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Poly(2-hydroxyethyl methacrylate) microspheres/liquid poly(dimethylsiloxane) composition for correction of small defects in face: Histological evaluation in animal experiment

D. HORÁK[1,*], A. ADAMYAN[2], O. GOLUBEVA[2], N. SKUBA[2], T. VINOKUROVA[2]
[1]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic; Center for Cell Therapy and Tissue Repair, 2nd Medical Faculty, Charles University, V Úvalu 84, 150 00 Prague 5, Czech Republic*
E-mail: horak@imc.cas.cz
[2]*Vishnevskii Institute of Surgery, Academy of Medical Sciences, 113 811 Moscow, Russia*

Two kinds of composition based on commercial liquid poly(dimethylsiloxane) and laboratory-made poly(2-hydroxyethyl methacrylate) (PHEMA) microspheres of different size fractions (30–40 or 125–180 μm) were prepared. Tissue reaction on injection of the compositions, optimum microsphere size and morphology were investigated in the experiments on rats. The microspheres induced foreign body reaction characterized by an increased content of fibroblasts and mild infiltration of injection field by inflammatory cells. The 125–180 μm microspheres seemed to be well covered with poly(dimethylsiloxane) and more uniformly distributed in the tissue than the 30–40 μm ones. As a result, the extent of foreign body reaction induced by the former microspheres was somewhat lower than that induced by the latter. Moreover, time-dependent degradation of 30–40 μm PHEMA microspheres was more pronounced than that of 125–180 μm ones, which can affect duration of the aesthetic effect after prospective facioplasty. Results of histological investigations demonstrate a good prospect of the proposed composition for contour and bulk facioplasty of small soft tissue defects and skin wrinkles.

1. Introduction

Correction of skin wrinkles in face and modeling of lip contours and lip bulk by surgical means is associated with many disadvantages. Simple and comfortable injection methods are therefore being developed allowing desirable aesthetic results to be achieved without postoperative scars [1]. Injection methods require insertion of a supplementary material into lips. This leads to a search for new materials to ensure a lasting aesthetic effect after correction of wrinkles and facial defects. Such materials have to satisfy the following requirements [2]: Simple application by injection, biocompatibility with the organism, long-term effect.

Initially, various synthetic and biological polymers, e.g., broken cartilage were used in injectable preparations for facioplasty. A number of injection materials currently used for correction of deep wrinkles, depression under the eyes, lip enhancements, and acne scars include both completely, or partly biodegradable and nonbiodegradable materials, of which none is fully satisfactory [3, 4]. Biodegradable materials include hyaluronic acid [5], collagen [6,7], partly degradable materials contain biodegradable collagen [3] or hyaluronic acid [8] and nondegradable poly(methyl methacrylate) microspheres 30–40 μm in diameter [9] (Artecoll, Rofil Medical International, Netherlands), 40–60 μm dextran beads (Reviderm Intra, Rofil Medical International, Netherlands) or poly(2-hydroxyethyl methacrylate-*co*-ethyl methacrylate) hydrogel [10] 45–65 μm in size (DermaLive, USA). After injection of partly

*Author to whom all correspondence should be addressed.

biodegradable materials, the biodegradable component (collagen or hyaluronic acid) completely disappears within several weeks and only the filler particles remain. This occasionally induces erratic location of hydrogel in derma and also formation of visible contours as a result of collagen or hyaluronic acid displacement by cicatricial tissue. Completely nonbiodegradable materials contain poly(dimethylsiloxane) (Biopolimeros-Spain, Intraderm-Russia) or polyacrylamide (Formakryl-Russia, Interfal). Disadvantage of polyacrylamide consists in its hardening (calcification) in the tissue. Liquid poly(dimethylsiloxane) has a variety of advantages for application in aesthetic surgery [11, 12, 13]. It is inert (does not induce inflammation and abruption), non-teratogenic, does not change with increasing temperature, i.e., it can be easily sterilized, maintains initial viscosity after injection allowing successful correction of facial skin wrinkles and modeling lip contours and bulk. However, its main shortage consists in its liquid nature evidenced by the loss of volume effect 2–3 years after injection [14], because it migrates from the injection site and accumulates in the cells of reticuloendothelial system. Poly(dimethylsiloxane) particles floating in liquid poly(dimethylsiloxane) were therefore developed; however, they suffer from the same shortcomings, i.e. displacement [15]. Similar problems, i.e. the loss of achieved bulk and aesthetic effect during several months, accompanies application of all collagen-based preparations [6]. Also hyaluronic acid resolves and disappears requiring repeated injection [16]. In addition, collagen and hyaluronic acid being of animal origin can induce allergic reactions and carry virus infections.

With the aim to improve the correction of wrinkles, facial defects, lip bulk and contours, in particular to prolong the effect of liquid poly(dimethylsiloxane), we have developed two-component non-biodegradable injectable preparation. Medical-grade liquid poly(dimethylsiloxane) was used as a medium (carrier) and poly(2-hydroxyethyl methacrylate) (PHEMA) microspheres as a filler. The advantage of PHEMA is that it has a long history of application in medicine (ophthalmology, endovascular surgery, soft tissue implants) [17]. *In vitro* cytotoxicity testing of the microspheres on the cell cultures proved their non-toxicity [18]. Also Teflon filler was preliminarily tested; however, it proved to be inapplicable because it sediments due to a high density [19]. Moreover, its irregular shape did not allow passage through the injection needle. The objective of this report is to investigate the tissue reaction on the injection of particulate PHEMA/liquid poly(dimethylsiloxane) composition in rats and to compare performance of 30–40 and 125–180 μm PHEMA microspheres.

2. Experimental

2-Hydroxyethyl methacrylate (HEMA; Röhm GmbH, Germany), ethylene dimethacrylate (EDMA; Ugilor S.A., France) were distilled in vacuum before use. 2,2'-Azobisisobutyronitrile (AIBN; Fluka) was twice crystallized from ethanol. Poly(vinylpyrrolidone) K90 (PVP; $M_w \sim 360{,}000$) was from BASF and poly(vinyl alcohol) (PVA) Polyviol W 25/140 ($M_w \sim 80{,}000$; 87% hydrolyzed) from Wacker Chemie GmbH, Burghausen, Germany. Liquid poly(dimethylsiloxane) (Intraderm, viscosity 380–420 cSt, $M_w \sim 20{,}000$; medical grade) from Plastis, Moscow, Russia, and all other chemicals, which were obtained from Aldrich, were used as received.

2.1. Preparation of PHEMA microspheres

PHEMA microspheres were prepared by suspension copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) initiated with 2,2'-azobisisobutyronitrile in the presence of cyclohexanol and dodecan-1-ol diluents. Briefly, 597 ml of 2% solution of PVP and 3 ml of 4% solution of PVA were charged in l-l Büchi reactor, a solution of 48 g HEMA, 32 g EDMA, 0.8 g AIBN, 109 g cyclohexanol and 11 g dodecan-1-ol was added, purged with nitrogen for 10 min and then stirred with an anchor stirrer (400 rpm) at room temperature for another 10 min. The reaction continued at 70°C for 8 h, the product was washed with water ten times, methanol ten times, acetone five times, diethyl ether three times and dried in air. Finally the particles were classified on Alpine MZR 100 Zigzag Luftstrahlsieb (Germany) to obtain a 30–40 μm fraction. The procedure for the preparation of 125–180 μm microspheres was the same as above, with the exception that only 600 ml of the PVP K90 stabilizer (no PVA) and the stirring rate 500 rpm were used. Particles were then classified on the respective sieves using a Fritch Analyzette apparatus (Germany).

2.2. HEMASIL

HEMASIL 30 or 130 consisted of 90 wt% Intraderm and 10 wt% PHEMA microspheres 30–40 or 125–180 μm in size, respectively. γ-Sterilization of the material was carried out on a Sterus – 1 apparatus with sterilization dose 15 kGy.

2.3. Animal experiments

Thirty white female rats of Wistar strain (Klyukovo breeding station, Moscow region, Russia) weighing between 200 and 300 g were kept on a standard diet and then used in the investigation. Before injection of the composition, the animals were anesthetized with ether; the area 3 × 4 cm was shaved on hindlimbs and 0.1 ml of a PHEMA suspension in poly(dimethylsiloxane) was intradermally injected. HEMASIL 130 was injected into the right hindlimb with a 1.2 × 40 mm needle; HEMASIL 30 was introduced into the left limb using a common insulin needle.

2.4. Histological preparation

With the aim to examine the tissue reaction on the implant with time, the animals were killed by ether exposure after various observation periods ranging from 7 days to 1.5 year. Tissue surrounding the injection site was removed and the samples were observed both macro- and microscopically for 7, 14, 21 days, and also 1, 3, 6, 12 and 18 months after implantation. The material was fixed with 10% formaldehyde and embedded in paraffin. The deparaffinized sections were stained with hematoxylin and eosin, according to van Gieson, and by resorcinol-fuchsin. Toluidine Blue was used to detect elastic fibers. The stained sections were monitored using an Opton optical microscope (Carl Zeiss AG, Germany).

3. Results and discussion

3.1. Preparation of composition

Preparation of poly(2-hydroxyethyl methacrylate) (PHEMA) microspheres by suspension polymerization is a well established process developed originally for chromatographic packing materials [20, 21] and later on for artificial emboli [22]. To obtain a macroporous (non-gel-like) product, suspension copolymerization of HEMA and EDMA proceeds typically in the presence of diluents (porogens) exemplified by the cyclohexanol/dodecan-1-ol mixture. With the aim to produce non-agglomerated microspheres of the size tens of micrometers, a relatively high crosslinking degree (40 wt%) had to be used. This made it possible to avoid stabilization by inorganic materials, such as colloidal magnesium hydroxide, which is needed for production of low-crosslinked PHEMA microspheres of the size in hundreds of micrometers [23]. A water-insoluble crosslinking agent and porogens present in the polymerization mixture extracted HEMA from water thus minimizing HEMA solubility. It is just to remind that if HEMA alone is polymerized in aqueous medium, inorganic compounds have to salt it out from water. To obtain the PHEMA particle size applicable to injectable preparation, polymeric suspension stabilizers were used in the synthesis. Microspheres of the size in tens of micrometers required application of two suspension stabilizers dissolved in aqueous phase, namely high-molecular-weight PVP K90 and a small amount of PVA which depresses interfacial tension. In contrast, microspheres larger than 100 μm necessitated the use of a single PVP stabilizer. As a result, regular spherical PHEMA particles were obtained. They were carefully washed to remove all admixtures, dried and size-classified into two fractions: 30–40 and 125–180 μm.

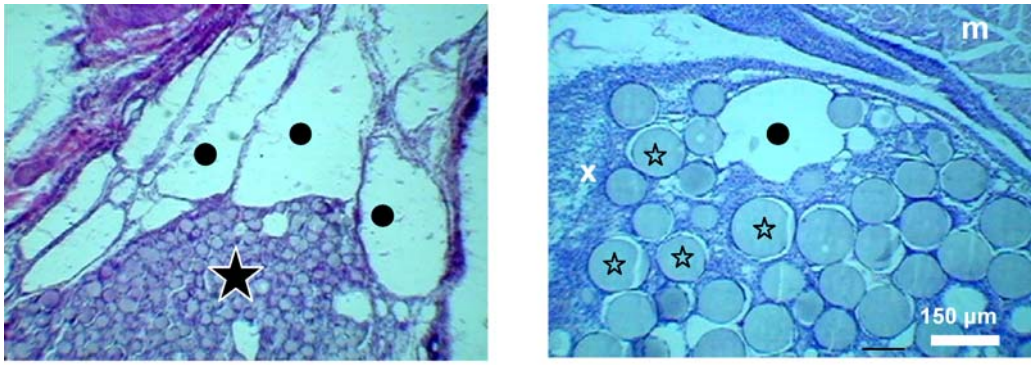
The PHEMA microspheres (filler) were then mixed with medical-grade liquid poly(dimethylsiloxane) (Intraderm; carrier) to form the dispersion. The dispersions containing 30–40 and 125–180 μm microspheres were denoted HEMASIL 30 and HEMASIL 130, respectively. Of great importance in the characterization of such compositions is the sedimentation time during that the PHEMA microspheres sediment to the bottom and the dispersion is separated into initial ingredients. The sedimentation time limits the period, during which the surgeon can introduce (inject) a completely dispersed composition. The sedimentation time was 15 min for HEMASIL 30 and 10 min for HEMASIL 130.

In the beginning, three carrier/filler weight ratios were used: 80/20, 85/15 and 90/10. During the control tests imitating the injection with a syringe, the 80/20 ratio was rejected since such a high concentration of the filler did not ensure a composition passing through a 1.2 $\times$ 40 mm needle. For the 85/15 ratio, only first 30% of the composition passed through the same needle, and then the needle was blocked by the accumulated filler. The composition with the carrier medium/filler ratio 90/10 was then found optimal and preferred in further experiments. Compared with the carrier medium, dynamic viscosity of composite materials increased, i.e. their liquidity decreased due to the presence of the filler. Liquidity of the composition decreased the more, the higher the filler (PHEMA microspheres) content in the carrier liquid (poly(dimethylsiloxane)). As a result, injection through the needle was more difficult. While the composition containing 30–40 μm PHEMA microspheres readily passed through the insulin needle (0.5 $\times$ 16 mm), 125–180 μm microspheres required needles used for intracutaneous injection (0.6 $\times$ 25 mm).

3.2. Morphological analysis of tissue reaction to investigated materials

To specify the tissue reaction to the polymeric material and its biocompatibility, morphological changes in the tissues surrounding the implant were investigated in animal experiments in various times after the injection (7, 14, 21 days, 1, 3, 6, 12 and 18 months). A peculiarity of the experiments consisted in that subcutaneous implantation of PHEMA/liquid poly(dimethylsiloxane) composition was realized for the first time. A second feature is the application of hydrogel microspheres differing in their diameters (HEMASIL 30 vers. HEMASIL 130). The effect of PHEMA particle size was analyzed and the formation of blood vessels also examined. As follows from the following morphological investigation, the tissue changes basically differed depending on the size of hydrogel microspheres.

After injection, both HEMASIL 30 and HEMASIL 130 compositions were subcutaneously dispersed in the region between the muscle fascia and skin (Figs. 1(a) and (b)). In some cases, the composition infiltrated the endomysial tissue of striated muscle. Poly(dimethylsiloxane) and hydrogel could be easily distinguished. While the poly(dimethylsiloxane) liquid was not stained by histological procedures due to its hydrophobicity and remained translucent, the hydrogel captured hematoxylin and eosin. Implanted HEMASIL typically consisted of a mixture of

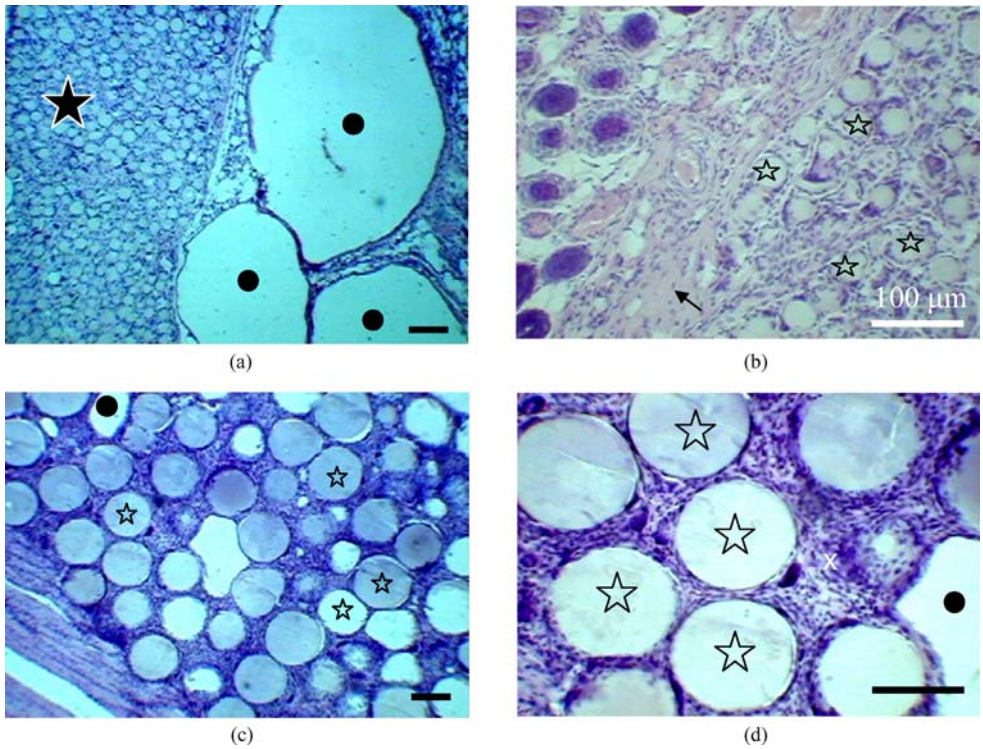


(a) (b)

Figure 1 (a) HEMASIL 30 and (b) HEMASIL 130 one week after intradermal injection. PHEMA microspheres (open asterisk), cluster of PHEMA microspheres (black asterisk), poly(dimethylsiloxane) droplets (circle), nuclei of fibroblasts and inflammatory cells at implantation site (cross), m (muscle tissue). Hematoxylin and eosin. Bar is 150 μm.

transparent poly(dimethylsiloxane) droplets and spherical PHEMA microspheres stained with hematoxylin (Figs. 1(a) and (b)). Hydrogel microspheres were usually closely packed. Poly(dimethylsiloxane) drops in histological sections of HEMASIL 30 had not only various size, but not seldom also different shape, mainly spherical and ellipsoidal (Figs. 1(a) and (b)). Partial mixing of poly(dimethylsiloxane) and 30–40 μm PHEMA microspheres in the tissues was confirmed also 3 weeks after the injection (Fig. 2(a)). Large poly(dimethylsiloxane) drops of various sizes prevailed even after 3 months.

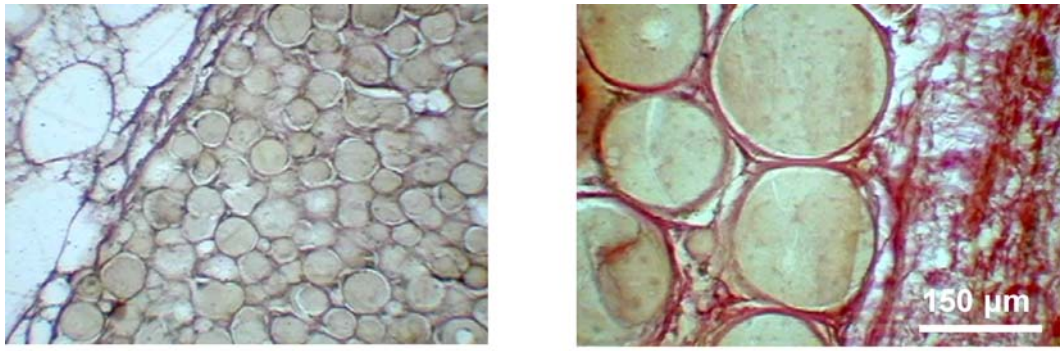
It is interesting to note that in contrast to HEMASIL 30 compositions, which rearranged to separate 30–40 μm microspheres and poly(dimethylsiloxane) droplets, large poly(dimethylsiloxane) drops were mostly absent even during longer observation periods (3 weeks—3 months) after subcutaneous injection of HEMASIL 130 composition (Figs. 2(c) and (d)) documenting thus rather uniform mixing



(a) (b)

(c) (d)

Figure 2 (a, b) HEMASIL 30 and (c, d) HEMASIL 130 three weeks after intradermal injection. PHEMA microspheres (open asterisk), cluster of PHEMA microspheres (black asterisk), poly(dimethylsiloxane) droplets (circle), nuclei of fibroblasts and inflammatory cells at implantation site (cross), fibrous connective tissue containing fibroblasts (arrow). Hematoxylin and eosin. Bar is 100 μm.



(a) (b)

Figure 3 (a) HEMASIL 30 and (b) HEMASIL 130 six months after intradermal injection. Fibrillar tissue with low cellularity prevails at site of implantation. Van Gison staining. Bar is 150 μm.

of both its components. Also the number of isolated poly(dimethylsiloxane) drops in voids between HEMASIL 130 microspheres (Fig. 2(d)) was smaller than with HEMASIL 30 (Fig. 2(b)). It may be anticipated that, compared with HEMASIL 30, the number of 125–180 μm microspheres enclosed in poly(dimethylsiloxane) was substantially higher.

Large microspheres might better retain poly(dimethylsiloxane) 6 months after implantation than the 30–40 μm ones (Fig. 3(b) vers. Fig. 3(a)). This can be ascribed to the contact surface area, which is smaller for 125–180 than 30–40 μm microspheres. A higher surface area of 30–40 μm microspheres then does not allow their complete coverage with poly(dimethylsiloxane).

Remarkable differences were found in the biological response of subcutaneous soft tissue to the microspheres until 3 weeks after their injection. The cellularity at implantation sites of HEMASIL 30 was very low with prevalence of fibroblasts (Fig. 2(b)). The situation after implantation of HEMASIL 130 was significantly different.

HEMASIL 130 induced a more pronounced tissue response (Fig. 2(d)) compared with HEMASIL 30, i.e., the cellularity of soft connective tissue was remarkably higher in the presence of 125–180 μm particles. Cytological analysis showed that a majority of these cells can be classified as fibroblasts. The volume of extracellular matrix produced by these cells was very low. According to the morphology of nuclei of cells located between the injected microspheres, a mild infiltration of soft tissue by lymphocytes and macrophages can be expected. The incidence of foreign body giant cells was somewhat higher at the implantation site of HEMASIL 30 than in the case of HEMASIL 130. This might be ascribed to incomplete encapsulation of 30–40 μm microspheres, i.e. to the absence of poly(dimethylsiloxane) protection against macrophage attack. The advantage of HEMASIL 130 composition consisted in that poly(dimethylsiloxane) surrounding most 125–180 μm microspheres thus alleviated possible initial reactive inflammation. Monitoring the tissue response in different time intervals, cellularity exhibited a decreasing tendency in both studied systems. Microspheres of HEMASIL 30 were surrounded by a very thin and incomplete capsules formed from extracellular matrix 6 months after injection (Fig. 3(a)). A similar phenomenon was observed also in the case of HEMASIL 130, where the connective tissue capsules were complete and somewhat thicker than in HEMASIL 30 (Fig. 3(b)). Poly(dimethylsiloxane) droplets were surrounded by discontinuous capsules of connective tissue containing a very thin bunch of collagen fibrils (Fig. 3(a)). No inflammatory reaction was detected after implantation of both HEMASIL 30 and 130 during observation periods (Figs. 2(b, d) and 3(a, b)). Partial degradation of hydrogel microspheres was observed in both applied systems beginning 12 months after implantation (Figs. 4(a) and (b)). Hydrogel degradation was accompanied by absorption of small amounts of calcium salts on its surface detected only by special microscopic techniques. The residues of hydrogel microspheres and abundant droplets of poly(dimethylsiloxane) were detected in the soft tissue, the cellularity of which again strongly increased in comparison with the observation period 6 months after microsphere injection (Figs. 4(a) and (b)). The giant cell reaction was observed only on destroyed hydrogel particles. Bundles of connective tissue were found in regions with accumulated degraded hydrogel (Fig. 4(a)). A large number of fat cells, which positively affect reparative processes, being mediators between the irritator (foreign body) and vascular bed were observed in the main capsule. It should be mentioned here that no signs of tumor formation were detected after implantation of both HEMASIL 30 and 130. Large hydrogel microspheres can be thus considered more acceptable for application in plastic surgery than the small ones, since the former seem to be better protected by poly(dimethylsiloxane) shell from macrophage attack (Fig. 3(b)). In fact, the implant integrated with the

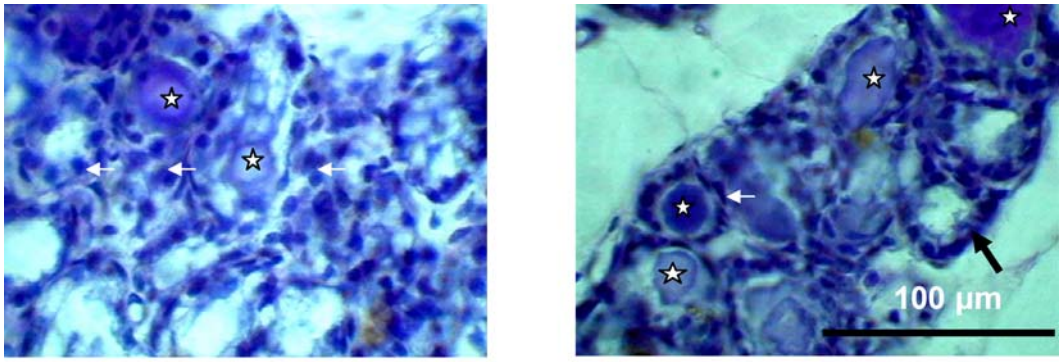


Figure 4 (a) HEMASIL 30 and (b) HEMASIL 130 eighteen months after intradermal injection. PHEMA microspheres at stage of degradation (asterisks). Numerous inflammatory cells, predominantly macrophages infiltrate the site of implantation, some of the cells (arrows) adhere to surface of microspheres. Hematoxylin and eosin. Bar is 100 μm.

surrounding tissue. This is advantageous since it helps in shaping tissue contours by the HEMASIL 130 composition even in prolonged periods after the treatment. Thus, the composition is superior to many injection materials recently used in aesthetic surgery. Even when the observed hydrogel degradation slightly decreased the implant volume, it retained the poly(dimethylsiloxane) bulk preventing its migration almost completely and ensuring the result of volume correction.

4. Conclusions

To correct lip defects and individual or multiple permanent perioral wrinkles by traditional means, repeated injections of conventional materials are often required. With the aim to obviate repeated injections, new HEMASIL composition was developed for deep wrinkles and defects of skin in face. In spite of the fact that HEMASIL 30 (containing PHEMA microspheres 30–40 μm in size) better passed through the needle of the syringe, which is used for its introduction into the rat limbs, than HEMASIL 130 (containing 125–180 μm microspheres), the latter composition demonstrated better mixing of liquid poly(dimethylsiloxane) with the hydrogel. Both compositions were observed subcutaneously in rats. Each 125–180 μm microsphere seemed to be enclosed in a poly(dimethylsiloxane) shell which protected it from macrophage attack in the first periods after injection. In late periods of observation, the protective role was then played by subsequently formed connective tissue capsules of 1–2 matured cells and 1–2 collagen fibrils. Small voids were filled with fine poly(dimethylsiloxane) droplets resembling honeycombs. Contrary to that, majority of 30–40 μm PHEMA microspheres did not seem to be able to retain poly(dimethylsiloxane). In the absence of poly(dimethylsiloxane) around the microspheres, an inflammatory reaction could occur, not receding with time. The microspheres became vulnerable to macrophages, which is disadvantageous in terms of PHEMA degradation. This could negatively affect stability of the accomplished aesthetic effect. Since the results of preliminary medical and biological investigation of HEMASIL 130 injection preparation confirmed its advantages for clinical application in facioplasty of contour deformities ensuring uniform distribution of hydrogel microspheres in liquid poly(dimethylsiloxane) and reducing macrophagic reaction and inflammation, its toxicity was investigated. Toxicological investigation included its intracutaneous implantation in white rats. They confirmed its nontoxicity and compliance with the requirements for medical products in a long-term contact with the internal environment of the organism [19, 24]. A stable and long-lasting bulk effect in facioplasty can be thus expected.

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