Experimental

Migration Studies and Histology of Injectable Microspheres of Different Sizes in Mice

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Injectable dermal filler materials consist of either fluids, biological fragments, or suspensions of particles or microspheres. Particles and microspheres are said to "migrate," but migration can occur only when they are injected into blood vessels. To evaluate biocompatibility and transport, five nonresorbable polymethylmethacrylate microspheres of various sizes, suspended in different carriers, as well as resorbable polylactic acid and dextran microspheres were injected subcutaneously into mice. The five implantation sites were the right cheek, right axilla, right groin, urethra, and the right quadriceps muscle of the thigh. These sites were excised along with the local lymph nodes, lungs, liver, and spleen at 1, 3, 6, and 9 months after injection. Polymethylmethacrylate microspheres of 4 μm and 8 μm were phagocytosed but not transported to lymph nodes or distant organs. Larger microspheres of 20, 40, and 100 μm were encapsulated by connective tissue, macrophages, and giant cells. Polylactic acid microspheres caused a mild inflammatory response and had disappeared at 6 months. Dextran microspheres caused a pronounced foreign-body reaction and were phagocytosed at 9 months. The extremely large carbon-coated spheres of 200 to 500 μm in diameter "migrated" up to 1 cm from the implantation site. With the exception of an erroneous intravenous injection, no migration or transportation of any of the injected microspheres to lymph nodes or filter organs was seen. Obviously, the collagen glue released no microspheres. After subdermal injection, the collagen carrier substance kept the microspheres apart as a scaffold for tissue ingrowth, whereas all other carrier substances, such as gelatin, hyaluronic acid, or alginate, separated soon after injection, thereby causing agglomeration of the microspheres. (*Plast. Reconstr. Surg.* 113: 1380, 2004.)

During the past decades, different dermal filler substances[1–3] consisting of high-viscosity fluids or polymer particle suspensions[4,5] have been injected beneath wrinkles and acne scars. These substances are useful for the correction of dermal, bony, and soft-tissue defects and have potential for applications as bulking agents in patients with gastroesophageal reflux[6] or urinary[7] and fecal incontinence.

Animal studies[8] and clinical trials[9] have shown good biocompatibility of microspheres and short- and long-term efficacy, as would be expected from their chemical structure and surface characteristics. Resorbable materials, such as collagen, hyaluronic acid, polymethylacrylate, dextran,[10] polylactic acid,[11] and alginates,[12] are removed by phagocytosis over a period of 3 to 9 months, depending on the amount and physical properties of the bulking agent implanted. Permanent fillers, such as paraffin, silicone fluid, Teflon, and silicone particles with irregular surfaces, can only be partly phagocytosed and eventually may form foreign-body granulomas.

Microspheres up to 15 μm in size were generally phagocytosed and were sometimes transported to local lymph nodes.[13,14] Larger microspheres from nonresorbable polymers with a smooth surface were soon encapsulated with fibrous tissue and escaped phagocytosis.[4,7] Clinically, all injected substances, including collagen, hyaluronic acid, microspheres, and particles, have been shown to cause foreign-body granulomas in a small percentage of patients.[4,15,16]

The ideal soft-tissue filler substance for skin defects and sphincters is histocompatible and safe, remains stable at the implantation site, keeps its volume and remains pliable, does not cause protrusion of the skin, evokes minimal foreign-body reactions, will not be removed by

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phagocytosis, has no potential for migration to distant locations, and causes no foreign-body granuloma.

The goal of this study was to evaluate and confirm the histocompatibility and eventual migration of the various filler substances. For some of these products, there are no scientific publications on biocompatibility or histology and no clinical studies. All histologic organ sections were searched carefully for macrophages, giant cells, and particles or microspheres to detect any migration to distant locations.

Materials and Methods

Animals

The animal experiments were approved by the Animal and Safety Committee of the Veterans Affairs San Diego Health Care System. Forty-four adult, female, white, wild-type ICR mice, 8 weeks of age, were purchased from Harlan (Indianapolis, Ind.). All subdermal and intramuscular injections with a 26-gauge needle were performed under general anesthesia using halothane. During the experiment, no signs of infection, weight loss, tumors, self-mutilation, or death occurred. All applied methods met American Veterinary Medical Association guidelines.

The filler substances were implanted subdermally into the right cheek, right axilla, and left groin (close to the lymph nodes), around the female urethra, and intramuscularly into the quadriceps muscle of the right thigh. The left side and left lymph nodes served as controls. Each injected dose was 0.1 ml per site, or 0.5 ml per animal. Groups of four animals were euthanized using carbon dioxide at 1, 3, 6, and 9 months. The following filler substances were used:

1. The 4.3-μm polymethylmethacrylate microspheres (batch B 157) were purchased from EMCM B.V., Nijmegen, Holland, and prepared as a 20% suspension in 3.5% bovine collagen solution, which was purchased from Dermasearch GmbH, Wald-Michelbach, Germany (batch 001-801-01/659).
2. The 8.3-μm polymethylmethacrylate microspheres (batch B 100) were prepared as a 20% suspension in 3.5% collagen solution (see above).
3. The 20.0-μm polymethylmethacrylate microspheres (batch L 610) were 20% suspension in 3.5% collagen solution (see above).
4. Artecoll in suspensions 20% polymethylmethacrylate microspheres (40 μm) in 3.5% collagen solution was obtained from Artes Medical Inc., San Diego, Calif. (lot 01B0901).
5. Zeraplast, a 20% suspension of 40-μm polymethylmethacrylate microspheres in 2% hyaluronic acid-1 (Rofilan), was obtained from Rofil Medical International N.V., Breda, Holland (lot 2-3201). Rofilan is hyaluronic acid of bacterial origin with a high molecular weight (2.5 million daltons) that is cross-linked with the help of a plant extract.
6. The 40-μm polymethylmethacrylate microspheres (lot I-203) were prepared as a 20% solution suspended in 3% cross-linked hyaluronic acid-2 (Juvederm 30) from L.E.A.Derm, Paris, France (lot N30.01.14). This hyaluronic acid is excreted from a genetically altered stem of *Streptococcus equi* (as is Restylane).
7. The 40-μm polymethylmethacrylate microspheres (lot I-203) were prepared as a 20% suspension in 10% alginate (Pronova SLM 100) donated by FMC Biopolymer, Oslo, Norway (batch 110064). Alginate is a polysaccharide derived from brown algae (phaecophycea).
8. The 100-μm polymethylmethacrylate microspheres (lot E-149) were purchased from EMCM and suspended in 3.5% collagen solution.
9. New-Fill powder with poly-L-lactic acid (L-PLA) microspheres (20 to 100 μm) was prepared as a 4.5% suspension in 2.7% methylcellulose. It was purchased from Biotech Industry S.A., Luxembourg (lot 99C18).
10. Reviderm intra, a suspension of 2.5% dextran microspheres (40 μm as in Sephadex) in 2.0% hyaluronic acid (2.5 million daltons) of bacterial origin (Rofilan), was donated by Rofil Medical (lot 1-2701).
11. Durasphere, a suspension of pyrolytic carbon-coated zirconium oxide beads of 212 to 500 μm in a 3% betaglucan gel, was obtained from Carbon Medical Technologies, Inc., St. Paul, Minn. It is used as a bulking agent in stress urinary incontinence.

After euthanasia, the implantation sites, adjacent lymph nodes, and distant organs (liver, spleen, lungs, muscle, and urethra) were excised, fixed in formaldehyde, and prepared for histologic examination.

From each implantation site and each organ,

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at least four sections were cut at different levels for histologic examination. These sections were stained with hematoxylin and eosin or with Masson's trichrome stain. A total of 880 sections of five implantation sites and 800 sections of lymph nodes, liver, spleen, and lungs were examined.

The black Durasphere beads at the implantation sites could be visualized during the autopsies. All five implanted sites were easily identifiable macroscopically at 1 and 3 months, but not all implants could be visualized at 6 and 9 months. In the latter cases, the whole area was excised and five to 10 different levels were sectioned to visualize microscopic traces of the implant.

Three study endpoints were investigated: implant site response, phagocytosis, and migration. For implant site response, the character or grade of local implant site response and the infiltration of the host response into the implantation site were evaluated at each time point for each test material. The local foreign-body response was graded according to the Duranti scale,[17] as follows: grade 0, no visible reaction; grade 1, slight reaction with few inflammatory cells; grade 2, clear inflammatory reaction with one or two giant cells in the 400× field; grade 3, fibrous tissue with inflammatory cells, lymphocytes, and giant cells; and grade 4, granuloma with encapsulated implant and a clearcut foreign-body reaction. If microspheres were found, histologic evidence of phagocytosis of microspheres was evaluated in all implantation sites and in off-site organs. Non–implant site organs (i.e., the local lymph nodes, lungs, spleen, and liver) were sectioned and evaluated for signs of migration of microspheres. At least four sections were evaluated for each organ. In case of doubt or positive findings, additional adjacent sections also were evaluated.

Results

4.3-μm Polymethylmethacrylate Microspheres in Collagen

Implant site response. Throughout the 9 months of observation, the implants were easily detectable because of an obvious capsule and its cream-like white/gray content. Microscopically, the 4-μm beads were encapsulated singularly or in groups of 10 to 20 with collagen fibers and were interwoven by a fine network of capillaries, rare macrophages, fibroblasts, and connective tissue. No giant cells were seen and no macrophages or microspheres were detected outside of the fine fibrous capsule of the implant.

At 1 month, tissue ingrowth occurred from the periphery to about 0.2 mm in depth, whereas the major portion of the implant consisted of beads suspended in cell-free injectable collagen. At 3 months, the ingrowth of capillaries and connective tissue was complete (Fig. 1, *above*), and at 9 months, the 4-μm polymethylmethacrylate implant was firm and permanently encapsulated (Fig. 1, *center*).

The Duranti scale was applied to all histological sections, which showed implanted material. At 1 month, the 4-μm implants showed grade 0, at 3 months, a beginning grade 1 (few inflammatory cells), at 6 and 9 months, grade 2 (one or two giant cells in a 400× field). The same evaluation applied to implants of different-sized polymethylmethacrylate microspheres; no true foreign-body reaction was seen.

Phagocytosis. Most macrophages aligned themselves along the cell infiltration zone, central to the fibrous encapsulation, and were filled with four to 22 microspheres.

Migration. Microspheres of 4 μm in diameter were detected in the lung of mouse 14 at 6 months. A bolus of hundreds of beads was found in a major lung artery, which showed no obvious ingrowth of connective tissue at that time. In another more peripheral artery of the same lung lobe, some macrophages had invaded the bolus (Fig. 1, *below*). No other 4-μm microspheres were detected at any time in the distant organs (i.e., liver, spleen, local lymph nodes, and lungs) of other animals.

It had been noted at the time of injection that the axillary vein of mouse 14 was punctured during implantation of the test substance close to the axillary lymph nodes. The subsequent bleeding was stopped using pressure. The puncture track to the urethra of the same animal showed a similar histological picture; the implant of 4-μm beads was only slowly invaded by macrophages at 6 months.

8.3-μm Polymethylmethacrylate Microspheres in Collagen

Implantation site. Macroscopically, the implants of these mice appeared well encapsulated and there was no dispersion into the surrounding tissue. Their content was white/gray and pasty. Histologically, at 1 month, the centers of most implants were not invaded by con-

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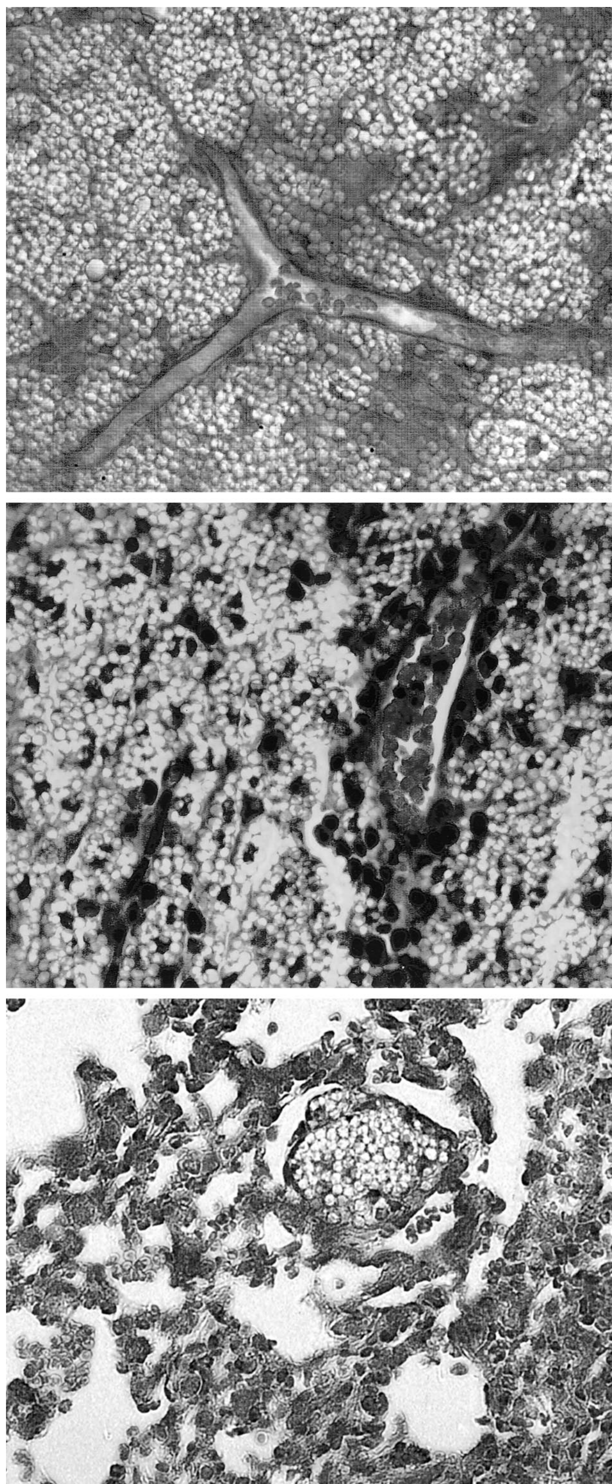

FIG. 1. (*Above*) A network of capillaries interweaves the 4-μm polymethylmethacrylate microspheres at 3 months, but little ingrowth of connective tissue is seen (hematoxylin and eosin stain, ×400). (*Center*) At 9 months, macrophages have invaded the 4-μm implant and have phagocytosed some of the beads (Masson's trichrome stain, ×400). (*Below*) A bolus of 4-μm microspheres in a distant lung artery of one mouse (mouse 14). Some macrophages have invaded the implant (hematoxylin and eosin stain, ×400).

nective tissue. However, the centers of these implants showed full cellular ingrowth at 3 months (Duranti grade 2). A strong capsule separated the implant from the subcutaneous fat at 6 months (Fig. 2).

Phagocytosis. No beads were phagocytosed within the implantation site. Interestingly, the solitary microspheres found in one lymph node were not phagocytosed.

Migration. Microspheres were noted in one axillary lymph node of mouse 21. In this lymph node at 1 month, 16 microspheres of 8 μm in diameter were found freely dispersed in the center in one section. The microspheres were not engulfed by macrophages but were lying free between the lymphocytes. The lungs, liver, and spleen were free of microspheres at all times. Local lymph nodes of all other animals were free of microspheres.

20-μm Polymethylmethacrylate Microspheres in Collagen

Implantation site. At 1 month, there was a seam of fibrous tissue of approximately 3 mm at the periphery (Fig. 3). The center (approximately 2 mm in diameter) consisted of microspheres suspended in cell-free collagen. The implant showed full ingrowth of connective tissue and encapsulation of the beads after 3 months.

Phagocytosis. At all times, macrophages were attached to the 20-μm microspheres, but true phagocytosis could not be verified in the histological slices of 7-μm thickness. No giant cells were observed, and the number of lymphocytes

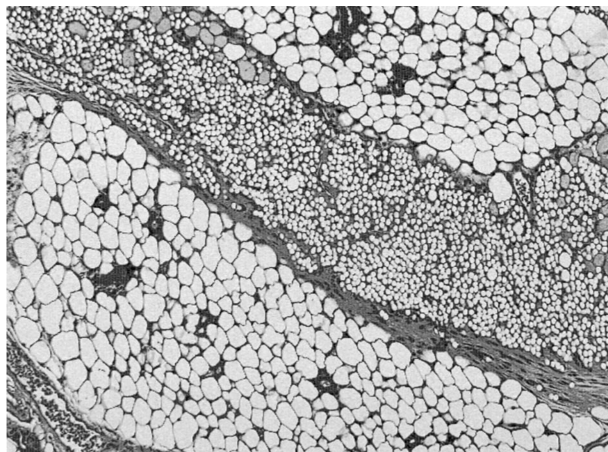

FIG. 2. An 8-μm polymethylmethacrylate implant between two strands of fat at 6 months. The implant is separated from the subcutaneous fat by a rather strong capsule of connective tissue (Masson's trichrome stain, ×100).

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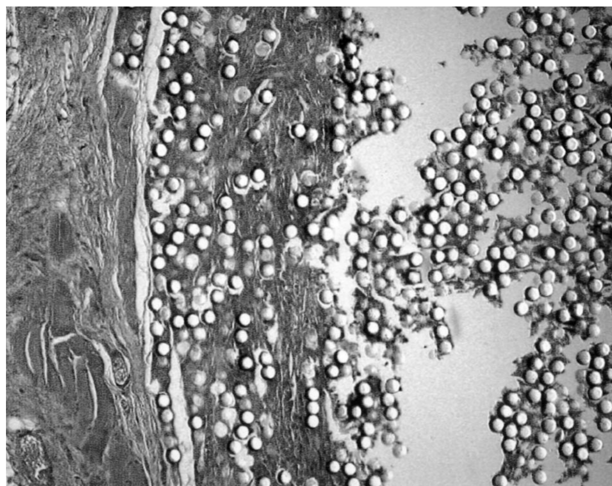

FIG. 3. At 1 month, the 20-μm polymethylmethacrylate microspheres are invaded by a seam of 2-mm connective tissue. The beads in the center are still embedded in cell-free collagen (hematoxylin and eosin stain, ×100).

was negligible (Duranti grade 2). No beads were found outside the implantation site.

Migration. All distant organs were free of obvious microspheres. The only shape corresponding to a 20-μm microsphere was detected in one of the lung sections of mouse 33 at 6 months. It appeared to be surrounded by a macrophage. Given the lack of beads within the lymph nodes and the location in the lung, it is likely that this bead was transported hematogenously and then phagocytosed once in situ.

40-μm Polymethylmethacrylate Microspheres in Collagen

Implantation site. The histological pictures of the 40-μm beads (Artecoll) were similar to those of the 20-μm beads. At 1 month, each individual microsphere was separated from adjacent microspheres by a thin layer of eosinophilic material representing collagen fibers. The foci were discrete and well circumscribed within the soft tissue. The peripheral regions of these foci were infiltrated with macrophages (approximately one per 15 beads) and a few multinucleated giant cells to a depth of 2 to 3 microspheres.

The centers of the lesions were cell free and were separated only by fibrin fibers. At the 3- and 6-month time points, giant cells had extended deeper into the lesions. The implanted denatured collagen solution appeared to be absorbed at 1 month. New collagen deposition was evident at 1 month. This collagen deposition increased the space between individual microspheres. The number of inflammatory cells was small at all times, suggesting that collagen and microspheres induce a minimal immune response. Strong tissue ingrowth and encapsulation of every single bead by fibrous tissue or some macrophages were obvious at 9 months (Fig. 4). Very rarely, multinucleated giant cells surrounded a microsphere (Duranti grade 3).

Phagocytosis. No evidence of microsphere phagocytosis was noted at the implantation site, even though macrophages 20 μm in diameter and giant cells 80 to 100 μm in diameter were attached to many of the 40-μm microspheres. This phenomenon was noted mainly at 3 and 6 months and less so at 9 months.

Migration. No 40-μm microspheres were detected outside the implants or in any of the excised filter organs. This is consistent with our first experiments in rats,[18] which demonstrated noninvolved lungs, liver, spleen, and local lymph nodes after intradermal injection of Arteplast (Artepharma GmbH, Frankfurt, Germany). It is also consistent with many histological examinations of Artecoll in human skin.

40-μm Polymethylmethacrylate Microspheres in Hyaluronic Acid-1

Implantation site. The polymethylmethacrylate beads and their carrier, hyaluronic acid-1 (Rofilan in Zeraplast), apparently separated soon after injection. The microspheres were packed in clusters and basophilic hyaluronic acid-1 was deposited in huge lakes. These lakes

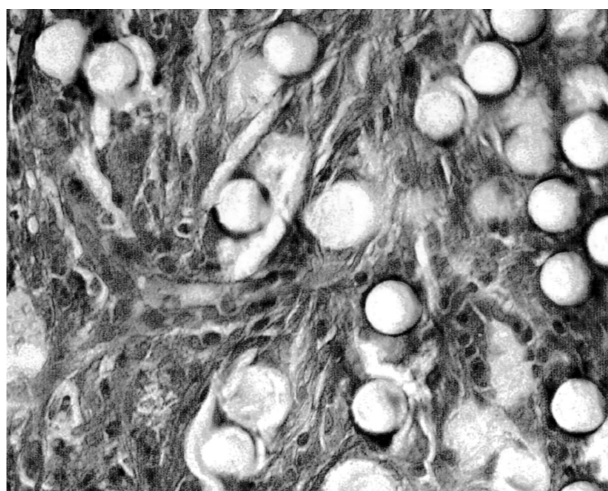

FIG. 4. At 9 months, the 40-μm polymethylmethacrylate implant (Artecoll) is fully interwoven with macrophages, fibroblasts, and, rarely, giant cells (Duranti grade 3). A fine fibrous capsule separates the implant from the fatty tissue and an adjacent lymph node (hematoxylin and eosin stain, ×400).

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were surrounded by a cascade of giant cells with foamy cytoplasm at 3 months (Fig. 5). The giant cells had disappeared at 6 months. At 1 and 3 months, scattered giant cells were seen between the microspheres, but these cells had disappeared in later sections (Duranti grade 3). At 6 and 9 months, the distance between single beads still was less than half the diameter of the microspheres (10 to 20 μm). It can be assumed that less than a quarter of the implant volume consisted of cellular and fibrous tissue. No phagocytosis or migration was seen outside the implantation site.

40-μm Polymethylmethacrylate Microspheres in Hyaluronic Acid-2

Implantation site. This cross-linked hyaluronic acid product from France (Juvederm 30) remained easily detectable in the skin for up to 3 months. Histologically, the hyaluronic acid-II had separated from the beads at 1 month and was found in light-blue lakes, invaded by macrophages and scattered giant cells (Duranti grade 3). No cascades of giant cells surrounded the hyaluronic acid-2 lakes. At 6 months, no residue of blue hyaluronic acid could be detected. The polymethylmethacrylate beads were well encapsuled but packed closer than the Artecoll implants (Fig. 4). No phagocytosis or migration was seen in any of the histological slides.

40-μm Polymethylmethacrylate in Alginate

Implantation site. At 1 and 3 months, the polymethylmethacrylate-alginate implants felt firmer than the polymethylmethacrylate-collagen implants. The polymethylmethacrylate-alginate implants had softened at 6 and 9 months.

At 1 month, the beads were separated from the alginate, which was found in large gray lakes in the vicinity of the packed beads. Macrophages and scattered giant cells had invaded the lakes and phagocytosed the alginate (Fig. 6). At 3 months, the microspheres had been pushed apart by invading fibrous tissue and a few giant cells (Duranti grade 3). At 6 months, the lakes had disappeared and the implant had an appearance similar to that of the Artecoll implant (Fig. 4). No phagocytosis or migration was seen in any of the histological slides.

100-μm Polymethylmethacrylate in Collagen

Implantation site. The implantation of the larger microspheres was as uneventful as that of the 40-μm polymethylmethacrylate. Histological examination of the implant showed similar encapsulation with very few macrophages and sporadic giant cells (Duranti grade 3). Overall, the amount of connective tissue surrounding the 100-μm beads was significantly smaller than the amount of connective tissue surrounding implants of bigger beads (Fig. 7).

Phagocytosis. No evidence of phagocytosis was noted in the implants, even when many macrophages were attached to the beads. Some scattered giant cells were attached to the beads.

Migration. No 100-μm beads were found outside the implants or its capsules or in any of the filter organs.

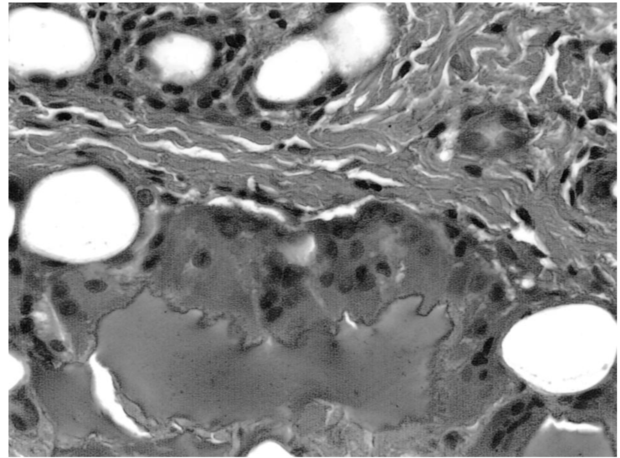

FIG. 5. Zeraplast: At 3 months, the 40-μm polymethylmethacrylate microspheres have separated from the hyaluronic acid carrier, which is phagocytosed by a seam of multinucleated giant cells (Masson's trichrome stain, ×400).

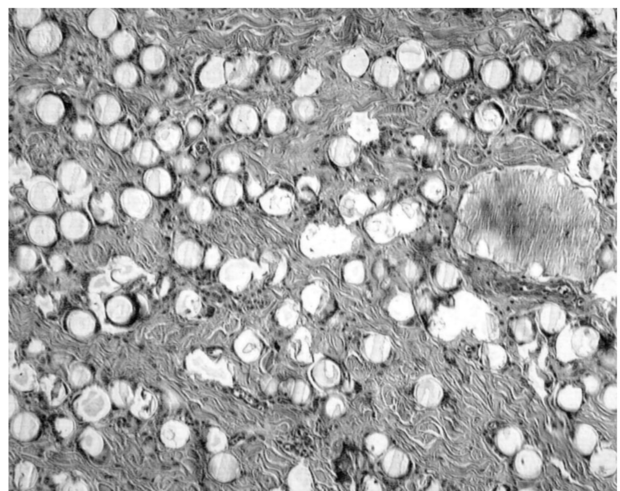

FIG. 6. The 40-μm polymethylmethacrylate beads suspended in alginate at 1 month. The alginate between the beads has been fully absorbed and replaced by strong fibrous tissue. A few macrophages and scattered giant cells can be seen (Masson's trichrome stain, ×100).

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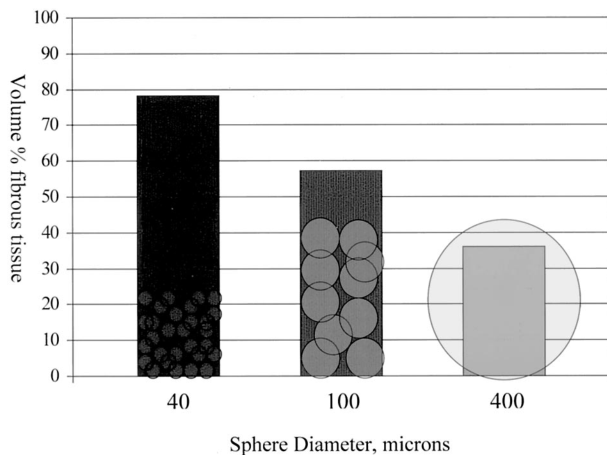

FIG. 7. The effect of microsphere diameter on fibrous tissue volume in all interstices. Calculations assume a 10-μm fibrous capsule surrounding each sphere, which accounts for 20-μm-wide interspaces.

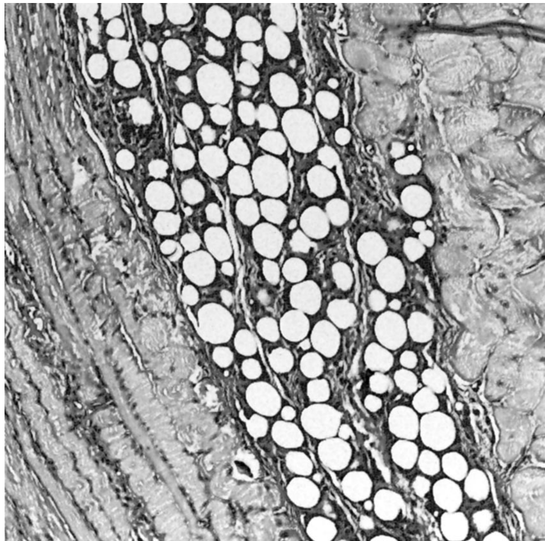

FIG. 8. At 1 month, the shape of the polylactic acid microspheres in New-Fill (1 to 80 μm) is still well defined; the foreign-body reaction in the thigh muscles is mild, with scattered giant cells (Duranti grade 2) (hematoxylin and eosin stain, ×100).

Polylactic Acid (20 to 100 μm) in Methylcellulose

Implantation site. The polylactic acid implants (New-Fill) were easily palpable until 3 months after implantation, but had disappeared from the mouse skin at 6 months. At all time points, a fine capsule could be observed around the implant. At 3 months, the transparent microspheres were still spherical and surrounded by a few macrophages and lymphocytes (Fig. 8). At 6 months, most microspheres revealed a porous surface structure, were fissured and sometimes misshapen, and were surrounded by small giant cells. Their pseudopodia infiltrated the surface of some of the microspheres, and these cells showed signs of active phagocytosis.[19] At 9 months, the degradation of the polylactic acid microspheres was complete. In the mice, no remnant of cicatricial fibrosis was found, despite total disappearance of polylactic acid residue after 6 months. This finding illustrates the excellent biocompatibility of polylactic acid.

Migration. No polylactic acid microspheres were found in lymph nodes or distant organs, despite a wide variety of bead size present in New-Fill. Macrophages that had engulfed many small microspheres and one 20-μm microsphere either were unable to migrate (e.g., enter a lymph or blood vessel) or were immobilized by the tight capsule of the implant.

Dextran (40 μm) in Hyaluronic Acid-1

Implantation site. The injection of dextran beads (Reviderm intra) caused swelling and redness that continued for almost 3 months, possibly a toxic effect of free dextranomers. The palpable deep dermal implant started to disappear at 4 months and was not palpable at 6 months. Histologically, the 40-μm dextran beads produced the greatest amount of granulation tissue among all injectables. At 1 month, the hydrophilic microspheres were swollen and measured up to 75 μm in diameter; some were broken apart and surrounded by foamy cells and giant cells. At 3 months, a few elastic fibers and a great number of giant cells were seen (Fig. 9). The surface of the dextran beads was still intact at that time but showed irregularities at 6 months and total disintegration at 9 months.

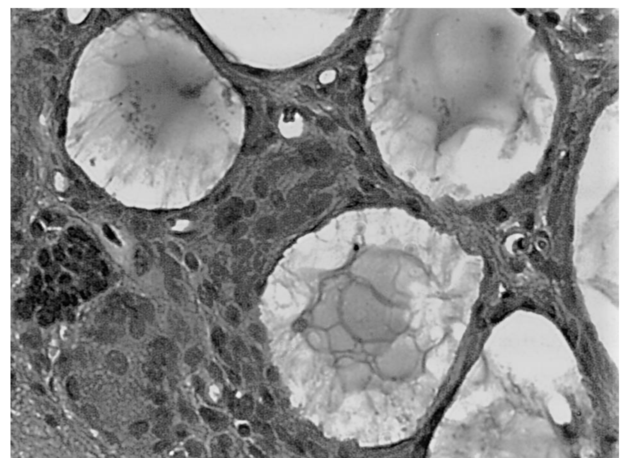

FIG. 9. At 3 months, the dextran beads in Reviderm intra are swollen and showing signs of disintegration within a strong foreign-body reaction, here with four giant cells (Duranti grade 4) (Masson's trichrome stain, ×400).

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TABLE I
Histological Reactions after Injection of Different Microspheres

Microsphere	Average Size (μm)	Carrier	Migration	Histological Reaction	Duranti Grade at 1/3/6/9 Months
PMMA	4	Collagen	Emboli in 1 lung	Mild	0/1/2/2
PMMA	8	Collagen	Single sphere in 1 lymph node	Mild	1/2/2/2
PMMA	20	Collagen	1 sphere in lung	Mild	1/1/2/2
PMMA/Artecoll	40	Collagen	No	Moderate	2/3/3/2
PMMA/Zeraplast	40	HA-1, Rofilan	No	Moderate	3/3/2/2
PMMA	40	HA-2, Juvederm 30	No	Moderate	3/3/2/2
PMMA	40	Alginate	No	Moderate	2/3/3/2
PMMA	100	Collagen	No	Mild	2/3/3/2
PLA/New-Fill	1–80	Methylcellulose	No	Mild	1/1/2/0
Dextran/Reviderm intra	40	HA-1, Rofilan	No	Strong	3/4/3/3
Carbon-coated Durasphere	200–500	Beta-glucan	Dislocation of all beads	Mild	0/1/1/0

PMMA, polymethylmethacrylate; PLA, polylactic acid; HA, hyaluronic acid.

Phagocytosis. Macrophages and giant cells were attached to all microspheres, but no ingested microspheres could be detected. At 3 months and later, most of these phagocytosing cells could be detected easily because of their foamy cytoplasm.

Migration. No dextran beads, bead fragments, or foamy macrophages were found outside the implant.

Durasphere

Implantation site. The big carbon-coated zirconium oxide beads, which resemble black caviar, required an 18-gauge syringe for subcutaneous or periurethral injection.[7] The black shadows disappeared from the subdermal implantation site within 4 weeks and at 3 months were found adjacent to the pectoral or abdominal fascia, which is up to 10 mm beneath the primary injection site.

Histology. Before histological slicing, the specimens were embedded in polymethylmethacrylate and stained with McNeil stain. The beads were encapsulated with one to two layers of fibrous tissue and apparently crossed the whole thickness of subcutaneous fat, where the beads were dispersed in an area of 2 cm^2 and were 2 to 4 mm apart. At 9 months, each single bead was encapsulated within loose fibrous tissue without macrophages or giant cells. No signs of decay of the carbon shell were seen.

Migration. There was obvious dislocation of up to 1 cm of the 0.2- to 0.5-mm big black beads from the implantation site.

Discussion

Biocompatibility

Microspheres are either resorbable or nonresorbable, depending on their chemical composition. The polymethylmethacrylate microspheres appeared to be the most stable microspheres in these experiments. Polymethylmethacrylate was synthesized in 1902 and has been widely used in the human body as bone cement, in artificial dentures, in intraocular lenses, and as a cover of pacemakers. Its excellent biocompatibility and lack of toxicity have been demonstrated in many studies since the 1930s.[18] Once injected, the polymethylmethacrylate microspheres cannot be broken down by enzymes because a methyl group in the alpha-position stabilizes the molecule. Polymethylmethacrylate has a specific weight of 1.18 gcm^{-3} and can only be dissolved by benzene and alcohol. Artecoll gives long-lasting, predictable results, but it is “nonforgiving” when implanted mistakenly in an incorrect plane or into facial muscles or lips.[5]

Similarly, polylactic acids do not occur naturally but were synthesized by French chemists in 1954. Polylactic acid and polyglycolic acid have been used safely in suture materials (Vicryl, Dexon), in resorbable plates and screws, in guided bone regeneration, in orthopedic surgery, neurosurgery, and craniofacial surgery, and as drug delivery devices.[19] Histologically, polylactic acid does not stimulate the natural production of collagen[11] but causes a foreign-body reaction, characterized by macrophages, giant cells, and some elastic fibers. The polylactic acid polymer New-Fill disappeared from the tissue within 3 to 6 months mainly because of extracellular hydrolysis, ester cleavage, and the catalytic effect of hydrosoluble acid monomers formed in the polymer matrix during degradation.[20] Whether the mild inflammatory response elicited by polylactic acid can be ascribed to the degradation activity of macrophages is not clear.

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The degradation rate of polylactic acid polymers in vivo is almost twice that in vitro.[19] Furthermore, degradation only appears when the molecular weight of the acid falls below 20 $\times 10^3$ daltons. Nine months after implantation, no polymer residues or remnant cicatricial fibrosis was found, confirming the good biocompatibility of polylactic acid microspheres.

Dextrans also are not natural compounds but were synthesized by English scientists in the 1940s. Dextran microspheres are the substrate in chromatography columns (Sephadex) used for the separation of proteins. Dextran molecules of 40,000 and 80,000 daltons are used as plasma expanders, since dextran molecules smaller than 20,000 daltons are filtered by the kidney. Dextran beads of 100 μm in diameter were found intact 2 years after implantation in the abdominal skin of rats,[10] despite the fact that the positive surface charges of dextran beads apparently attract macrophages. The macrophages in turn release transforming growth factor beta and interleukins, which stimulate fibroblasts to produce collagen fibers.

After extensive resorption of the dextran beads at 9 months, little or no cicatricial residue was detected at the implantation sites in these studies. Dextran beads of 100 μm in diameter (Deflux, from Q-Med, Uppsala, Sweden) are currently used in clinical trials for the treatment of stress urinary incontinence.

Migration

Migration was seen macroscopically only with the rather large Durasphere beads. They had elicited only a very thin fibrous capsule, consisting of one or two layers, which is necessary for the fixation of these beads at the implantation site. Due to constant activity of the mice, the beads may have passively "migrated" through the whole thickness of the subcutaneous fat, from the subdermal space to the epifascial space of the neck, chest, and abdomen, as well as to the axilla and groin. This kind of dislocation has been described in the anterior pelvis after periurethral injection of Durasphere in humans, but it can be called neither migration nor transportation.[7]

Histologically, no phagocytosed polymethylmethacrylate or polylactic acid microspheres of 4 and 8 μm were detected outside the capsules surrounding the implants in local lymph nodes or in any other organs examined in the mice. It appeared that they were transported as single beads to the lymph nodes via the lymphatics. When macrophages have phagocytosed large numbers ($>$10) of particles, they, as well as giant cells ("frustrated macrophages"[10]), apparently are unable to move and migrate. Furthermore, the average life span of a macrophage is a few hours and that of a giant cell is not more than a day.[21] This implies that the turnover rate of cells at the site of all injected materials is especially high.

In contrast to most previous animal studies,[7,10,12,22] only small amounts (0.1 cc) of the different injectables were implanted into five sites in mice. Since only small quantities will be injected beneath facial wrinkles, this study better represents the actual clinical situation. A depot of 1.0 cc implanted beneath the forehead skin[22] or the abdominal skin[10] of a rat may take much longer to absorb than 0.1 cc injected into a mobile area of the body, such as the murine face, axilla, or groin.

Dermal Blood Vessels

The blood supply to and from the skin is managed through three distinct networks: the superficial papillary plexus, the lower subcutaneous plexus, and the deep epifascial plexus.[23] In addition, a lymphatic system drains the skin's interstitial fluid through three parallel plexuses. Animal experiments and perfusion studies of human skin revealed venules in the dermis with outer diameters of up to 35 μm, collecting venules in the subcutis with diameters of around 50 μm but some up to 120 μm, and veins in the epifascial plane with diameters of up to 300 μm. Similarly, lymph capillaries with diameters of up to 30 μm were developing in the dermis, lymphatic precollectors with diameters of up to 100 μm were present in the subcutis, and lymph collectors with diameters of up to 100 to 600 μm were found in the epifascial plane.

The plane where most of the dermal filler substances are implanted is the deep reticular dermis and the adjacent dermal-subdermal junction. There, the venules and collecting venules have a diameter of 35 to 120 μm, and the lymph collectors have a diameter of 30 to 100 μm. In the skin, the network of vessels is so dense that, theoretically, every iatrogenic puncture will enter some of these vessels.

CONCLUSIONS

There are three mechanisms by which microspheres can be transported:

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1. Hematogenous. If an injection inadvertently enters or damages a vein at the time of implantation, it is possible for beads to be transported from the implantation site. The lungs are the most likely endpoint of this type of migration. Beads of 8 and 20 μm in diameter found within arterioles of the lung were most likely transported there after inadvertent puncture of a large vessel.
2. Lymphatic. As with veins, large lymph vessels in the injection sites, if injured, may result in some transportation. The local lymph nodes are the most likely endpoint of migration, followed by the lungs.
3. Phagocytosis. If beads are phagocytosed and taken from the implantation site, they are likely to travel within the macrophages through the lymphatic system to the local lymph nodes.

Intradermal and subcutaneous implantation of 40-μm polymethylmethacrylate microspheres suspended in 80% collagen has been clinically proven to be safe in more than 200,000 patients worldwide.[5,24] In contrast to other injectable materials with lower viscosity,[17,21] it is impossible to inject microspheres through a resting needle. Within the dense tissue of the deep dermis, the needle has to be moved back and forth to fill the created tunnels with this substance. The gel state of the collagen at body temperature keeps the microspheres in suspension and prevents transportation or migration.

In humans, the carrier substance is collagen with a melting point above 40°C. Therefore, the collagen keeps its gel state during intradermal and subdermal injection and prevents the microspheres from moving or being drawn into open venules. Some of the venules of the subcutaneous plexus may have a diameter slightly larger than the diameter of the microspheres. If such a bigger venule has been opened during the implantation of 40-μm polymethylmethacrylate microspheres, a few microspheres may theoretically enter the circulation. Microspheres are extruded through 27-gauge needles in 200-μm-wide strands; a possible embolus of 200 μm^3 will contain only about six microspheres of 40 μm in diameter. It is highly unlikely that even multiple doses of beads of this magnitude would pose a significant risk to the patient.

Migration of particulated implant materials has been of concern since the migration of Teflon, silicone, and other injectable bulking agents[7] was widely published in the urology literature.[25] Because of the relatively early development of the local fibrotic encapsulation response, it is reasonable to conclude that migration—or more correctly, transportation—is an event that occurred at the time of implantation and before the development of a robust fibrous capsule surrounding the implantation site.

While aqueous carriers like hyaluronic acid or alginate dissipate into the tissue soon after the implantation and leave the microspheres agglomerated, the only true gel, collagen, keeps the microspheres embedded until the collagen is phagocytosed. At the same time, the microspheres act like a scaffold for the ingrowth of the patient's own connective tissue, which comprises up to 80 percent of the 40-μm polymethylmethacrylate implant.[5] This process (i.e., cellular ingrowth in a nonresorbable scaffold to create new tissue for repair or replacement) is called tissue engineering.[26] Thus, the injection of a particulate sphere-like filler substance such as 40-μm polymethylmethacrylate is a novel form of tissue engineering.

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