Endoscopic lower esophageal sphincter bulking for the treatment of GERD: safety evaluation of injectable polymethylmethacrylate microspheres in miniature swine

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Background: Endoscopic therapy for GERD is an appealing, minimally invasive alternative to medical treatment and surgery. Various materials have been tested to augment the lower esophageal sphincter (LES), with limited success. To our knowledge, safety and migration of polymethylmethacrylate (PMMA) microspheres has never been evaluated.

Objective: To assess the safety, migration, inflammatory reaction, and durability of PMMA injected into the LES of miniature swine to create a reflux barrier.

Design: Animal study.

Setting: Approved animal research facilities.

Intervention: Injection of the LES of miniature swine with PMMA. Histopathology of the injected site at certain intervals and postnecropsy microsphere counts of various organs.

Main Outcome Measurements: Minimal inflammatory reaction at the injection site, persistent bulking effect of the material, and no migration of microspheres.

Results: Injection of LES with PMMA caused a mild inflammatory reaction. The bulking effect of the injected material was persistent. Migration of microspheres was eliminated with the use of larger-sized microspheres.

Limitations: Animal model.

Conclusion: Our phase I study documented that 40-μm polymethylmethacrylate microspheres are biocompatible and that PMMA microspheres are resistant to degradation when injected submucosally into the wall of the esophagus. The detection of 40-μm PMMA microspheres in local lymph nodes, liver, and lungs of some animals in the phase I study clearly documented transport of PMMA away from the injection site. This finding was eliminated by increasing the size of microspheres to 125 μm. The potential therapeutic effects of these larger microspheres for humans with GERD remains to be evaluated. (Gastrointest Endosc 2010;72:337-42.)

GERD presents a significant medical problem in Western societies. It is the third most common GI disorder in the United States.[1] Epidemiological studies have shown that approximately 50% of the U.S. population experience heartburn monthly, 15% to 20% weekly, and about 10% daily.[2] Medical treatment with proton pump inhibitors or H2 blockers is highly effective but treats symptoms only and does not affect the lower esophageal sphincter (LES), which plays a major role in the etiology of GERD. Surgical fundoplication restores the natural reflux barrier, but the procedure is associated with risks and complications. Endoscopic therapy of GERD is an appealing, minimally invasive alternative to medical treatment and surgery. Unfortunately, to date, none of the endoscopic methods to

Abbreviations: G40, 40-μm polymethylmethacrylate microspheres; G125, 125-μm polymethylmethacrylate microspheres; LES, lower esophageal sphincter; PMMA, polymethylmethacrylate.

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treat GERD presented since 1988 have gained wide adoption by practicing gastroenterologists.[3]

Polymethylmethacrylate (PMMA) was synthesized in 1902 and has been widely used in the human body as bone cement, in dentures and intraocular lenses, and as covers for pacemakers. Its excellent biocompatibility and lack of toxicity have been documented in many studies since 1930.[4,5] PMMA microspheres are round and uniform with a smooth surface. When placed in soft tissue, they cannot be broken down by enzymes and, if larger than 15 μm, cannot be phagocytized.[6] Bovine collagen serves as a temporary carrier material for the PMMA microspheres and is soon completely replaced by the body's own fibrous tissue, which encapsulates each individual microsphere. Encapsulation prevents migration of spheres and provides persistent bulking at the implant site. Intradermal and subcutaneous implantation of 40-μm PMMA microspheres suspended in 3.5% bovine collagen to correct facial wrinkles has been clinically proven to be safe and effective in more than 400,000 patients worldwide.[4,5] In 2006, ArteFill received U.S. Food and Drug Administration approval as the first and only permanent injectable wrinkle filler for the correction of nasolabial folds, whereas 125-μm PMMA microspheres are not yet approved for human use.

In 2001, Feretis et al[7] reported on the injection of 100-μm PMMA microspheres into the LES of miniature swine. The microspheres were suspended in bovine gelatin, and a total of 5 to 10 mL per animal was injected submucosally into the LES through an open gastrotomy. One month after injection, microspheres were found in the cardia, grouped into clusters that were surrounded by connective tissue strands. In the specimens that were retrieved at 4, 5, and 6 months, the density of collagen fibers had increased, whereas the number of foreign body giant cells remained stable. No PMMA microspheres were found in lymph nodes, and liver histology was normal. Subsequently, bulking of the LES with PMMA in humans was reported by the same group, and no complications were observed.[8] The mean GERD symptom severity score, total acid reflux time, and the mean DeMeester score improved significantly after treatment.

In a recent experimental study from Brazil,[9] the LESs of 8 miniature pigs were injected endoscopically with PMMA microspheres (range 1.9-72.4 μm, mean diameter 40 μm). The augmentation of the antireflux barrier was measured by LES manometry and gastric yield volume and pressure studies, which was maintained at the 6-month follow-up. Microspheres were detected in local lymph nodes, probably because of the relatively small particles.[9] Our study was undertaken to further evaluate migration, local tissue reaction, safety, and durability of PMMA microspheres of two sizes injected around the LES in miniature swine.

Take-home Message

- At present, there is no reliable bulking agent used for treatment of GERD. Safety and effectiveness of polymethylmethacrylate (PMMA) microspheres injected beneath wrinkles have been documented for 20 years. This study confirms biocompatibility and durability of injected PMMA microspheres in the lower esophagus of swine.

MATERIALS AND METHODS

The phase I study was conducted in collaboration with the Sierra Biomedical research facility in Fresno, California (Protocol no. R-136). Eight healthy, male, Yucatan miniature swine (miniswine) weighing between 27 and 34 kg were used. The animals were fasted overnight and then sedated with a cocktail of ketamine, xylazine, and atropine. They were then intubated and anesthetized via isoflurane in 100% oxygen. A single-channel, forward-viewing sigmoidoscope (Olympus CF140; Olympus America, Inc, Melville, NY) was used. Submucosal injections under direct visualization were performed with a specially designed, 42-inch, elongated, flexible injector with a 25-gauge needle and a stopper at 3 mm (Paragon Medsystems, San Diego, Calif).

Six animals were injected with 40-μm polymethylmethacrylate microspheres (G40) consisting of nonabsorbable PMMA microspheres (approximately 6 million spheres per 1 mL) 40 μm in diameter, suspended in 3.5% bovine collagen solution. Two additional animals were injected with the collagen carrier alone. A total of 6 injections (1 mL each) were performed: 2 at the level of the cardia just below the Z-line, 2 at the Z-line, and an additional 2 injections 2 cm above the Z-line. All injections were performed by clinical gastroenterologists experienced in submucosal injections. All procedures were videotaped. Animals were fed a soft diet on the first postoperative day and were started on a regular diet the second day. Serum chemistry, hematology, and urine analyses were performed on postoperative days 1, 7, 29, 55, and 83, and endoscopic examination was done before the swine were killed. Half of the animals were killed on day 8 and the rest on day 84. The swine were killed by use of an approved veterinary euthanasia cocktail after sedation with telazol/xylazine solution. Gross necropsy of all internal organs was conducted by qualified personnel on all animals, and sampling of organs was performed for histology and microsphere detection.

An unaffiliated veterinary pathologist (P.B.L.) performed histological examination of the injection sites and random samples of liver, lung, spleen, thymus, and regional (thoracic and upper abdominal) lymph nodes. The histological analysis focused on inflammatory response to the implanted material (the subjective grading scale was: none = 0, minimal = 1, mild = 2, moderate = 3, marked = 4) and on microsphere migration. Microsphere counts were performed on representative samples of thoracic lymph nodes and on 50 g each of liver, lung, and spleen.

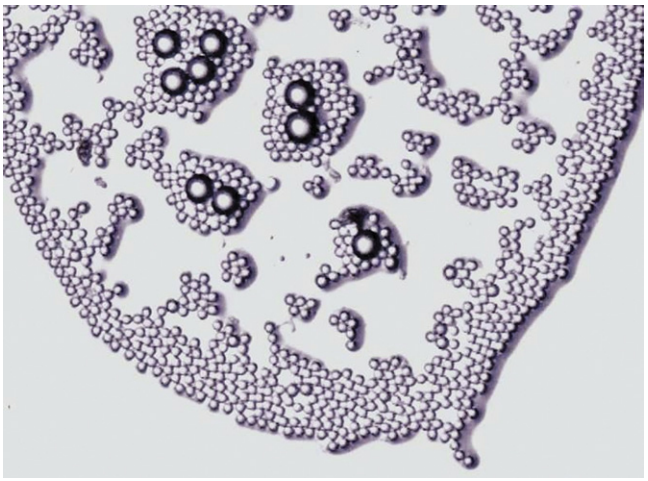

Figure 1. Mix of 40-μm and 125-μm polymethylmethacrylate microspheres (orig. mag. × 20).

The method was based on dissolution of the specimens with 1 M potassium hydroxide (KOH), centrifugation, and microscopic examination of the solid residual for microspheres.

Because some migration was seen with 40-μm spheres and because blood vessels (venules) of the cardia are larger than those above the squamocolumnar junction, a second series of animal testing was done to further assess tissue effect and migration of larger and smaller microspheres.

The phase II study was conducted in collaboration with the Perry Scientific research facility in San Diego, California (Protocol no. 02-0023). Seven healthy, female, Yucatan miniswine (approximately 40 kg each) were used. Each animal was injected under the same conditions as in the phase I protocol. Two different sized PMMA microspheres were used (Fig. 1), suspended in 3.5% bovine collagen: G40 and 125-μm PMMA microspheres (G125).

Six animals were injected submucosally with 1 mL of both G40 and G125 suspended in collagen in 4 separate blebs, 1 to 2 cm proximal to the LES (8 mL total bulking volume). The cardia was not injected. One animal was injected with 2 mL epinephrine (1:10,000) in the 6 injection sites 10 minutes before injection of the bulking agent. Recovery and follow-up of animals, killing on postprocedure day 8, and necropsy were identical to those of the phase I protocol. Representative samples of organ tissues (50 g each) were collected for microsphere counts.

RESULTS

Phase I

At gross necropsy, all injected blebs were visible at day 8 and day 84 (except for control animals), and the overlying mucosa was normal (Fig. 2A-C). Surrounding tissues and all major visceral organs appeared to be normal, and there were no changes consistent with blood vessel embolization or tissue infarction.

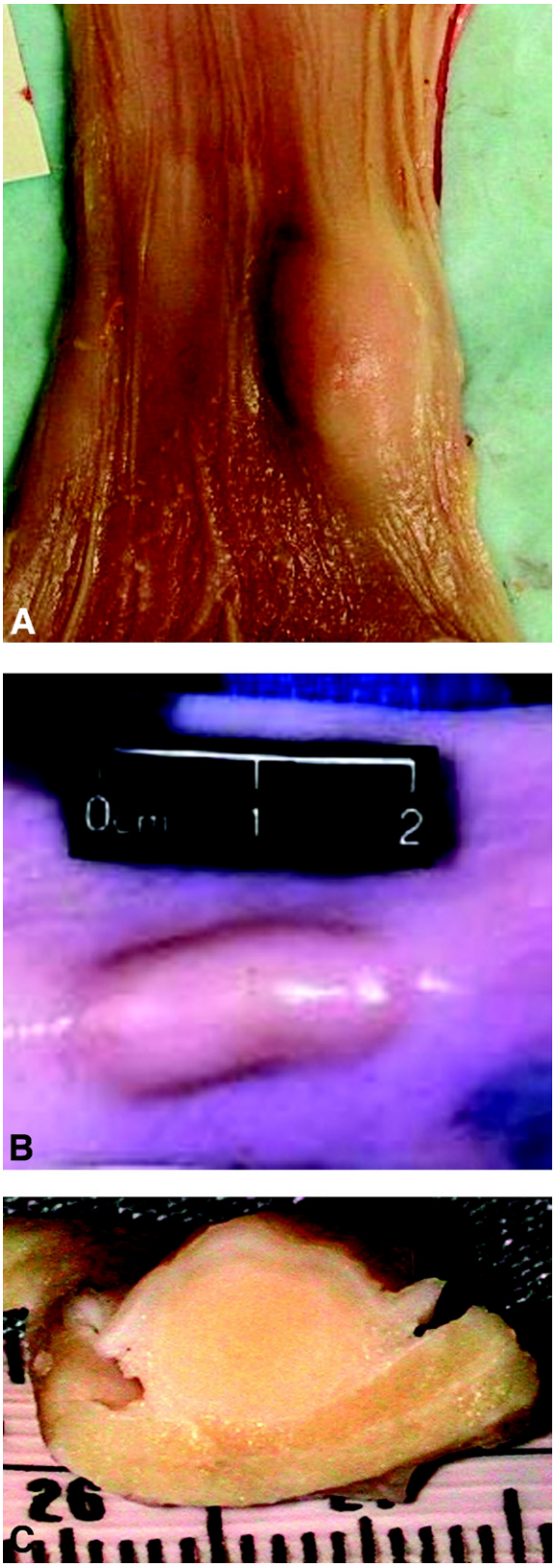

Figure 2. A and B, At 84 days, the bulking implant is still of the same size and at the same location, soft and pliable. No ulceration or inflammation is detectable. **C,** A macroscopic cross section shows the completely integrated submucosal polymethylmethacrylate implant.

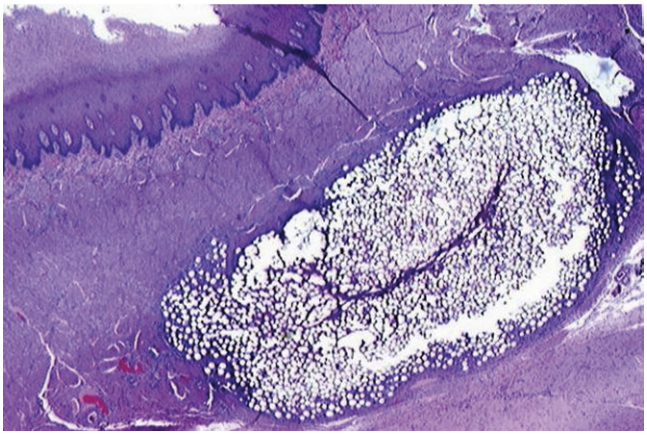

Figure 3. At 8 days, the submucosal implant with a thin layer of homogenous eosinophilic material (H&E, orig. mag. × 20).

At day 8, histomorphology of the injected area of the esophagus showed mild foreign body inflammation (grade 2 in all 3 animals treated with G40) and the presence of clusters of uniformly sized, empty, circular spaces where the PMMA beads were dissolved by alcohol during tissue processing. In the center of larger implant lesions was a thin layer of homogenous eosinophilic material representing the collagen component of G40 or fibrin deposits (Fig. 3). Macrophages and multinucleated giant cells surrounded and infiltrated the lesions to a depth of approximately 100 μm (2-3 microspheres). The presence of microbead aggregates and associated inflammation caused mild distortion of the myofibers making up the inner muscularis layer. In the control animal, multiple foci of hemorrhage mixed with fibrin were found in the area of collagen deposits.

At day 84, the lesions appeared to be similar to those noted at day 8, except the aggregates were surrounded by a fine, fibrous capsule (Fig. 4). The foreign body inflammation and collagen deposition around and within the microbead aggregates in the submucosa were mild, and adjacent myofiber degeneration was minimal. The number of inflammatory cells relative to the size and number of microbeads was small, suggesting that microbeads induced a minimal inflammatory response. Increased trichrome-stain-positive fibrosis was seen between microbeads in all lesions.

The histology of local lymph nodes, spleen, liver, and lung showed scattered, mild, eosinophilic and macrophage infiltrates in some animals but no PMMA microspheres. Organ dissolution, however, clearly demonstrated that there were microspheres washed away from the injection site and filtered in the liver, lung, and local lymph nodes (Table 1). Histological evaluation of thymus and spleen specimens was normal, and no microspheres were detected in these dissolved organs.

Phase II

Gross necropsy at day 8 revealed distal esophageal implant blebs without mucosal edema, erythema, or ulcerations. In one animal (no. 11), the bulk of one injection was found outside the esophageal muscle but still beneath the adventitia (Fig. 5). Surrounding tissues and all major visceral organs appeared to be within normal limits in all animals. There were no changes of the organs suggestive of tissue infarction. The results of the microsphere counts in dissolved tissue samples of the phase II study are summarized in Table 2.

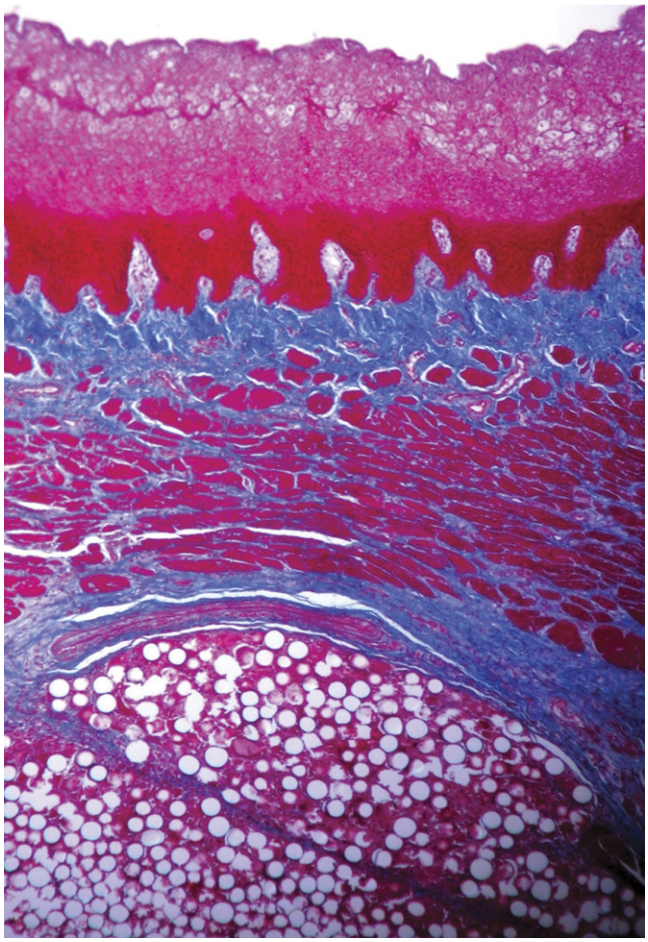

Figure 4. After 84 days, the submucosal implant encapsulated with a fine, fibrous capsule (Masson trichrome, orig. mag. × 40).

We also have documented that injected PMMA could be removed easily from the submucosal space by using EMR, should overinjection occur. Removed G40 implants were intact and elastic and could be squeezed and bent by finger manipulation.

DISCUSSION

Endoscopic augmentation of the LES is an appealing alternative treatment to medical and surgical options for GERD, especially in view of the possible prevention of esophageal cancer.

Our phase I study documented that G40 is biocompatible and that PMMA microspheres are resistant to degradation

TABLE 1. Polymethylmethacrylate microsphere count after G-40 injection and organ dissolution*

Animal	Termination day	Lung	Liver	Spleen	Lymph nodes
Esophagus G-40					
78425	8	5.3	0	0	5.7
78426	8	5.7	57.3	0	0.3
78430	8	2.3	>100	0	>100
78423	84	8.0	4.0	0	0
78424	84	>200	0	0	0
78428	84	31.7	0	0	0
Esophagus collagen control					
78427	8	0	0	0	0
78422	84	0	0	0	0

G40, 40-μm polymethylmethacrylate microsphere.
*Each value represents the average number of spheres found in 3 microscopic fields (×10).

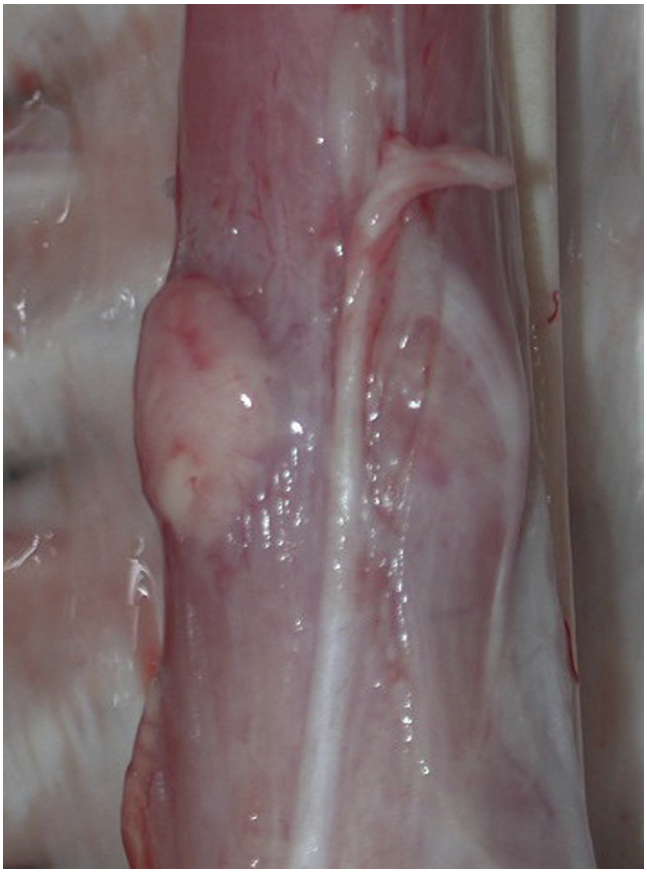

Figure 5. Incorrect extramural implantation in animal 11.

when injected submucosally into the wall of the esophagus. During the 3-month period, injected microspheres remained as discrete, well-circumscribed foci in the submucosa, and blebs bulged into the lumen of the esophagus without any change in the overlying mucosa.

TABLE 2. Polymethylmethacrylate microsphere counts in dissolved tissue samples after esophageal injection with both G-125 and G-40

Animal no.	Lung 125 μm	Liver 125 μm	Lymph nodes 125 μm
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
8	0	0	0
25	1	0	0
23 Epinephrine pretreatment	0	0	0

The detection of 40-μm PMMA microspheres in local lymph nodes, liver, and lungs of some animals in the phase I study clearly documented transport of PMMA away from the injection site. This finding was eliminated by increasing the size of microspheres to 125 μm. Transport of microspheres most likely happens via disrupted veins or lymphatic vessels during injection. Although slow transport of PMMA into lymph nodes or the thoracic duct is of minor clinical concern, a larger quantity of microspheres acutely entering the portal or systemic circulation could theoretically cause infarction of distal tissues/organs. The veins of the distal third of the esophagus are arranged in 3 plexuses and drain into the chest veins and portal circulation. The veins of the mucosal plexus and the plexus in the tunica muscularis propria have diameters between 35 and 40 μm. There are only a few veins of the submucosal plexus, and they do not exceed 90

μm in diameter.[10,11] This could explain the different results in microsphere transport between 40-μm and 125-μm microspheres in phases I and II and also corroborates results of the Feretis study,[8] in which no transport of 100-μm PMMA microspheres was documented.

The finding of a single 125-μm PMMA microsphere in lung tissue in the phase II study is of uncertain etiology and significance. The review of the video documentation on the injection showed minor spillage of the material from the injection site in the esophagus. We speculate that some of the G125 material may have been aspirated after the procedure because of the physiologic reflux in miniswine. Alternative microembolization remains a rare possibility, which has been shown by Lemperle et al (unpublished data, 2009) after injection of particles into the urethra. Studies are needed to determine the long-term migration potential.

However, even if some PMMA microspheres were to enter the portal circulation, they would be trapped in the hepatic "filter" of the portal vein. This filter is represented by the sinusoids between interlobular veins and central veins and has the same diameter as the hepatic cells (20-40 μm).[11] Unlike arteries in many organs, the interlobular veins have no terminal ramifications. Therefore, closure or embolization of a small number of sinusoids would not cause an infarction of the liver tissue, and microspheres would be permanently encapsulated. The lungs and spleen have filter properties similar to those of the liver. Theoretically, some beads could end up in the brain, skin, or other organs through an open atrial septal foramen, but by that time they would be dispersed in the bloodstream, and occlusion of single arterioles most likely would not cause any obvious clinical symptoms.

Our study shows that the diameters of the single branches of the submucosal venous plexus and the extramuscular esophageal veins at the level of the lower esophagus play a crucial role in the determination of a safe size of PMMA microspheres.

CONCLUSION

PMMA microspheres suspended in bovine collagen meet nearly all criteria of an ideal injectable implant material.[12] The material has relatively low viscosity, it does not have to be refrigerated, and it can be injected through a specially designed, 25-gauge needle. It is biologically inert at the implantation site, causing only minimal foreign body reaction. It is nonallergenic and nonimmunogenic. PMMA microspheres are nonbiodegradable and mostly remain at the implantation site, forming soft, pliable blebs as reflux barriers, if properly injected. The blebs are capable of resisting mechanical strain and have a good degree of elasticity and plasticity. The submucosal blebs can be removed by EMR if necessary. The larger size of the 125-μm microspheres reduces the risk of transportation from the injection site, because almost all veins of the esophageal venous plexuses are less than 90 μm in diameter. The potential therapeutic effects of these larger microspheres for humans with GERD remains to be evaluated.

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