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Polymethylmethacrylate dermal fillers: evaluation of the systemic toxicity in rats

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Abstract. This study evaluated local and systemic reactions after an intravascular injection of polymethylmethacrylate (PMMA) at two concentrations in a murine model. Thirty rats were divided equally into three groups: 2% PMMA, 30% PMMA, and a control group (normal saline only injection). The filler was injected into the ranine vein. The rats were sedated at 7 and 90 days and a clinical evaluation performed. After euthanasia, the right lung, liver, and right kidney were removed, weighed, and microscopically analyzed. The submandibular lymph nodes and tongue were removed and examined microscopically. Serum was subjected to liver and kidney function tests. No groups showed clinical alterations. Microspheres were not observed at any distant organ. Two samples from the 2% PMMA group showed a local inflammatory response at day 7 and another two samples from the 30% PMMA group at day 90. The group injected with 30% PMMA presented higher levels of alanine aminotransferase ($P = 0.047$) after 90 days when compared with the other groups. The data obtained in this study demonstrate that intravascular injections of PMMA fillers show potential health risks such as chronic inflammation at the implantation site.

Key words: oral medicine; polymethylmethacrylate; adverse effects; toxicology.

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Introduction

The use of facial fillers is one of the options to minimize the signs of facial ageing and to correct skin imperfections. They are increasingly preferred over conventional plastic surgery due to their low financial cost and painless and noninvasive application.^{1–5}

Each type of soft-tissue filler has its own technique of implantation, period of permanence in the tissues, and adverse effects that are inherent to its composition.^{1,2}

Several chemical compounds can trigger toxic reactions, and there are different ways to evaluate the possible outcomes.⁶

Facial fillers containing polymethylmethacrylate (PMMA) can be classified as non-biodegradable or permanent, since they consist of non-absorbable microspheres that are suspended in an aqueous carrier or bovine collagen.⁷ Permanent fillers must be carefully monitored due to the severity of possible complications that can occur late and can present a difficult or impossible resolution.^{8–10}

The term ‘migration’ is found in the literature to define the displacement of PMMA microspheres in two different ways: when they are injected into blood vessels,^{11,12} or when they are transported through phagocytosis.¹³

PMMA has a specific implantation technique and must not be injected into blood vessels, to avoid particle migration and to achieve a satisfactory result. However, there are several published case reports of disastrous local complications due to injections being intravascular or very

close to a blood vessel.^{14–17} A study on the systemic manifestations caused by intravenous injection of PMMA fillers in humans or animals has not been published previously.

The aim of this study was to identify and analyze fast-occurring as well as latent systemic and local tissue reactions and the occurrence of particle migration to distant organs after intravascular injection of PMMA at different concentrations in a murine model. It is necessary to better understand the mechanism of a possible toxicity induced by this material in order to improve the safety of this treatment. The ventral surface of the rat tongue was selected as the site of injection owing to the fact that it is less vulnerable to traumatic factors¹⁸ and also as it is highly vascularized, comprising a plexus composed of a superficial vascular network and the ranine veins.¹⁹

Materials and methods

This research was initiated after approval from the scientific and ethics committee

and the ethics committee for animal use; the procedures were carried out in accordance with institutional guidelines for animal care and use.

The material used for this experiment was PMMA microspheres suspended in hydroxyethylcellulose gel. Two extremes of concentration (2% and 30%) were chosen to compare the possible systemic toxic effects when the product is injected into a blood vessel. The materials were injected at the beginning of the experiment. Clinical and histological evaluations were performed at 7 and 90 days (Fig. 1).

Animal model

The sample comprised 30 female Wistar rats (*Rattus norvegicus*) from the same breeder. Each animal weighed about 200 g and had an average age of 2 months at the beginning of the experiment. They were obtained from the animal facility of the State Foundation of Production and Health Research (FEPPS), Porto Alegre, Brazil. Animals were individually numbered on the tail and housed in plastic

cages placed in ventilated racks at a temperature of 22 °C with a 12-h light–dark cycle. Animals were fed a standard diet of rat chow (Nuvilab-CR1) and given water ad libitum. The animals were randomly allocated to one of three groups, according to the treatment received: group 1 ($n = 10$): 2% PMMA; group 2 ($n = 10$): 30% PMMA; and group 3 ($n = 10$): control, 0.9% NaCl; each group was divided equally into experimental periods of 7 days (A) and 90 days (B).

Anaesthesia

Initially, each rat was weighed on a digital scale (Urano model UDI 2500/0.5; Urano, Canoas, RS, Brazil) to calculate the dose of anaesthetic. This procedure was performed with an intraperitoneal injection of a mixture of 20 mg/ml xylazine hydrochloride (0.05 ml/100 g), a sedative, analgesic, and muscle relaxant, with 50 mg/ml ketamine hydrochloride (0.1 ml/100 g), an anaesthetic for veterinary use. Animals from the respective groups, chosen randomly, were successively anesthetized.^{4,18}

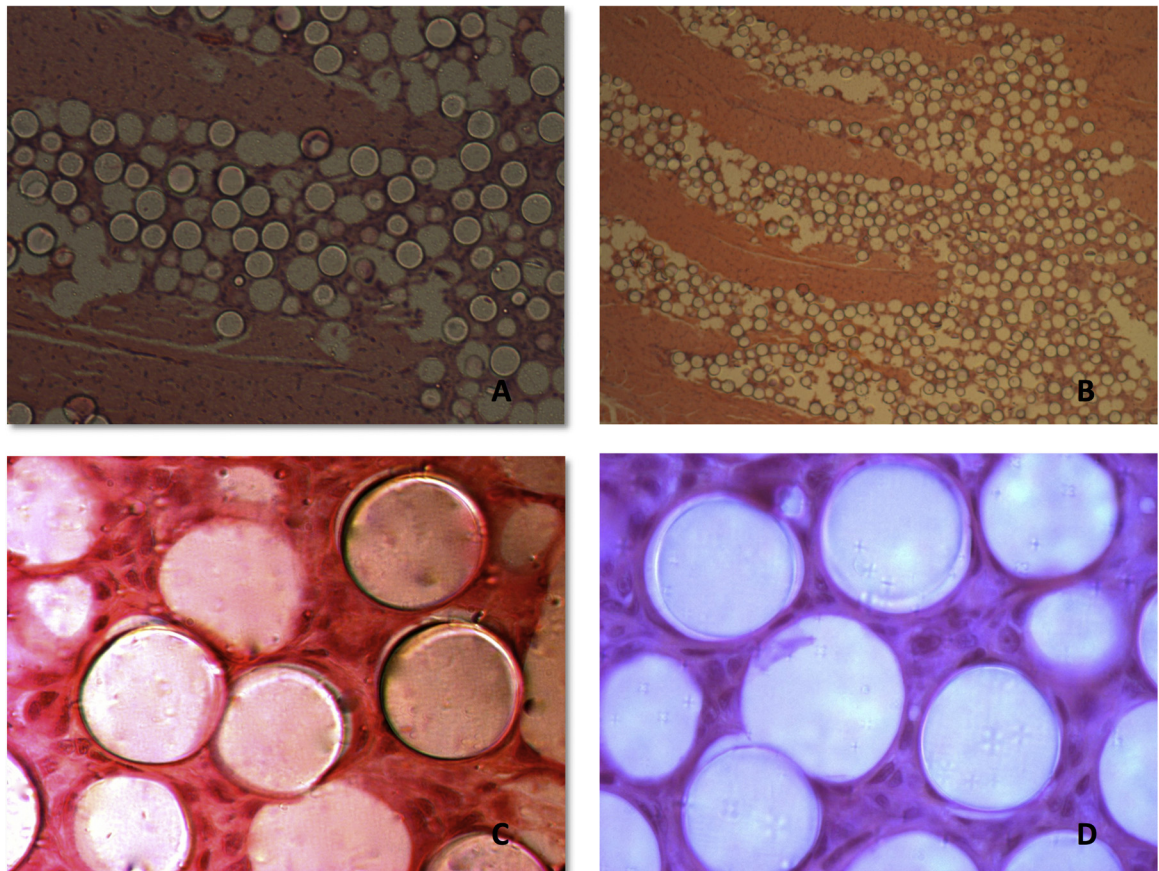


Fig. 1. Phase contrast microscopy showing PMMA microspheres in the tongue with different manipulations and magnifications: (A) 200 \times , (B) 100 \times , (C) and (D) 400 \times .

Injection of the material

When sedation was evident, the animal was placed on a surgical table in supine position and its paws tied with the use of elastic strips. The tongue was pulled out with tweezers to expose the ventral tongue region. Using a disposable insulin syringe (1/2 in. 26G; 13 mm × 0.45 mm), 0.05 ml of each material (group 1: 2% PMMA; group 2: 30% PMMA; and group 3: 0.9% NaCl) was injected into the right ranine vein (lingual vein), which is located lateral to the lingual frenulum.¹⁹ The needle was inclined as parallel as possible to the mucosa, with the bevel facing up.

Clinical evaluation

Before euthanasia, the animals were weighed and sedated in such a way that the tongues could be clinically evaluated. The clinical examination sought to identify possible tissue alterations, such as swelling, nodules, ulceration, necrosis, and/or suppuration.

Euthanasia

After injection of the material, animals were sacrificed and terminally bled by cardiac puncture at the respective monitoring periods.

Sample processing

Immediately before euthanasia, the animals were anesthetized via isoflurane inhalation. After anaesthesia, a thoracotomy was performed and blood samples collected without haemolysis by cardiac puncture. Samples were centrifuged at 8000 rpm at 4 °C to separate serum. The animals underwent necropsy; their right kidney, right lung, and liver were removed, weighed, and microscopically analyzed. The submandibular lymph nodes and the tongue were removed and examined microscopically. Sample fixation was carried out with the use of 10% neutral-buffered formalin for a minimum of 24 h. Samples of the tongue, lymph nodes, right lung, right kidney, and liver were sectioned longitudinally into two fragments. The inclusion was performed so that the edge of the sample had its long axis parallel to the paraffin block section. For each specimen, there was one histological section of 6 μm per slide, which was stained with haematoxylin and eosin (H&E).

Histological evaluation

The analysis of the slides was carried out in a blinded fashion (using masks for all

the evaluated slides) by an examiner who had previously been calibrated. The analysis of histological sections was conducted in the pathology unit using a biological microscope (Zeiss Axioskop 40, Carl Zeiss, Jena, Germany) coupled to a camera (Cool SNAP-Pro cf, Media Cybernetics, Bethesda, MD, USA) connected to a Dell Optiplex GX620 computer (Dell, Round Rock, TX, USA), at 100×, 200×, and 400× magnifications. Images of the tongue samples were transferred to Image-Pro Plus, version 4.5.1 (Media Cybernetics, Inc.; 2005). Some slides were analyzed with a fixed stage microscope for electrophysiological research (ECLIPSE FN1, Nikon, Tokyo, Japan) for phase contrast microscopy.

Inflammatory reaction of the tongue

Histological evaluation of the tongue was performed with an analysis of the presence or absence of lymphocytes, plasma cells, macrophages, giant cells, neutrophils, eosinophils, oedema, and hyperemia, at 200× magnification.

Migration

The microscopic evaluation of migration was based on the presence or absence of microspheres in the submandibular lymph node, right kidney, right lung, and liver of each animal.

Histological scoring of liver injury

Liver tissue was scored for histological necrosis and inflammation according to the modified activity index (HAI) grading.^{20,21}

Histological scoring of kidney and lung injury

Kidney and lung were evaluated based on the presence or absence of an inflammatory reaction.

Serum analysis

Serum was subjected to liver and kidney function tests. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in serum was quantitatively measured using a VITROS DT60 Chemistry System (Ortho Clinical Diagnostics, Rochester, NY, USA) by a kinetic reaction with multiple measuring points. The serum creatinine level was determined by two-point kinetic method (Labtest Diagnostics, Lagoa Santa, MG,

Brazil). Samples were processed in an automatic biochemical system (Vitros Fusion 5.1; Ortho Clinical Diagnostics, Rochester, NY, USA), using specific reagents for each test. The tests were performed in the clinical laboratory.

Statistical analysis

All data were tabulated and analyzed using SPSS 17 software (SPSS Inc., Chicago, IL, USA), with a two-way analysis of variance (ANOVA) parametric test, complemented by the Bonferroni correction test, at a significance level of 5%.

Results

Clinical alterations

No group showed any clinical alteration at 7 and 90 days.

Migration

Microspheres were not observed in any distant organs (lung, liver, and kidney) or lymph nodes.

Inflammatory reaction of the tongue

Two samples from the 2% PMMA group showed moderate inflammation at 7 days, while another two samples from the 30% PMMA group also showed inflammation at 90 days—one being moderate with an infiltrate of mononuclear cells and sparse neutrophils and eosinophils, and the other being severe with a lymphoplasmacytic infiltrate and granuloma formation. Mastocytosis was observed during the histological evaluation of several samples of the tongue, lymph nodes, and submandibular gland at all times of the study (Fig. 2).

Liver function tests

At 90 days, there was a significant difference in ALT levels ($P = 0.047$) between the group injected with 30% PMMA and the other two groups (2% PMMA and control) (Fig. 3). The levels of AST were not significantly different between the groups at any time of the study.

Creatinine

At 90 days, all groups showed a significant increase in creatinine levels ($P = 0.001$), but there was no difference between the PMMA groups and the control group (Fig. 3).

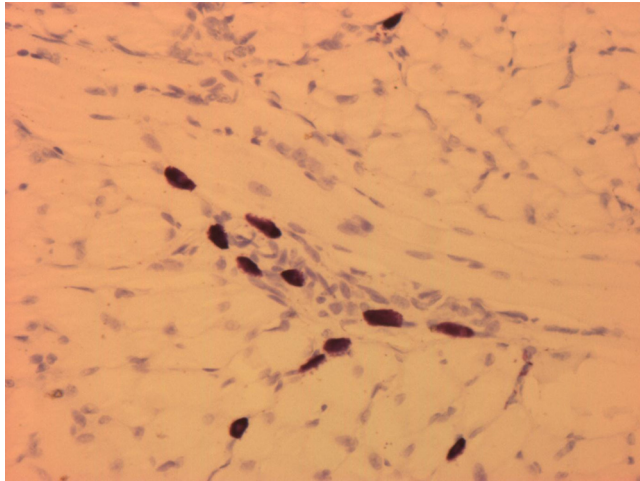


Fig. 2. Mastocytosis was evident after staining with toluidine blue.

authors that PMMA particles could migrate to distant organs, this study evaluated the aspects involved in a possible systemic reaction to this material using a murine model. The experiments were conducted through the methodology used in most toxicity studies.

Kidney and liver are considered target organs for drug metabolism. The kidney plays an important role in the filtration of plasma and metabolic homeostasis, and therefore medications can induce some level of toxicity by interacting directly with the organ's structure or indirectly inducing some damage to the electrolyte balance or blood circulation.^{22,23} Serum creatinine levels should be measured to identify possible toxic effects on the kidney that are influenced by the following factors: drug pharmacokinetics, dose tolerance, drug interactions, physiological variations, pathological factors, and genetic factors.²⁴

Due to the major role of liver in the metabolism and excretion of drugs and xenobiotics, it will frequently manifest signs of toxicity. In the USA, the principal cause of death due to acute liver failure is drug-induced liver injury.^{23,25} Serum levels of AST and ALT are considered an important sign of hepatocellular

Organ weight evaluation

Changes in liver weight did not differ significantly between the groups at any observation time. Lung weight was statistically different between the PMMA groups ($P = 0.048$) only at 7 days, while the kidney weight increase was associated with the animals' gain in body weight during the study period (Fig. 4).

Discussion

The lack of published studies on the possible toxic effects that could be caused by the systemic distribution of PMMA microspheres prompted this study. Since it has been reported widely that needle misplacement due to professional inexperience may result in an intravascular injection and because it has been suggested by some

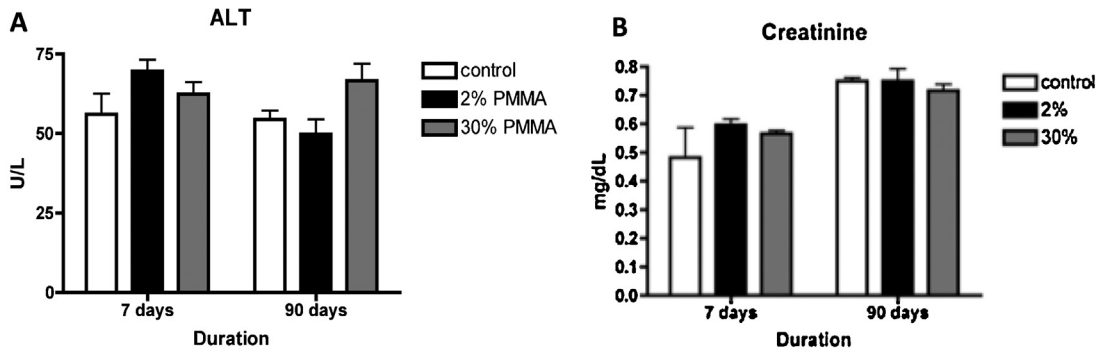


Fig. 3. Serum levels of alanine aminotransferase (ALT) (A) and creatinine (B). There was a difference in ALT between the PMMA groups and the control group over time, while for creatinine the differences were only due to time.

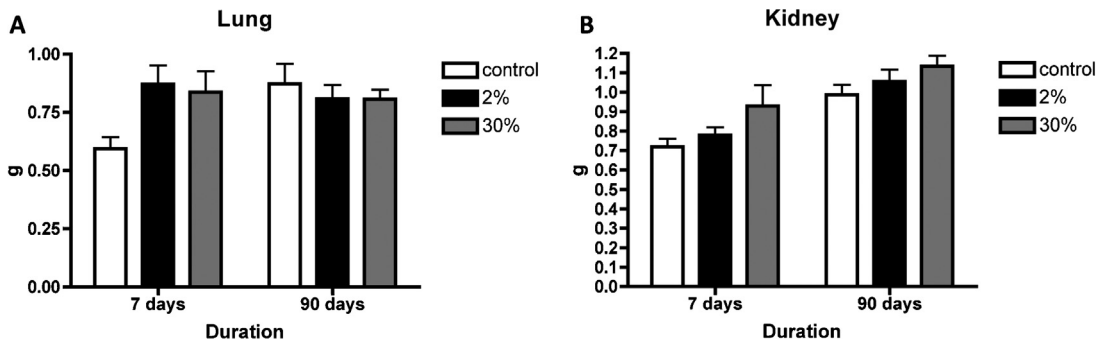


Fig. 4. The lung weight of samples from the 2% and 30% PMMA groups was statistically higher ($P = 0.048$) than that of the control group at 7 days (A). There was a proportional increase in kidney weight in all groups at 90 days (B).

damage.²⁶ ALT is present basically in the liver, while AST is also involved in other organs.

Although histological signs of liver and kidney damage were not found in this study, the level of ALT activity in serum was higher in the group injected with 2% PMMA than in the other two groups at 7 days, while at 90 days the 30% PMMA group showed higher levels of ALT. This indicates that for each group, the effect of PMMA differed from the control group over time. Previous studies have demonstrated that ALT levels higher than 51 U/l could be considered a sign of hepatotoxicity.²⁷ However, no difference was found when the AST levels were assessed, and in comparing creatinine levels, only time seemed to account for increases. This fact could be related to the ageing process in the animals.

In toxicology experiments, a comparison of the organ weights of the treated group and control group is conventionally used to evaluate the toxic effect of the test product, and changes may represent a sensitive indicator of chemically induced toxicity. However, organ weight data must be interpreted with caution and considered in combination with clinical and histopathology findings, since the changes may not be related to the treatment.^{28,29} Our evaluation of weight changes was not able to determine whether they were the result of toxicity induced by PMMA since a correlation between clinical and histopathology findings was not established, despite the statistically different lung weight data between the PMMA groups and the control group.

The systemic distribution of the injected material was evaluated microscopically in all experimental groups on the basis of the presence or absence of an inflammatory response and/or PMMA particles in the right submandibular lymph node, lung, kidney, and liver of each animal. Microspheres were not observed in this study at any distant organ and inflammation was also not present at any distant organ. The submandibular glands of some animals were removed by accident and surprisingly microspheres were found in two specimens of the 2% PMMA group after 7 days. Our findings are in line with most previous studies, indicating that PMMA microspheres are not able to migrate to distant organs, in contradiction to the findings of Rosa and de Macedo,³⁰ who observed hepatic and renal inflammation after PMMA injection in mouse ears. However, the microspheres were observed in the submandibular gland, so the results could indicate a different situation if the

organs were entirely examined, or if other organs were also assessed, such as the heart and spleen. Surprisingly thromboembolic complications were not detected at any observation time, and also unexpected is the fact that inflammation in the tongue was observed in only four animals, two in the 2% PMMA group at 7 days and two animals in the 30% PMMA group at 90 days, with just one case being a granuloma formation. These findings differ from the results obtained by Moure et al.⁴; the authors performed an injection at the ventral surface of the tongue of a greater amount of PMMA (0.07 ml) and found an intense inflammatory reaction with polymorphonuclear neutrophil infiltration after 7 days and chronic inflammation with moderate intensity at 60 and 90 days, represented by the presence of a lymphoplasmacytic infiltrate and giant cells next to the PMMA particles at all observation times.

Mastocytosis was observed during the histological evaluation of several samples of the tongue and lymph nodes at all times of the study. In an attempt to establish a correlation between their presence and a possible foreign-body reaction that could be mediated by this type of cell, mast cells were scored in the tongue samples. However, the statistical analysis demonstrated that mast cells decreased in number in the tongue samples after 90 days and that there was no difference between PMMA groups and the control group. It can be assumed that since mast cells are found in larger quantities in acute wounds, participating in the inflammatory reaction, angiogenesis, and extracellular matrix reabsorption and remodeling,^{31,32} even the trauma caused by needle insertion could result in an increased presence of mast cells at the injection site. It should also be highlighted that recent studies have demonstrated that plasma estradiol levels increase in the presence of mast cells in several tissues in a dose-dependent way,³³ and since female rats were employed in our study, we have to consider the possibility that our findings may be related to changes in the animals' estrous cycle.

Considering our methods and the data obtained in this study, intravascular injection of PMMA fillers shows potential health risks, such as chronic inflammation at the implantation site. Furthermore, the group injected with 30% PMMA had elevated ALT levels at 90 days and both PMMA groups showed increased lung weight at 7 days, indicating a non-specific systemic reaction. Considering our present results and the lack of previous studies

examining the possible reactions of systemic toxicity and genotoxicity caused by cosmetic fillers containing PMMA, further research on this issue is warranted.

Funding

None.

Competing interests

None declared.

Ethical approval

This study was carried out with the approval of the Scientific and Ethics Committee (protocol 0060/11) and the Ethics Committee for Animal Use (protocol 11/00261) of the Pontifical Catholic University of Rio Grande do Sul, PUCRS, Brazil.

References

1. Cox SE. Clinical experience with filler complications. *Dermatol Surg* 2009;**35**:1661–6.
2. Dastoor SF, Misch CE, Wang H. Dermal fillers for facial soft tissue augmentation. *J Oral Implantol* 2007;**33**:191–204.
3. Medeiros CC, Cherubini K, Salum FG, de Figueiredo MA. Complications after polymethylmethacrylate (PMMA) injections in the face: a literature review. *Gerodontology* 2013. <http://dx.doi.org/10.1111/ger.12044>. March 7 [Epub ahead of print].
4. Moure SP, de Vargas KF, Borghetti RL, Salum FG, Cherubini K, da Silva VD, de Figueiredo MA. Clinical and pathological characteristics of polymethylmethacrylate and hyaluronic acid in the rat tongue. *Int J Oral Maxillofac Surg* 2012;**41**:1296–303.
5. De Maio M. The minimal approach: an innovation in facial cosmetic procedures. *Aesthetic Plast Surg* 2004;**28**:295–300.
6. Borzelleca JF. Paracelsus: herald of modern toxicology. *Toxicol Sci* 2000;**53**:2–4.
7. Caldellas AV, Castro CC, Aboudib JH, Guimarães LA, Geissler P, Cedrola J. The polymethylmethacrylate effects on auricle conchal cartilage: report of 21 cases. *Aesthet Surg J* 2010;**30**:434–8.
8. Zielke H, Wölber L, Rzyany B. Risk profiles of different injectable fillers: results from the Injectable Filler Safety Study (IFS Study). *Dermatol Surg* 2008;**34**:326–35.
9. Lemperle G, Knapp TR, Sadick NS, Lemperle SM. ArteFill permanent injectable for soft tissue augmentation. I. Mechanism of action and injection techniques. *Aesthetic Plast Surg* 2010;**34**:264–72.
10. Duffy DM. Complications of fillers: overview. *Dermatol Surg* 2005;**31**:1626–33.
11. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. *Aesthetic Plast Surg* 2003;**27**:354–66.

12. Lemperle G, Morhenn VB, Pestonjamas V, Gallo RL. Migration studies and histology of injectable microspheres of different sizes in mice. *Plast Reconstr Surg* 2004;**113**:1380–90.
13. Tomazic-Jezic VJ, Merritt K, Umbreit TH. Significance of the type and the size of biomaterial particles on phagocytosis and tissue distribution. *J Biomed Mater Res* 2001;**55**:523–9.
14. De Castro ACB, Collares MVM, Portinho CP, Dias PC, Pinto RA. Extensive facial necrosis after infiltration of polymethylmethacrylate. *Braz J Otorhinolaryngol* 2007;**73**:850.
15. Kubota T, Hirose H. Permanent loss of vision following cosmetic rhinoplastic surgery. *Jpn J Ophthalmol* 2005;**49**:535–6.
16. Silva MT, Curi AL. Blindness and total ophthalmoplegia after aesthetic polymethylmethacrylate injection: case report. *Arq Neuropsiquiatr* 2004;**62**:873–4.
17. De Figueiredo JC, Naufal RR, Zampar AG, Mélega JM. Expanded median forehead flap and abbé flap for nasal and upper lip reconstruction after complications of polymethylmethacrylate. *Aesthetic Plast Surg* 2010;**34**:385–7.
18. Borghetti RL, De Vargas KF, Moure SP, Salum FG, De Figueiredo MA. Clinical and histologic evaluation of effects of hyaluronic acid in rat tongue. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;**113**:488–94.
19. Verli FD, Marinho SA, Rossi-Schneider TR, Yurgel LS, de Souza MA. Angioarchitecture of the ventral surface of the tongue from Wistar rats. *Scanning* 2008;**30**:414–8.
20. Lee S, Kim S, Le HD, Meisel J, Strijbosch RS, Nose V, Puder M. Reduction of hepatocellular injury after common bile duct ligation using omega-3 fatty acids. *J Pediatr Surg* 2008;**22**:2010–5.
21. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**:696–9.
22. Choudhury D, Ahmed Z. Drug-associated renal dysfunction and injury. *Nat Clin Pract Nephrol* 2006;**2**:80–91.
23. Cui Y, Paules RS. Use of transcriptomics in understanding mechanisms of drug-induced toxicity. *Pharmacogenomics* 2010;**11**:573–85.
24. Short A, Cumming A. ABC of intensive care: renal support. *BMJ* 1999;**3**:41–4.
25. Baillie TA, Rettie AE. Role of biotransformation in drug-induced toxicity: influence of intra and inter-species differences in drug metabolism. *Drug Metab Pharmacokinet* 2011;**26**:15–29.
26. Strom SC, Davila J, Grompe M. Chimeric mice with humanized liver: tools for the study of drug metabolism, excretion, and toxicity. *Methods Mol Biol* 2010;**640**:491–509.
27. Lenaerts AJ. Significant increases in the levels of liver enzymes in mice treated with anti-tuberculosis drugs. *Int J Antimicrob Agents* 2005;**26**:152–8.
28. Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, et al. Society of toxicologic pathology position paper: organ weight recommendations for toxicology studies. *Toxicol Pathol* 2007;**35**:751–5.
29. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicol Pathol* 2007;**35**:742–50.
30. Rosa SC, de Macedo JL. Reações adversas a substâncias de preenchimento subcutâneo. *Rev Soc Bras Cir Plast* 2005;**20**:248–52.
31. Babaei S, Bayat M. Effect of pentoxifylline administration on mast cell numbers and degranulation in a diabetic and normoglycemic rat model wound healing. *Iran Red Crescent Med J* 2012;**14**:483–7.
32. Hebda PA, Collins MA, Tharp MD. Mast cell and myofibroblast in wound healing. *Dermatol Clin* 1993;**11**:685–96.
33. Jing H, Wang Z, Chen Y. Effect of oestradiol on mast cell number and histamine level in the mammary glands of rat. *Anat Histol Embryol* 2012;**41**:170–6.

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