were diffuse IHC stained. Following this, images were deconvoluted through the Fiji Image J software, Version 2.1.0/1.53c (National Institutes of Health; Bethesda, MD, USA), and analysed in a standard colour threshold by a single evaluator. The IHC staining for MIP-3α was localised and the stained cells per field were counted. An average value of IHC staining was obtained for each target.

The Statistical Package for the Social Sciences (SPSS), Version 21.0 (IBM Inc., Chicago, Illinois, USA) was used for data analysis. Quantitative data obtained from the evaluation of inflammatory process (HE

staining) and IHC analysis were submitted for two-way analysis of variance and Tukey's test with a 5% significance level. Qualitative data were submitted to visual comparison.

The study was approved by the Research Ethics Committee of the School of Dentistry, São Paulo State University, Araçatuba, Brazil (Process number 00594-2016).

Results

The specimens stained by HE were analysed to evaluate the inflammatory response generated by AR samples implanted in the rats' subcutaneous tissue. There was a moderate inflammatory infiltrate for the MW and AP groups and a light infiltrate for the WB group after seven days. The characteristics indicative of an inflammatory response gradually decreased over time. No necrosis or formation of granulomas was observed in any of the tested groups [Figure 1]. This result is more evident when evaluating the mean number of cells that were attracted to the focus of the inflammatory process. In the first seven days, a larger number of cells (mast cells, eosinophils, lymphocytes and neutrophils) were observed compared to other durations, except for macrophages, which had a greater quantity in 30 days. The MW group exhibited the highest number of inflammatory cells [Figures 2–6], while the WB group presented a higher number of eosinophils [Figure 3] and lymphocytes [Figure 4] after 15 days. In the AP group, a higher number of macrophages and neutrophils were observed after 15 [Figure 5] and 60 days [Figure 6], respectively.

The immunolabeling of different targets was identified through the IHC analysis. In general, when analysed over time, a higher response was verified after seven days of implantation. However, exceptions were verified for the IL-1β immunolabeling of the MW (numerical only) and AP groups, where the response was higher after 15 days of implantation, for the IL-6 immunolabeling of the AP group, where the response was higher after 30 days and for the MIP-3α immunolabeling of the WB and AP groups, where the response was higher after 30 days. However, there was

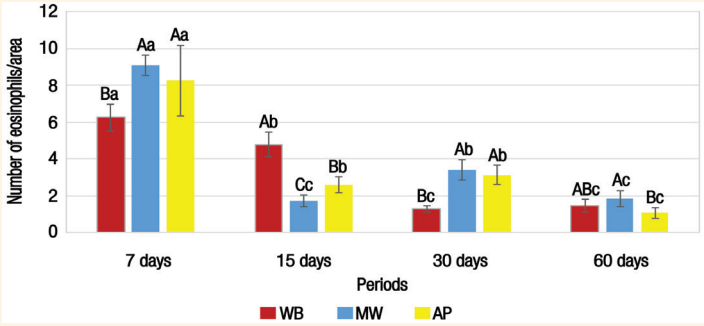

Figure 3: Number and standard deviation of eosinophils per area for each group of male Wistar rats (water bath, microwave energy and autopolymerisation) at four different points of time with different uppercase letters indicating statistical difference between groups at the same time (P <0.05) and different lowercase letters indicating statistical difference between durations within the same group (P <0.05).

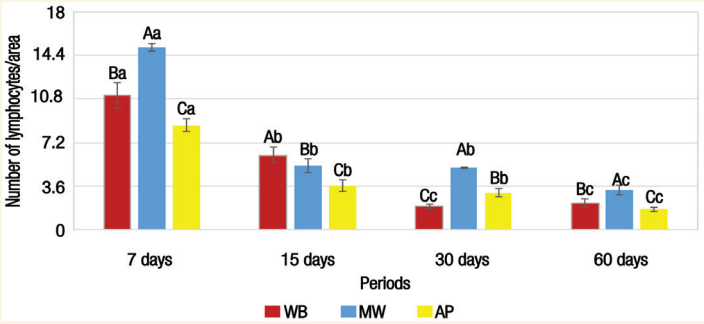

Figure 4: Number and standard deviation of lymphocytes per area for each group of male Wistar rats (water bath, microwave energy and autopolymerisation) at four different points of time with different uppercase letters indicating statistical difference between groups at the same time (P <0.05) and different lowercase letters indicating statistical difference between durations within the same group (P <0.05).

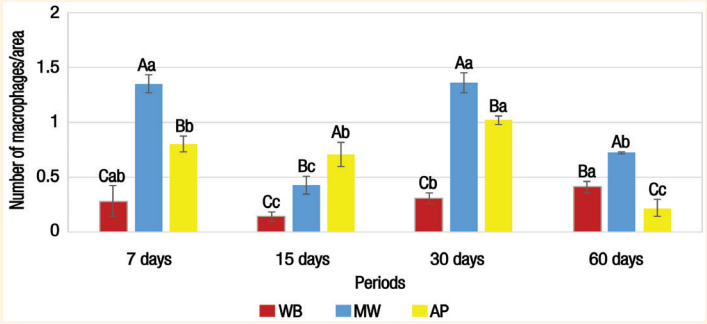

Figure 5: Number and standard deviation of macrophages per area for each group of male Wistar rats (water bath, microwave energy and autopolymerisation) at four different points of time with different uppercase letters indicating statistical difference between groups at the same time (P <0.05) and different lowercase letters indicating statistical difference between durations within the same group (P <0.05).

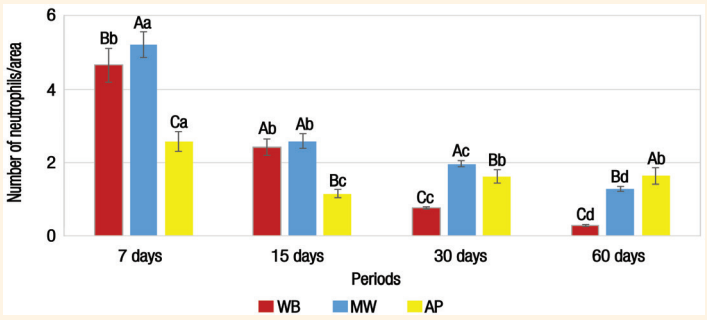

Figure 6: Number and standard deviation of neutrophils per area for each (water bath, microwave energy and autopolymerisation) at four different points of time with different uppercase letters indicating statistical difference between groups at the same time (P <0.05) and different lowercase letters indicating statistical difference between durations within the same group (P <0.05).

a decrease in the immunolabeling for all the tested targets in 60 days compared to the previous durations.

After seven days of implantation, it was verified that the WB and AP groups presented a higher IL-1β immunolabeling, with a statistically significant difference when compared to the MW group. However, a higher immunolabeling was verified for the AP group after 15 and 30 days. No statistically significant difference was observed between groups compared to IL-6 immunolabeling after seven days of implantation. The AP group presented higher immunolabeling after 30 and 60 days, significantly different from other groups. Although the IL-6 immunolabeling was reduced after 60 days for this group, it was statistically similar to the number recorded at seven days. The AP group showed the highest IL-17 immunolabeling, statistically different from the other groups after 15 and 30 days. Regarding TNF-α, the AP group had the highest immunolabeling after seven days of implantation, with a statistically significant difference from the MW group. However, the highest immunolabeling was verified for the WB and MW groups after 15 and 30 days. After 60 days, the MW group exhibited greater immunolabeling than the other groups. For the MIP-3α immunolabeling, no statistically significant difference was observed between groups at different durations.

Discussion

The null hypothesis that the different polymerisation methods of white AR do not produce inflammatory tissue responses in the subcutaneous tissue was tested and rejected since differences were observed between groups regarding the number of cells associated with inflammation and immunolabeling of the inflammatory mediators evaluated.

In vivo assays involve the complexity of cell-cell and cell-tissue interaction and a variety of cell types and hormonal and systemic effects.[8] Therefore, it is essential to analyse the local tissue response after the implantation of samples of AR for ocular prostheses on the subcutaneous tissue of rats. Considering that biocompatibility is directly dependent on the components released by the AR, a decrease of the

inflammatory response was observed over time.[19] Mast cells, eosinophils, lymphocytes and neutrophils were observed in a greater quantity in the first seven days after implantation, especially for the MW group [Figures 1–4 and Figure 6]. During the microwave polymerisation, the resin is confined inside the flask and the process is carried out in a dry environment, making it difficult to leach residual monomers and other substances.[3] In addition, the microwave energy acts only on the monomer molecules.[20] These factors may explain the results found. Corroborating these results, da Silva *et al*.found unsatisfactory *in vitro* results for the MW group, which exhibited slight cytotoxicity on a conjunctival cell line.[3]

Mediators secreted under acute inflammatory conditions include several pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α. These cytokines are present in the first hours at the inflammatory site, responsible for the chemotactic activity for different cells in the first days of inflammation, corroborating the present study's results from the present study.[12,13] The WB and AP groups showed a higher IL-1β immunolabeling after seven days, whereas the AP group showed a higher TNF-α immunolabeling. These mediators are synthesised by lymphocytes and macrophages, among other cells, and act chemotactically by attracting cells such as lymphocytes to the inflammatory site.[21] Lymphocytes, whose primary functions are to produce antibodies (B lymphocytes) and induce the inflammatory response (T lymphocytes), were the most recruited cells during the process, mainly in the first seven days [Figure 4], followed by mast cells [Figure 2] and eosinophils [Figure 3].[21]

Smaller amounts of neutrophils and macrophages were found after seven days [Figures 5 and 6]. The absence of significant neutrophil invasion in the tissue indicates that there was no severe inflammatory response or necrosis associated with the subcutaneous AR sample, corroborating the findings reported by Yokoyama *et al*.[22] These authors found similar results with the implantation of carbon nanofibers in the subcutaneous tissue, stating that a low amount of neutrophils is associated with the presence of a non-acutely toxic material.[22] Macrophages, on the other hand, were observed in a higher quantity after 30 days [Figure 5]. It is possible that macrophage cells were 'alternatively' activated (M2) from the stimulus they received and were later attracted to the implantation site.[23] In this condition, M2 macrophages promote a Th2-type immune response, inducing the process of tissue repair, angiogenesis, extracellular matrix remodelling and anti-inflammatory activity.[23]

However, it is interesting to note that the AP group showed greater immunolabeling of different inflammatory mediators in greater durations of analysis as follows: IL-1β (at 15 and 30 days); IL-6 (at 30 and 60 days); IL-17 (at 15 and 30 days). This fact can be explained by the low degree of conversion of the AR during the chemically activated polymerisation, resulting in the continuous release of free radicals and unreacted residual monomers during polymerisation.[19,24] These are potential tissue irritants and may have influenced the continuous release of pro-inflammatory mediators.[24] IL-17 has an important role in inducing local tissue inflammation and MIP-3α, also known as CCL20, is an inflammatory chemokine that can be secreted or expressed by endothelial cells. Further, neutrophils, natural killer cells, Th17, B lymphocytes and a variety of other immune system cells are related to a pro-inflammatory or cytotoxic response.[15]

The WB and MW groups showed a higher TNF-α immunolabeling after 15 and 30 days. These findings corroborate those of the study by Gretzer *et al*.[25] These authors observed a greater TNF-α quantity after 21 days and associated this factor with a biphasic response of the TNF-α since there is an initial increase in its amount associated with the sample implantation and an increase of this mediator in the repair stage.[25]

After 60 days, there was a decrease in the immunolabeling for all IHC targets. However, it is important to note that the MW and AP groups showed a higher immunolabeling of IL-6 and TNF-α, respectively, at this time. Thus, although the inflammatory response had decreased, its intensity was higher these two groups.

One of the limitations of this study was that the durations shorter than seven days were not included in the analysis of the inflammatory response. However, the intent was to avoid the interference of the surgical procedure factor in the results and to allow a sufficient amount of tissue adjacent to the sample for analysis.[25] Going forward, studies to verify the leaching of residual monomers and other substances after polymerisation of the studied materials should be performed to understand the polymerisation process better.

Conclusion

According to the present study's results, the different polymerisation methods of white coloured acrylic resin for ocular prostheses resulted in an inflammatory response in the subcutaneous tissue of rats. The polymerisation by microwave energy and by chemical activation resulted in a higher inflammatory response.

ACKNOWLEDGEMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel and FAPESP (Foundation for Support to Research of the State of Sao Paulo; #2015/20221-7) for the scholarship granted to the first author, Emily V.F. da Silva. The authors thank FAPESP (#2016/03455-7) for the financial support granted to Daniela M. dos Santos.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

This work was supported by the Coordination for the Improvement of Higher Education Personnel and Foundation for Support to Research of the State of Sao Paulo (#2015/20221-7 and #2016/03455-7, respectively).

AUTHORS' CONTRIBUTION

All authors contributed equally to the work. MCG, DMS and SHPO participated in the concepts and coordination of the study, performed the study design, and drafted the manuscript. EVFS, SBB, PAP and VGBB conceived the study, fabricated the samples, and participated in assays, as well helped draft the manuscript. EVFS, AST and SBB performed the statistical analysis and participated in interpretation of the data. All authors read and approved the final version of the manuscript.

References

1. da Silva EVF, Goiato MC, Bonatto LDR, de Medeiros RA, Santos DMD, Rangel EC, et al. Toxicity analysis of ocular prosthesis acrylic resin with or without pigment incorporation in human conjunctival cell line. Toxicol In Vitro 2016; 36:180–5. https://doi.org/10.1016/j.tiv.2016.08.005.
2. Canadas MD, Garcia LF, Consani S, Pires-de-Souza FC. Color stability, surface roughness, and surface porosity of acrylic resins for eye sclera polymerized by different heat sources. J Prosthodont 2010; 19:52–7. https://doi.org/10.1111/j.1532-849X.2009.00522.x.
3. da Silva EV, Goiato MC, Dos Santos DM, Bonatto LD, Brito VG, de Oliveira SH. Effect of different methods of polymerizing ocular prosthesis acrylic resin on a human conjunctival cell line. J Prosthet Dent 2016; 116:818–23. https://doi.org/10.1016/j.prosdent.2016.06.001.
4. Ata SO, Yavuzyilmaz H. In vitro comparison of the cytotoxicity of acetal resin, heat-polymerized resin, and auto-polymerized resin as denture base materials. J Biomed Mater Res B Appl Biomater 2009; 91:905–9. https://doi.org/10.1002/jbm.b.31473.
5. Pine KR, Sloan BH, Jacobs RJ. A proposed model of the response of the anophthalmic socket to prosthetic eye wear and its application to the management of mucoid discharge. Med Hypotheses 2013; 81:300–5. https://doi.org/10.1016/j.mehy.2013.04.024.
6. Goiato MC, Freitas E, dos Santos D, de Medeiros R, Sonego M. Acrylic resin cytotoxicity for denture base--Literature review. Adv Clin Exp Med 2015; 24:679–86. https://doi.org/10.17219/acem/33009.
7. Goldberg M. In vitro and in vivo studies on the toxicity of dental resin. Clin oral Investig 2008; 12:1–8. https://doi.org/10.1007/s00784-007-0162-8.
8. Ukelis U, Kramer PJ, Olejniczak K, Mueller SO. Replacement of in vivo acute oral toxicity studies by in vitro cytotoxicity methods: Opportunities, limits and regulatory status. Regul Toxicol Pharmacol 2008; 51:108–18. https://doi.org/10.1016/j.yrtph.2008.02.002.
9. Gomes-Filho JE, Gomes AC, Watanabe S, Oliveira SH, Bernabé PF, Percinoto C. Evaluation of tissue reaction, cell viability an cytokine production induced by Sealapex Plus. J Appl Oral Sci 2011; 19:329–36. https://doi.org/10.1590/S1678-77572011005000006.
10. Gomes-Filho JE, Silva FO, Watanabe S, Cintra LT, Tendoro KV, Dalto LG, et al. Tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution. J Endod 2010; 36:1698–1702. https://doi.org/10.1016/j.joen.2010.07.007.
11. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, LeRoux MA. Concise review: Role of mesenchymal stem cells in wound repair. Stem Cells Transl Med 2012; 1:142–9. https://doi.org/0.5966/sctm.2011-0018.
12. Berger A. Th1 and Th2 responses: What are they? BMJ 2000; 321:424. https://doi.org/10.1136/bmj.321.7258.424.
13. Fernandes AU, Portugal A, Veloso LR, Goiato MC, Santos DM. Assessment of the flexural strength of two heat-curing acrylic resins for artificial eyes. Braz Oral Res 2009; 23:263–7. https://doi.org/10.1590/S1806-83242009000300007.
14. Goiato MC, Dos Santos DM, Moreno A, Iyda MG, Rezende MCRA, Haddad MF. Effect of disinfection and storage on the flexural strength of ocular prosthetic acrylic resins. Gerodontology 2012; 29:e838–44. https://doi.org/10.1111/j.1741-2358.2011.00570.x.
15. Zhao L, Mei S, Wang W, Chu PK, Wu Z, Zhang Y. The role of sterilization in the cytocompatibility of titania nanotubes. Biomaterials 2010; 31:2055–63. https://doi.org/10.1016/j.biomaterials.2009.11.103.
16. Zaharia C, Zecheru T, Moreau MF, Pascaretti-Grizon F, Mabilleau G, Marculescu B, et al. Chemical structure of methylmethacrylate-2-[2',3',5'-triiodobenzoyl]oxoethyl methacrylate copolymer, radiopacity, in vitro and in vivo biocompatibility. Acta Biomater 2008; 4:1762–9. https://doi.org/10.1016/j.actbio.2008.06.009.
17. Silva GF, Tanomaru-Filho M, Bernardi MIB, Guerreiro-Tanomaru JM, Cerri PS. Niobium pentoxide as radiopacifying agent of calcium silicate-based material: Evaluation of physicochemical and biological properties. Clin Oral Investig 2015; 19:2015–25. https://doi.org/10.1007/s00784-015-1412-9.
18. da Fonseca TS, da Silva GF, Tanomaru-Filho M, Sasso-Cerri E, Guerreiro-Tanomaru JM, Cerri PS. In vivo evaluation of the inflammatory response and IL-6 immunoexpression promoted by Biodentine and MTA Angelus. Int Endod J 2016; 49:145–53. https://doi.org/10.1111/iej.12435.
19. Urban VM, Machado AL, Oliveira RV, Vergani CE, Pavarina AC, Cass QB. Residual monomer of reline acrylic resins. Effect of water-bath and microwave post-polymerization treatments. Dent Mater 2007; 23:363–8. https://doi.org/10.1016/j.dental.2006.01.021.
20. Jorge JB, Giampaolo ET, Vergani CE, Machado AL, Pavarina AC, Carlos IZ. Cytotoxicity of denture base resins: Effect of water bath and microwave postpolymerization heat treatments. Int J Prosthodont 2004; 17:340–4. https://doi.org/0.1016/j.prosdent.2004.07.003.
21. Abbas AK, Lichtman AH, Pillai S. 2011. Cellular and Molecular Immunology, 7th ed. Saint Louis, USA: Elsevier Saunders. pp. 560.

22. Yokoyama A, Sato Y, Nodasaka Y, Yamamoto S, Kawasaki T, Shindoh M, et al. Biological behavior of hat-stacked carbon nanofibers in the subcutaneous tissue in rats. Nano Lett 2005; 5:157–61. https://doi.org/10.1021/nl0484752.

23. de Carli ML, Miyazawa M, Nonogaki S, Shirata NK, Oliveira DT, Pereira AA, et al. M2 macrophages and inflammatory cells in oral lesions of chronic paracoccidioidomycosis. J Oral Pathol Med 2016; 45:141–7. https://doi.org/10.0.4.87/jop.12333.

24. Urban VM, Machado AL, Vergani CE, Giampaolo ET, Pavarina AC, de Almeida FG, et al. Effect of water-bath post-polymerization on the mechanical properties, degree of conversion, and leaching of residual compounds of hard chairside reline resins. Dent Mater 2009; 25:662–71. https://doi.org/10.0.3.248/j.dental.2008.10.017.

25. Gretzer C, Emanuelsson L, Liljensten E, Thomsen P. The inflammatory cell influx and cytokines changes during transition from acute inflammation to fibrous repair around implanted materials. J Biomater Sci Polym Ed 2006; 17:669–87. https://doi.org/10.1163/156856206777346340.