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Polymethylmethacrylate injections for augmentation and strengthening of abdominal muscles: an experimental model

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Abstract

Introduction: Polymethylmethacrylate (PMMA) is a synthetic, inert, biocompatible and nontoxic polymer, the applications in the medical field are numerous, and indications are increasingly broad. **Objectives:** To evaluate whether the fibrous response to injection of PMMA microspheres into striated muscles confers increased volume and resistance to muscle stretch. **Methods:** A total of 20 Wistar rats were injected with, 30% PMMA gel into the right rectus abdominis muscle, while the left rectus muscle received SHAM injections. After 4 weeks, all abdominal rectus muscles were measured and stretched on a bench dynamometer until rupture. The samples were analyzed histologically to verify the tissue response to PMMA. **Results:** The right rectus abdominis muscles of rats that received PMMA gel injection had increased volume and the breaking strength was greater than 100% compared to the left rectus muscles that received SHAM injections. There was a significant deposition of type I and III collagen fibers around the PMMA injection sites. **Conclusion:** PMMA microspheres implanted into the rectus abdominis muscle of Wistar rats promote neocollagenesis and significantly increase the breaking strength when subjected to stretch test. The mild and desired foreign body type tissue reaction around the PMMA is not objection to the augmentation of the rectus abdominis muscle.

Keywords: Polymethylmethacrylate. PMMA. Abdominal muscle. Rectus abdominis. Muscle Augmentation. Muscle strength. Neocollagenesis. Rats.

Introduction

Polymethylmethacrylate (PMMA) is a synthetic, inert, biocompatible and nontoxic polymer, the applications in the medical field are numerous, and indications are increasingly broad [1,2]. PMMA is used in cured solid form as bone cement, for treating bone fractures and osteomyelitis [3] and in the treatments of bone tumors [4]. PMMA microspheres suspended in gel form are used in injections to correct congenital and acquired deformities such as Scleroderma en coup de sabre [5], in vocal cord medialization [6], in tissue engineering for the manufacture of heart valves [7], for treating urinary and fecal incontinence and gastroesophageal reflux [8], for treating acne scars [9], and in subcutaneous and juxta-dermal collagen biostimulation obtaining satisfactory results, with low complication rates [10].

The results obtained in the filling of wrinkles and depressions in lipodystrophy resulting from the use of antiretroviral drugs in HIV confirm the safety and efficacy of the use of PMMA microspheres in the subcutaneous plane [11,12].

The intramuscular application of PMMA microspheres is the scope of this study, since the physical presence of the microspheres in the tissue stimulates neocollagenesis and neoangiogenesis, reactions that result in localized tissue-volume increase and then, the possibility of treating muscle asymmetries [13].

To date, there are no publications of experimental studies confirming the increase in volume and strength of muscle tissue after intramuscular injection of polymethylmethacrylate.

Methods

Study Design

Experimental, preclinical, nonrandomized, open-label, animal study followed the rules of the ARRIVE guidelines [14]. Evidence-based medicine (EBM) level: NA - Animal research.

Settings

This manuscript was performed in Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Ethical approval

The study was approved by the animal ethics committee, under number 82/09, of the Centro de Cirurgia Experimental, Faculdade de Medicina, Centro de Ciências da Saúde, UFRJ, Rio de Janeiro, Brazil.

Sample Size and Procedures

Twenty male Wistar rats (6 months old and weighing between 200 and 250g) were used. All rats were anesthetized and operated through a 6cm midline incision for median access to the rectus abdominis muscles.

The injectable used was Metacrill (Nutricel, Brazil), which contains 30% PMMA microspheres suspended in carboxymethylcellulose gel. The right rectus abdominis muscle of each rat received 0.3mL 30% PMMA gel along its entire length in a retroinjection movement. The injections were performed with 6 microdoses of 0.05mL per cm2. The left rectus abdominis muscle received, in the same areas, 6 saline injections of 0.05mL each injection.

Follow-up and Macroanalysis

After four weeks of the intervention, the 20 animals had their rectus abdominis muscles removed for study. Samples were evaluated macroscopically, and thickness was determined with a digital caliper in millimeters and standard deviation of the mean presented, and muscle strength was measured by a calibrated bench dynamometer (Instrutherm DD).

Histology

Microscopy was used to evaluate the tissue reaction in both groups, with emphasis on the analysis of the type and intensity of fibrous response in each case. Parallel to the morphological study of the sections stained with Hematoxylin-Eosin (HE), a histochemical study was performed using Sirius red (Picrosirius method) to visualize type I and type III collagen fibers. For further study, the fibers, and the histological preparations stained with Sirius red were also examined under polarized light.

Statistical Analysis

Data on thickness and strength were analyzed using SigmaStat® for Windows® version 3.1 (Jandel Corporation, San Raphael, CA, USA), with a significance level of 1% ($p<0.01$). Differences between the PMMA group and the SHAM group were analyzed by paired t-test and using One Way Repeated Measures ANOVA. Data were expressed as standard deviation of the mean.

Results

The procedure was performed **(Figure 1)**, and there was no ischemia or tissue necrosis. The collected specimens showed a difference in thickness visible to the macroscopic observation. The thickest samples were in the PMMA group, while the samples in the SHAM group were less thick. The right muscles showed greater thickness with statistical significance *($p<0.01$) **(Figure 2).**

Figure 1. PMMA injection in right rectus abdominis muscle.

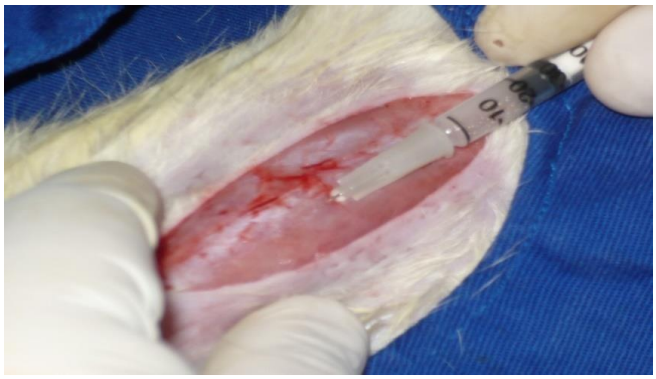
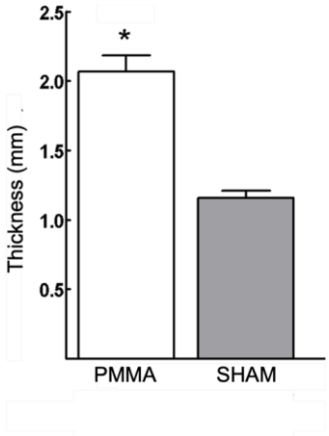


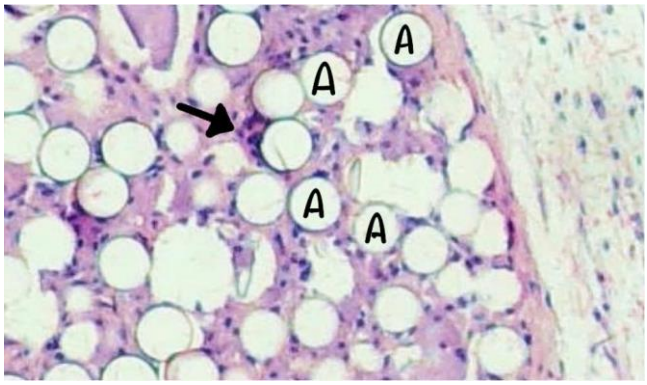
Figure 2. The thickness of the rectus abdominis muscles measured four weeks after injection. *Significantly difference between right and left rectus muscle ($p<0.01$).



On slides stained with HE, four weeks after injection, **Figure 3** shows a typical implant of PMMA microspheres with stable epithelioid histiocytes interspersing the implanted microspheres with a well-defined capsule and fibrous septa, without any

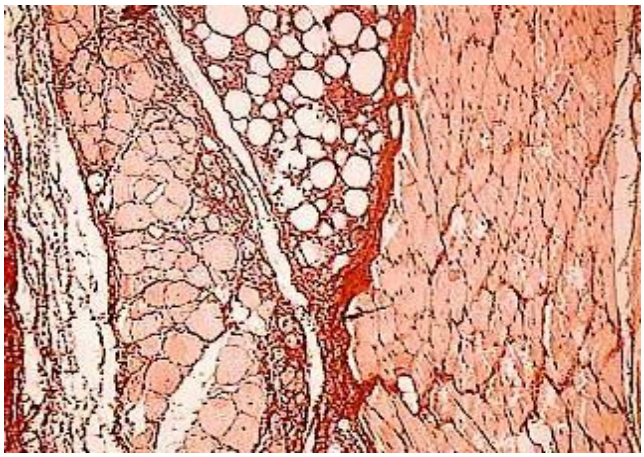
lymphocytic reaction or granuloma (without an immune hyperreaction with many giant cells).

Figure 3. Photomicrograph of Group PMMA. No vehicle residue is observed, showing PMMA microspheres (A) surrounded by macrophages and foreign bodygiant cells (arrow) (HE x400).



It is also observed that when PMMA is injected into the muscle plane, it does not dissect the muscle fibers, but follows the perimysium and lodges between the muscle fiber bundles without permeating the myocytes **(Figure 4).**

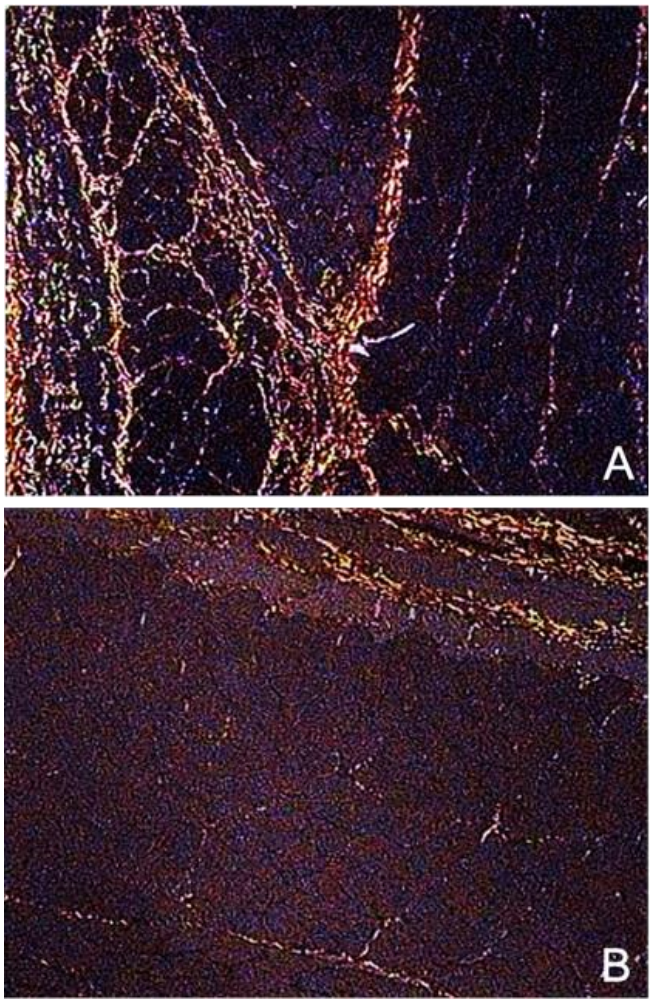
Figure 4. Photomicrograph of a right rectus muscle at 4 weeks. The void fibrina envelops of the dissolved PMMA microspheres are seen within the perimysium and attached to viable muscle tissue. The volume of the implant is about half of the size it will be after more tissue ingrowth at 3 months. (Sirius red x100).



In the PMMA group, Sirius Red staining under polarized light, it was possible to confirm the mentioned thickening of the capsule formed around the implant, always at the expense of the deposition of collagen fibers with a predominance of type I collagen. We also observed tenuous septa permeating the microspheres, formed by reticular fibers rich in type III collagen, which are shown in green **(Figure 5A).**

Microscopic images of the muscle tissue studied in the SHAM group showed a normal histological pattern, with the presence of collagen fibers in the epimysium and perimysium, and reticular fibers in the endomysium, which permeates the bundles, between the myocytes **(Figure 5B).**

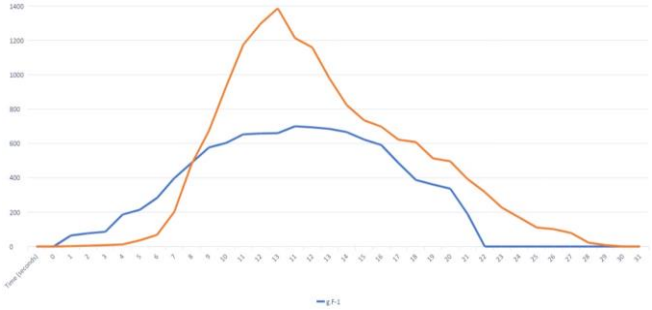
Figure 5A. Photomicrograph of a right rectus muscle. Fiber deposition is observed, with a predominance of type I collagen, in the capsule surrounding the injection. It is also possible to notice the appearance of tenuous septa of reticular fibers between the particles, being particularly interesting to observe that these fibers are like the endomysium, making the structural aspect of the injection like that of native muscle tissue. (Sirius red, polarized light x100). **Figure 5B.** Representative photomicrograph of Group SHAM. The normal aspect is observed, with collagen fibers in the epimysium and perimysium, and reticular fibers in the endomysium. (Sirius red, polarized light x100).



The resistance of the abdominal rectus muscles was measured until complete rupture **(Figure 6).** The PMMA group showed an average resistance of 1387g/F in 13s, while the SHAM group, 700g/F in 14s **(Figure 7).**

Figure 6. Measurement of the resistance of the rectus abdominis muscles. Demonstration of rupture of the rectus abdominis muscles.

Figure 7. Graph recorded by the dynamometer, comparing the distension of the muscle fibers, the beginning of the rupture, and the total time of the separation of the fibers. Right injected muscles (orange curve) at 1387 g/f in 13s, while SHAM injected left muscles (blue curve) ruptured already at 700 g/f in 14s. Significantly different between groups ($p<0.01$).



Discussion

The histological structure of the striated muscles of rats (Figure 1) is similar to that of man and common to all vertebrates [15]. The skeletal muscle tissue has longitudinal muscle fibers grouped in bundles bounded by the perimysium. Four weeks after the intervention, PMMA deposition in the right rectus abdominis muscles followed the perimysium in linear arrangements, and there was complete absorption of the gel vehicle.

The image presented in Figure 4 shows the muscle tissue interspersed by the PMMA microspheres, i.e., the coexistence of viable myocytes and no signs of cell damage among the injected synthetic microspheres, which reaffirms the histocompatibility of the product with striated muscle tissue.

Around the PMMA microspheres, on microscopy stained by Hematoxylin and eosin, there is the expected fibrous response, with the presence of macrophages, few foreign body giant cells, and deposition of collagen fibers. Type I collagen is the strongest, thickest, most densely packed, has a variable diameter (from 1 to 20 µm), and is characterized by its tensile strength, present in the perimysium and muscle tendons. The fibers composed of collagen type III form a finer network, measuring 0.5 to 2 µm, and are known as reticular fibers. These are present in various organs and form the structure that supports the extracellular matrix of the tissues. More than support, the reticular arrangement and the fine caliber of these fibers give the tissues the appropriate expansion capacity for organs that suffer volume changes, such as arteries, spleen, liver, and uterus, for example [16]. Then, it was possible to observe that the structural arrangement assumed by the new fibrous tissue in the implanted area resembles that of the muscle itself, with reticular septa (Figure 5A), similar to the endomysium, permeating the particles and joining the capsule, rich in collagen fibers (Figure 5B) that are arranged longitudinally, along the perimysium. These data are the same as presented by Teixeira et al. in 2021 [1].

As for the volume of muscle tissue, measuring the thickness of the samples studied in the different groups allowed us to state that the injection of PMMA microspheres in a 30% suspension in a gel vehicle promotes a volumetric increase due to the presence of biocompatible synthetic material plus the expected reaction: the formation of a foreign body-type reaction rich in fibers of the collagen system and mediated by macrophages, without a hyperimmune response like the pathological granuloma (Figure 3).

Morhenn et al. in 2002 [17] presented a study in which particles with 20micra or smaller are naturally phagocytized by macrophages. Microspheres around 40micra, such as those of PMMA in this study, suspended in a carboxymethylcellulose gel, are recognized by macrophages [9] but are not phagocytosed and transported through veins or lymph nodes. To isolate larger or irregular particles, or those with a rough surface, activated macrophages take on an epithelioid appearance and fuse around the particles, forming multinucleated giant cells. This phenomenon is the first step in the formation of a foreign body granuloma [1,18]. Thus, the foreign body reaction is the natural immune response to the injection performed, while the foreign body granuloma is a hyperimmune response to larger or irregular particles, also called LOIR (Late-Onset Inflammatory Response) [18]. No foreign body granuloma to PMMA microspheres has been reported when these are injected into deeper tissues,

such as epiperiosteal or intramuscular.

The right rectus abdominis muscles of rats that received PMMA gel injection (Figure 6) had an increase in breaking strength of more than 100% compared with the left rectus abdominis muscles that received SHAM. The statistically significant increase in muscle thickness (Figure 2) and rupture strength (Figure 7) are the new and relevant data of the study.

Conclusion

Polymethylmethacrylate implanted into the rectus abdominis muscle of Wistar rats promoted neocollagenesis and significantly increased the volume and burst strength of this muscle when subjected to stretch test. These findings open the possibilities for further studies on the clinical use of PMMA in muscle strengthening and treatment of muscle asymmetries.

Acknowledgement

Not applicable.

Funding

Own financing.

Ethical Approval

The study was approved by the animal ethics committee, under number 82/09, of the Centro de Cirurgia Experimental, Faculdade de Medicina, Centro de Ciências da Saúde, UFRJ, Rio de Janeiro, Brazil.

Data sharing statement

No additional data are available.

Conflict of interest

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

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