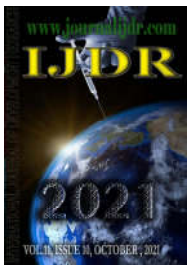
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 11, Issue, 10, pp. 51034-51039, October, 2021
https://doi.org/10.37118/ijdr.22999.10.2021



RESEARCH ARTICLE OPEN ACCESS

BIOCOMPATIBILITY AND FIBROUS RESPONSE OF POLYMETHYLMETHACRYLATE IN SKELETAL MUSCLES

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ARTICLE INFO

Article History:
Received 03rd August, 2021
Received in revised form
19th September, 2021
Accepted 17th October, 2021
Published online 30th October, 2021

Key Words:
Polymethylmethacrylate; Granuloma, Foreign Body/Chemically Induced; Biocompatibility.

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ABSTRACT

Polymethylmethacrylate (PMMA) is a synthetic polymer produced in microspheres form, used in many biomedical areas. **Objective:** To determine the PMMA injections biocompatibility in striated muscle tissue of Wistar rats. **Methods:** In vivo biocompatibility and fibrous tissue healing were assessed by histopathological evaluation at 2, 4, 8, and 16 weeks after implantation in 3 groups of adult rats. Group 1, with 20 adult rats, was injected with 30% PMMA gel in the ventral muscle. Other two groups with 20 and 5 rats were studied as control subjects. Group 2 was injected with the same amount of saline solution in the ventral muscle, while the subjects in group 3 underwent anesthesia and surgical incision. **Results:** After two weeks, a histiocytic inflammatory response was observed in group 1. The capsule formation where type I collagen predominated and a septum between the spheres, rich in collagen type III, which resulted in a stable foreign-body granuloma. No evidence of lymphocytic local reaction, tissue suffering, or necrosis was observed. **Conclusion**: PMMA is biocompatible when injected into the striated muscle tissue of Wistar rats. There was a fibrous response induced by PMMA showing microspheres surrounded by stable histiocytic granuloma without evidence of toxic or antigenic activities.

Citation: ***Eduardo da Costa Teixeira, Fernanda Bortolozo and Honório Sampaio Menezes.*** "Biocompatibility and fibrous response of polymethylmethacrylate in skeletal muscles*", International Journal of Development Research*, 11, (10), 51034-51039.

INTRODUCTION

Polymethylmethacrylate (PMMA) is a microsphere-shaped, industrially developed polymer. It has been used in the biomedical field for nearly a century, ever since the first use of bone cement. During the last decades, due to its physical, chemical, and biological features such as sphere proportion, which prevents phagocytosis; gel solubility; and inertness, PMMA has been used as soft tissue filling in different concentrations with both aesthetic and restorative purposes.
In a study using a commercial PMMA-based product, Lemperle *et al*. (Lemperle, 2003), after injecting the polymer in human subcutaneous tissue, categorized the result as a foreign-body immune reaction with the formation of macrophagic granuloma, which was limited and stabilized after approximately four weeks. This reaction goes through a stage of great influx of macrophages which trap the microspheres, producing a granuloma. Subsequently, fibroblasts, producers of collagen fibers, envelop and permeate the spheres, allowing for an increase in volume and a permanent anchoring of the material to the tissue.

Some research (Lemperle, 1991; McClelland *et al*., 1997; Lemperle*et al*., 2004) on PMMA subcutaneous injections have demonstrated the biocompatibility of the material with this tissue. Moreover, the authors also describe its effectiveness as a stimulant of fibrous response, which is responsible for the deposition of a great number of collagen fibers, especially type I collagen, which have thicker and stronger bundles, and reticulate fibers, predominantly of type III collagen. Clinical studies on PMMA microspheres in colloids have corroborated the product efficacy in subcutaneous cellular tissue augmentation (Lemperle, 1995; Carruthers, 2005) with the results got after filling congenital or acquired grooves and depressions, including lipodystrophy from the use of anti-HIV drug cocktails (Kraus, 2016). The clinically observed and histologically proven response to PMMA in subcutaneous cellular tissue suggests that it is possible to get limited, fibrous, stable, and beneficial reactions in different tissues without significant adverse results. Thus, PMMA gel solutions may be used in the muscular layer, causing volume augmentation and proliferation of collagen fibers, resulting in the reinforcement of thinning and weakening tissues. Therefore, the present study aimed to evaluate the biocompatibility of PMMA microspheres in gel injected into the striated muscle of rats and the possible fibrous response induced by the product.

MATERIALS AND METHODS

This study was carried out with a sample of 45 6-month-old Wistar rats weighing 240g-280g. The subjects were randomly assigned to three major groups. Group 1 received 0.3 ml of 30% PMMA into rectus *abdominalis* muscle under direct view, was divided into four subgroups (1A; 1B; 1C; and 1D, 5 rats each group) according to biopsy times (biopsies to histological study performed after 2, 4, 8, and 16 weeks, respectively). Group 2 consisted of 20 rats received 0.3 ml of saline solution (SS) at 0.9%, was also divided into four subgroups according to biopsy times, like group 1. And group 3 comprised 5 rats which underwent anesthesia and had their skin cut, being biopsied after 16 weeks. The samples collected were of the full thickness of the abdominal wall and included the surgical scar and underlying musculature, stained with hematoxylin-eosin (HE) and picrosirius (*Sirius red*- for collagen and reticular fibers identification). To assess the biocompatibility, the following criteria were established: 1) the type of inflammatory response; 2) the intensity and evolution of the inflammatory process; and 3) the existence or not of histopathological signs indicative of damage to the local muscular tissue. The fibrous response, the distribution and type of collagenous fiber during granuloma formation was observed. Thickness variation of the injected tissue, measured using a micrometric glass slide, was submitted to statistical analysis using the non-parametric Kruskal-Wallis and Mann-Whitney tests. Differences of $p < 0.05$ were considered statistically significant. This study was approved by the Research Ethics Committee of the UFRJ University.

RESULTS

All specimens were similarly processed. After being stained with picrosirius red and examined under polarized light, the collagenous and reticular fibers stand out. The former, rich in type I collagen and with high birefringence, can be seen in white or reddish, while the latter, rich in type III collagen and with small birefringence, can be observed as less shiny and in a greenish tone. The muscular tissue from all 20 animals in group 1 shows PMMA microspheres grouped among muscular fiber bundles. When injected in the muscular plane, the polymer promotes fiber dissection, running along the perimysium, and then sets among the bundles. In subgroup 1A, rats biopsied 2 weeks after implantation, the PMMA microspheres can be seen enveloped by multinucleated giant cells (Figure 1).

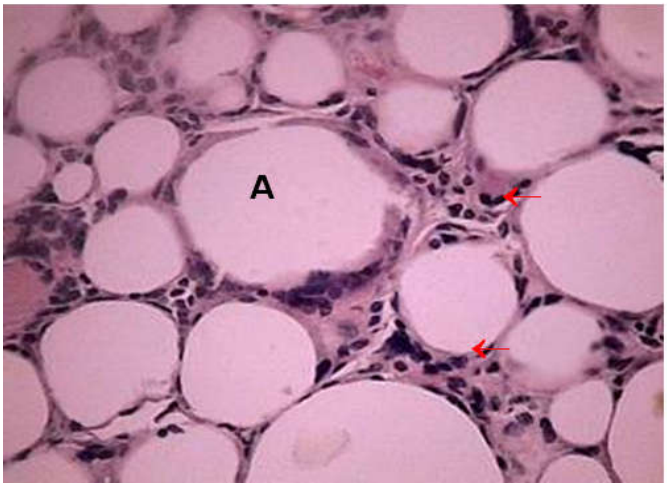

Figure 1. Photomicrograph of the material from a rat in subgroup 1A after 2 weeks. No gel delivery vehicle residue is observed and PMMA (A) microspheres can be seen surrounded by multinucleated giant cells (red arrows), a typical response to foreign bodies (HE – x400)

Samples from subgroups 1B, 1C, and 1D, biopsied, respectively, after 4, 8, and 16 weeks, showed a similar aspect to the ones from subgroup 1A. Thus, it confirms that the response is limited and formed a stable macrophagic granuloma with a well-defined capsule and fibrous septa interweaving the particles. When observed under polarized light, the histological preparations stained with picrosirius, in subgroup 1A (after two weeks), a slight deposition of collagen fibers, which are rich in fibrils type I collagen, into the granuloma being formed could be seen. In subgroup 1B, the thickening of the granuloma capsule could be confirmed, in addition to the presence of tenuous septa permeating the microspheres, which are formed by reticular fibers rich in type III collagen (Figures 2 and 3).

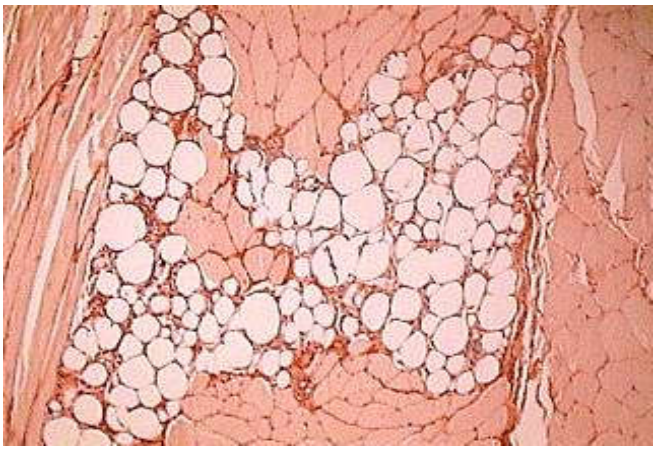

Figure 2. Histological preparation obtained from rat number 3 in subgroup 1B showing the PMMA microspheres grouped at the perimysium. The presence of viable muscle tissue among the implanted particles is noteworthy (picrosirius red – x100)

Figure 3. Under polarized light, surrounding the implant, the disposition of the capsule fibers, mainly of type I collagen, can be seen. Tenuous reticular fibers can also be observed among the PMMA particles (picrosirius red under polarized light – x100)

Subgroup 1C samples showed no signs of inflammatory response or lesions in adjacent tissues, being slightly different when compared to subgroup 1B. Under polarized light, granuloma evolution could be observed with the accumulation of collagen fibers in the capsule, especially of type I. This characteristic was also seen in subgroup 1D (Figures 4-7). No evidence of an immune response or significant inflammatory process was observed in all subgroups of group 1. The muscle tissue adjacent to the microspheres remained viable and showed no signs of suffering. None of the animals had any clinical manifestations or dead during the study period. Samples from group 2, rats injected with SS, appeared normal when stained with HE. Even in subgroup 2A, biopsied after two weeks of injections, no traces of the solution or any residual inflammatory response were found (Figure 8). When stained with picrosirius red and observed under polarized light, the muscular tissue seems histologically normal. There was no mortality or any systemic manifestation in the rats from group 2.

The group 3 (control group), stained with both HE and picrosirius red, appeared histologically normal, as observed in group 2. Similarly, there was no mortality or other complications during the observation period.

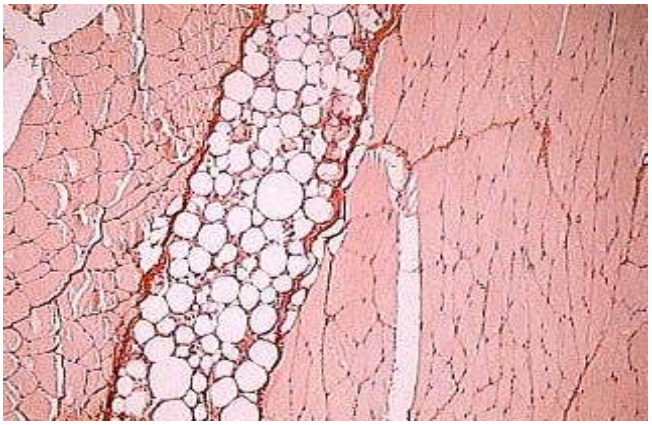

Figure 4. In subgroup 1D, 16 weeks after injection, the PMMA particles remain grouped between the muscle fibers and contained by a now fully formed fibrous capsule rich in type I collagen (pricosirius red – x100)

Figure 5. The same slide presented in Figure 4 (subgroup 1D, rat number 4) seen under polarized light. The granuloma capsule formed by collagen fibers and the discrete septa of reticular fibers can be seen among the microspheres (picrosirius red under polarized light – x100)

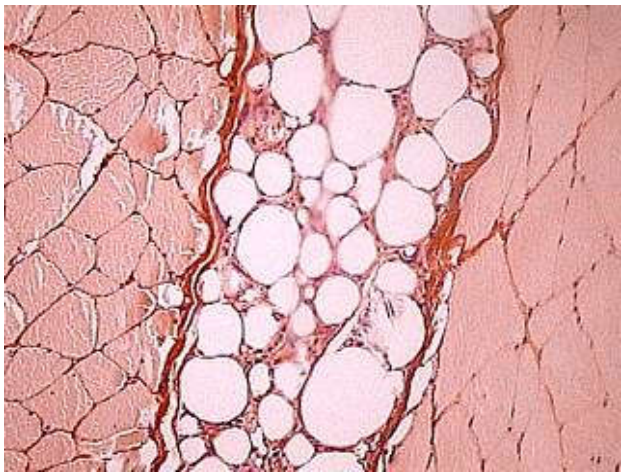

Figure 6. Under greater magnification (x200), a foreign body granuloma with its fibrous capsule and the adjacent muscular tissue with no signs of residual inflammatory response can be seen in the histological preparation (picrosirius red – x200).

All subgroups in group 1 shows PMMA dissecting the tissue (rectus abdominis), along a perimysium, in a way that the microspheres are spread between the membrane and the muscle fibers. This linear disposition of the polymer results in a flat granuloma which macroscopically forms a fibrous layer among the muscle fiber bundles. The presence of PMMA microspheres, as well as the cellular material and fibers deposited in the foreign body granuloma, promote the muscle thickening seen in the rats from group 1 (Table 1).

Fig. 7. Under polarized light, the mature capsule, composed mainly of type I collagen fibers, can be observed. The septa among the microspheres remain tenuous, formed by reticular fibers, mostly of type III collagen (picrosirius red under polarized light – x200)

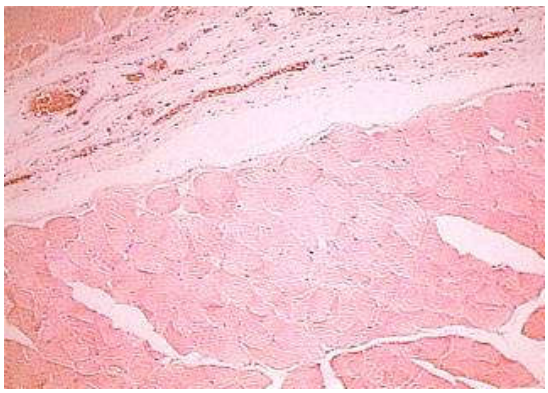

Figure 8. Photomicrograph showing the normal appearance of the muscle tissue biopsied 16 weeks after being injected with SS of rat number 5 in subgroup 2D (HE – x100)

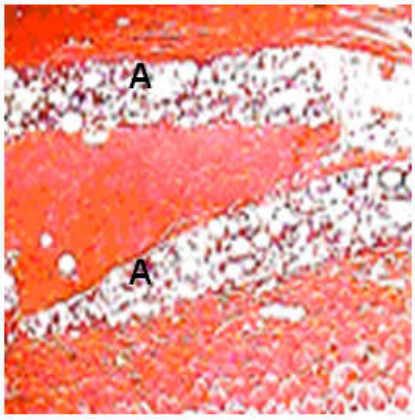

Figure 9. Photomicrograph of the rectus abdominis muscle of a rat in subgroup 1D showing the presence of multiple PMMA (A) microspheres arranged in layers between normal muscle fibers (HE – x40)

The animals' muscular layer from Group 1 can be visualized along with the clear presence of PMMA microspheres, these were found in layers, differing from the muscles of the rats belonging to the other groups (Figures 9 and 10). The measurements of muscle thickness in each groups, after 16 weeks, is shown in millimeters in Figure 11 and the data of each group can be seen in Table 1. Muscle thickness showed statistically significant difference ($p < 0.05$) among group 1D (PMMA group biopsied after 16 weeks) and the others; however, no difference was observed when group D2 (saline) and D3 (anesthesia only) were compared (Table 2).

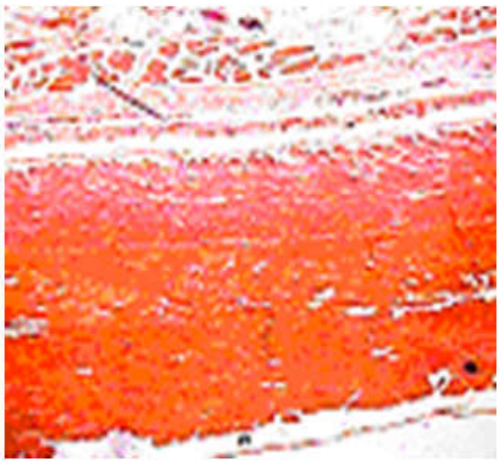

Figure 10. Glass slide mounted with normal-looking muscle tissue from rats in subgroup 2D showing no tissue thickness modification (HE – x40)

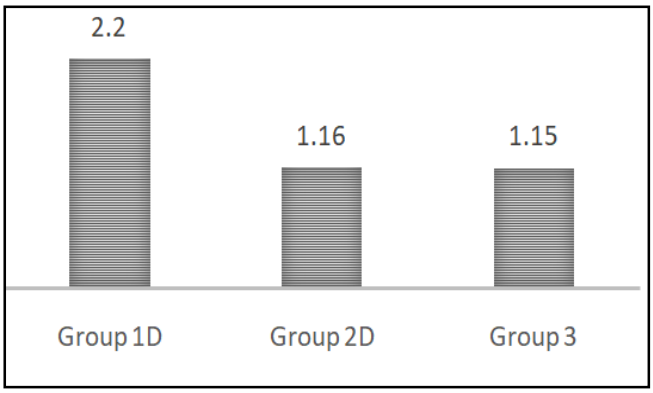

Figure 11. Muscle layer thickness of rats in groups 1D, 2D, and 3

Table 1. Muscle thickness (mm)

	n	*Mean (± SD)*	*Median*	*Amplitude*
Group 1D	5	2.20 (± 0.20)	2.18	1.96-2.50
Group 2D	5	1.16 (± 0.20)	1.18	0.90-1.43
Group 3	5	1.15 (± 0.14)	1.16	0.98-1.33

Table 2. Muscle thickness according substance exposure

	P
Group 1D (PPMA) x Group 2D (SS)	< 0.05 *
Group 1D x Group 3 (Anesthesia)	< 0.05 *
Group 2D x Group 3	> 0.05

*Kruskal-Wallis test

Table 3. Muscular thickness PMMA x SS

	N	*Mean (± SD)*	
Group 1D	5	2.20 (± 0.20)	*
Group 2D	5	1.16 (± 0.20)	

*Mann-Whitney teste p=0.008

A more thorough analysis was carried out on the animals from groups 1D and 2D, respectively, with PMMA and SS. The measurements of the muscle layer showed a significant difference ($p = 0.008$)* between both groups, Table 3.

DISCUSSION

The results obtained show histological homogeneity among the animals muscle within each group (Table 1). Nevertheless, when compared to the specimens from Group 1, a significant difference was found between these and those from other groups (Table 2 and 3). The control groups showed complete saline absorption at second week (Fig. 8). All subgroups in Group 1 (PMMA) showed complete absorption of the gel at second week (Fig 1) and the beginning of the formation of a foreign body granuloma around the PMMA microspheres forming a fibrous layer among muscular bundles (Fig 9). All previous histological studies on PMMA gel injections were carried out in subcutaneous tissue (Lemperle *et al*., 1995; Carruthers, 2005; Kraus, 2016), where there is no great resistance to the diffusion of microspheres, which tend to be arranged circularly from a gel "bubble". Thus, the disposition observed in the muscle is an original finding which takes special importance when clinical applications for the intramuscular use of PMMA gel are proposed (Figs 4 – 7). PMMA implant confirmed the biocompatible properties attributed to the material more than four decades ago, namely, it is inert, non-poisonous, and non-antigen. In two articles (DiMaio, 2002; Smith, 2005) the authors carried out reviews on the evolution of the use of PMMA as bone cement, these characteristics were reiterated. Nevertheless, they describe as the main adverse effect of the use of the product in arthroplasties the exothermic reaction which occurs during the polymerization of the product after a catalyst is added. It is noteworthy that, in PMMA gel implants, the microspheres remain in their original state and there is no heat generation. Experimental studies on histological aspects of the response to PMMA microsphere implants injected in the subcutaneous tissue (Lemperle, 2003; Lemperle, 2004) did not find evidence suggesting toxic or antigenic activities of the material. Moreover, no acute inflammatory response mediated by lymphocytes was described either. The same occurs in our experiment with no acute inflammatory or lymphocytic responses, but a mild inflammatory response with chronic evolution and mediated by macrophages, forming a foreign-body type granuloma (Fig 2 and 3).

Figures 2 and 3 are particularly interesting for evidencing the muscle tissue included in the granuloma among the PMMA particles. This is a significant finding because the presence of viable myocytes among the microspheres demonstrates the product's compatibility with striated muscle tissue. The foreign body reaction occurs due to the inability of macrophages to phagocytize PMMA particles. The microspheres have a diameter of approximately 40µm, that is, they are too large to be enveloped. Morhenn *et al*. (2003), in a study on the phagocytosis of different particles of products used as dermal implants, demonstrated that, while particles measuring 20µm or less are spontaneously phagocyted by macrophages, those of 40µm cannot be enveloped by mononuclear phagocytic cells. Lemperle *et al*. (2004) also studied the migration of injectable particles in rats and confirmed that PMMA microspheres do not migrate to the analyzed organs, including regional lymph nodes. When phagocytosis is impossible, the activated macrophages assume an epithelioid appearance and fuse, forming multinucleated giant cells that surround the implanted microspheres (formation of a foreign body granuloma). This phenomenon, observed in our study and during experimental studies (Lemperle, 2003) and described in every clinical-histological research (Morhenn, 2002; Rudolph, 1999; Requena, 2001; Caballero, 2003; Thaler, 2003; Kim, 2004; Lombardi, 2004). The same type of response induced by the polymer in the subcutaneous tissue was seen in the muscular plane, the intensity of the response in the latter was remarkably lower. Granulomas formed subcutaneously (Lemperle, 2003), despite being of the same type (foreign body) and morphologically similar, shows greater fiber density, richer in cellular material and fibers than those developed in striated muscular tissue.

This difference might be explained by the nature of the tissues. The subcutaneous layer has a greater number of tissue macrophages and, as a result, its responses to external stimuli must be more intense when compared to the muscle tissue. Nevertheless, in a clinical review performed 10 years after the use of the first product with PMMA in a colloidal delivery vehicle (Artecoll®) as an injectable subcutaneous implant for restorative and aesthetic purposes, Lemperle *et al*. (2002) confirmed the non-degradable nature of the product, as well as the stability of the granuloma formed. When performed subcutaneously, more intense reactions to the implant can explain the formation of aesthetically unpleasant granulomas (Rudolph, 1999; Lombardi, 2004), which result from excessive fibrous proliferation. As we demonstrate its do not occur when the implant is intramuscular, suggesting greater PMMA biocompatibility with muscle than the subcutaneous tissue. In spite of being less intense, the response induced by the material in the muscle promoted the formation of a stable granuloma with capsule rich in collagen fibers (Fig. 4 - 7), these findings were study also by Sun *et al*. (2006). Since these are stronger and larger fibers, it can be suggested that such response provides, in addition to increases in volume, greater tissue resistance (Fig 9 and 10). One factor known to be related to the intensity of the response is the surface of the particles. Several studies have shown that a smoother surface causes weaker macrophagic responses. Evolution in product manufacturing and quality control resulted in particles of regular size, shape and smooth surface. Thus, microsphere implants became viable as the patients' responses are less intense and more predictable. The safety and efficacy of PMMA implants are noteworthy, as it can be observed from the results obtained with the treatment of secondary facial lipodystrophy caused using antiretroviral drugs in HIV+ patients who benefited from the aesthetic effects of the technique (Kraus, 2016). The increase in muscular thickness is due to the PMMA injection that promotes an increase in tissue and granuloma volumes. This finding is especially important for the current clinical uses of PMMA, as well as for future techniques which may be developed. The volume augmentation also confirms the clinical observations previously mentioned. With the dissemination of the aesthetic results from subcutaneous PMMA injections, there has been an increase in the number of physicians who use such technique and patients being treated with it. Increasingly larger works have been carried out, including multicenter studies such as the one by Cohen *et al*. (2004), which was performed under the supervision of the Food and Drug Administration (FDA) and confirmed the favorable results reached with few complications. More articles (Fagien, 2004; Rullan, 2004) have demonstrated similar success, thus, encouraging the search for new uses of the product. Many uses for PMMA implants have been proposed, such as those for treating female urinary incontinence (Kobashi, 2002) or for substituting testicles (Karademir, 2004). Endoscopic injections of PMMA at the gastroesophageal junction to treat reflux have produced encouraging results (Feretis, 2001; Iqbal, 2006) with minimal complications. Furthermore, PMMA may also be used in the medialization of the vocal cords aiming at improving phonation due to paralysis. Although it is still restricted to isolated groups (Stein, 2000; Kwon, 2004; Bihari, 2006), this technique has been used with positive results. In several medical areas, PMMA is a safe and effective intramuscular, subcutaneous, and intradermal filler. It is used to treat Polland Syndrome (Chacur, 2019), Progressive Hemifacial Atrophy (Chacur*et al*., 2020), acne scars (Karnik, 2014), and gluteal muscle augmentation and subcutaneous remodeling (Karnik, 2014; Chacur, 2019). PMMA has been proved a very useful scaffold in tissue engineering for manufacturing cardiac valves (Oveissi, 2019), muscles (Cui, 2019), bones (Leão, 2018), and nervous tissues (Mattotti, 2012). The significant presence of collagen fibers, constituted by fibrils rich in type I and III collagen which form the thickest, densest, and strongest bundles of collagen, in the granuloma capsule suggests an increase in the resistance of the treated muscle (Fig. 5, 7 and 9). Therefore, PMMA implants might be useful in the treatment of conditions resulting from the weakening of muscle tissues, such as hernias and diverticula, or muscular volume loss. Our findings have special importance when implant is intramuscular because the PMMA microspheres form a fibrous layer among muscular bundles enhancing volume augmentation. This feature isn´tduetoneedle trauma or saline volumization (Fig 10). It allows clinical use in aesthetic surgery and/or recovers muscular volumes.

CONCLUSION

According to data from this study PMMA is biocompatible when injected into the striated muscle tissue of Wistar rats. There was a fibrous response induced by PMMA showing microspheres surrounded by fibrous material rich in collagen types I and III, with no evidence suggesting toxic or antigenic activities, nor acute inflammatory response mediated by lymphocytes was found.

ACKNOWLEDGMENTS: Augusta Ketzer Menezes for the English assistance.

REFERENCES

Bihari A, Meszaros K, Remenyi A, Lichtenberger G. Voice quality improvement after management of unilateral vocal cord paralysis with different techniques (abstract). Eur Arch Otorhinolaryngol. Epub 2006 Aug 9.

Caballero CR, Requena L, Sanmartin O, Botella R. Histopathologic findings of granuloma caused by polymetilmethacrylate microspheres. Arch Dermatol. 2003;139(11):1505.

Carruthers A, Carruthers JDA. Polymethylmethacrylate microspheres/ collagen as a tissue augmentin agent: personal experience over 5 years. Dermatol Surg. 2005 Nov;31(11)1561-5.

Chacur R, Menezes HS, Chacur NMBS, *et al.* A Treatment of Progressive Hemifacialatrophy (Parry Romberg Syndrome) with Polymethyl Methacrylate: A Case Report. Indian Journal of Applied Research. Volume -10 | Issue - 5 | May – 2020.

Chacur R, Menezes HS, Chacur NMBS, *et al*. Correction of Poland Syndrome (Chest Hypoplasia) Using Polymethylmethacrylate Implant. Biomed J Sci & Tech Res 14(1)-2019. BJSTR. MS.ID.002493.).

Chacur R, Menezes HS, Chacur NMBS, *et al*. Gluteal Augmentation with Polymethyl Methacrylate: A 10-year Cohort Study. PlastReconstr Surg Glob Open. PlastReconstr Surg Glob Open. 2019 May; 7(5): e2193

Chacur R, Menezes HS, Dias DA, *et al*. Cellulite Treatment Using Subcision and Polymethyl Methacrylate Filling – Goldinicision Case Report. Indian Journalof Applied Researc, Vol 9, Issue 9, September 2019.

Cohen SR, Holmes RE. Artecoll: a long-lasting injectable wrinkle filler material: report of a controlled, randomized, multicenter clinical trial of 251 subjects. PlastReconstr Surg. 2004 Sep 15;114(4):964-76.

Cui C, Wang J, Qian D, *et al*. Binary Colloidal Crystals Drive Spheroid Formation and Accelerate Maturation of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes. ACS Appl Mater Interfaces. 2019 Jan 30;11(4):3679-3689.

DiMaio FR. The science of bone cement: a historical review. Orthopedics. 2002 Dec;25(12)1399-1407.

Fagien S, Born TM. Artecoll: a long-lasting injectable wrinkle filler material: report of a controlled, randomized, multicenter clinical trial of 251 subjects (Discussion). PlastReconstr Surg. 2004 Sep 15;114(4):977-9.

Feretis C, Benakis P, Dimopoulos C, Dailianas A, Filalithis P, Stamou KM, Manouras A, Apostolidis N. Endoscopic implantation of Plexiglas (PMMA) microspheres for the treatment of GERD. GastrointestEndosc. 2001;53(4):423-6

Iqbal A, Salinas V, Filipi CJ. Endoscopic therapies of gastroesophageal reflux disease. World J Gastroenterol. 2006 May 7;12(17):2641-55.

Karademir K , Senkul T , Demir S , Erden D , Iseri C , Baykal K. A new testis prosthesis material: polymethylmethacrylate. Urol Int. 2004;72(1):71-5.

Karnik J, Baumann L, Bruce S, *et al*. A double-blind, randomized, multicenter, controlled trial of suspended polymethylmethacrylate microspheres for the correction of

atrophic facial acne scars. Journal of the American Academy of Dermatology, Volume 71, Issue 1, 2014, Pages 77-83.

Kim KJ, Lee HW, Lee MW, Choi JH, Moon KC, Koh JK. Artecoll granuloma: a rare adverse reaction induced by microimplant in the treatment of neck wrinkles. Dermatol Surg. 2004 Apr;30(4):545-47.

Kobashi KC, Leach GE. Injection therapy for female stress urinary incontinence. Infect Urol. 2002;15(3):9-19.

Kraus CN, Chapman LW , Korta DZ, Zachary CB. Quality of life outcomes associated with treatment of human immunodeficiency virus (HIV) facial lipoatrophy Int J Dermatol. 2016 Dec;55(12):1311-1320.

Kwon TK, Buckmire R. Injection laryngoplasty for management of unilateral vocal fold paralysis. CurrOpinOtolaryngol Head Neck Surg. 2004 Dec; 12(6):538-42.

Laeschke K. Biocompatibility of microparticles into soft tissue fillers. Semin Cutan Med Surg. 2004;23:214-7.

Leão RS, Maior JRS, Lemos CAA, *et al*. Complications with PMMA compared with other materials used in cranioplasty: a systematic review and meta-analysis. Braz Oral Res. 2018 Jun 7;32:e31.

Lemperle G, Hazan-Gaúthier N, Lemperle M. PMMA microspheres (Artecoll) for skin and soft-tissue augmentation. Part II: clinical investigations. PlastReconstr Surg. 1995 Sep;96(3):627-34.

Lemperle G, Morhenn V, Charrier U. Human Histology and Persistence of Various Injectable Filler Substances for Soft Tissue Augmentation. Aesth. Plast. Surg. 27:354–366, 2003

Lemperle G, Morhenn VB, PentonjamaspV,Gallo RL. Migration studies and histology of injectable microspheres of different sizes in mice. PlastReconstr Surg. 2004 Apr;113(5):1380-90.

Lemperle G, Ott H, Charrier U, Hecker J, Lemperle M. PMMA microspheres for intradermal implantation: Part I. Animal research. Ann Plast Surg. 1991 Jan;26(1):57-63.

Lemperle G, Romano JJ, Busso M. Soft tissue augmentation with Artecoll: 10-year history, indications, technics, and complications. Dermatol Surg. 2003 Jun;29(6):573-87.

Lombardi T, Samson J, Plantier F, Husson C, Küffer R. Orofacial granulomas after injection of cosmetic fillers. Histopathologic and clinical study of 11 cases. *J Oral Pathol Med.* 2004; 33:115-20.

Mattotti M, Alvarez Z, Ortega JA, *et al*. Inducing functional radial glia-like progenitors from cortical astrocyte cultures using micropatterned PMMA. Biomaterials. 2012 Feb;33(6):1759-70.

McClelland M, Egbert B, Hanko V, Berg, RA,.DeLustro F. Evaluation of Artecoll Polymethylmethacrylate Implant for Soft-Tissue Augmentation: Biocompatibility and Chemical Characterization. PlastReconstr Surg. 1997 Nov;100(6):1466-74.

Morhenn VB, Lemperle G, Gallo RL. Phagocytosis of different particulate dermal filler substances by human macrofages and skin cells. Dermatol Surg. 2002 Jun;28(6):484-90.

Oveissi F, Naficy S, Leefacturing A, *et al*. Materials and manufacturing perspectives in engineering heart valves: a review Mater Today Bio. 2019 Dec 5;5:100038.

Requena C, Izquierdo MJ, Navarro M, Martínez A, Vilata JJ, Botella R, *et al*. Adverse reactions to injectable aesthetic microimplants. American Journal of Dermatopathology. 2001;23(3):197-202.

Rudolph CM, Soyer HP, Schuller-Petrovic S, Kerl H. Foreign body granuloma due to injectable aesthetic microimplants. American Journal of Surgical Pathology. 1999 Jan;23(1):113-7.

Rullan PP. Soft tissue augmentation using Artecoll: a personal experience. Facial Plast Surg. 2004;20(2):111-6.

Smith DC. The genesis and evolution of acrylic bone cement. Orthop Clin North Am. 2005;36(1):1-10. 8- Morhenn VB, Lemperle G, Gallo RL. Phagocytosis of different particulate dermal filler substances by human macrofages and skin cells. Dermatol Surg. 2002 Jun;28(6):484-90.

Stein J, Eliachar I, Myles J, Munoz-Ramirez H, Strome M. Histopathologic study of alternative substances for vocal fold medialization. Ann OtolRhinolLaryngol. 2000 Feb;109(2):221-6.

Sun SG, Ma BA, Zhou Y, Zhang MH, Fan QY. Effects of bone cement particles on the function of pseudocapsule-derived fibroblasts. Acta Orthop, 2006;77(2):320-8.

Thaler MP. Foreign body granulomas caused by polymethylmethacrylate microspheres: an added perspective. *Arch Dermatol.* 2003;139(11):1505-6.

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