Urethral Bulking With Polymethylmethacrylate Microspheres for Stress Urinary Incontinence: Tissue Persistence and Safety Studies in Miniswine

Gottfried Lemperle, Patrick B. Lappin, Corbett Stone, and Stefan M. Lemperle

OBJECTIVES
To evaluate the safety and persistence of injectable polymethylmethacrylate (PMMA) microspheres as a long-lasting urethral bulking agent in pigs. PMMA microspheres of 2 different diameters (40 and 125 μm) were tested to investigate the potential for migration and dislocation after injection. A similar product containing 40-μm PMMA microspheres has been used as an injectable wrinkle filler for >25 years and received Food and Drug Administration approval in 2006 (ArteFill).

METHODS
A total of 22 female pigs received 4 submucosal implantations of PMMA microspheres, using either a cystoscope or a newly developed urethral injection device (UroScope). At death and necropsy at 8 days and 1, 3, and 6 months, the urethral injection site, liver, lung, spleen, and pelvic and iliac lymph nodes were processed for histologic examination and microsphere count using organ dissolution and microscopy.

RESULTS
All injected submucosal blebs were still present at 6 months and showed no signs of inflammation. Tissue dissolution of the local lymph nodes and major organs demonstrated the transport of some of the 40-μm microspheres to the local lymph nodes and lung but not to the liver or spleen. In contrast, no 125-μm microspheres were detected in any distant organ.

CONCLUSIONS
The submucosal implantation of 125-μm PMMA microspheres into the urethra provided a safe and persistent bulking effect in pigs. The positive results of the present study encourage additional investigation of 125-μm PMMA microspheres as a long-lasting bulking agent for the treatment of female stress urinary incontinence. Furthermore, a newly developed urethral injection device (UroScope) proved beneficial and cost-effective to facilitate the transurethral injections of 125-μm PMMA microspheres.


Stress urinary incontinence is a common medical condition affecting approximately 1 in 3 postpartum women. More than 100 different treatments have been proposed since the late 1930s. Some of the minimally invasive techniques attempted for stress urinary incontinence have included retropubic and transobturator sling placement. At present, the most widely used treatment option is the midurethral tension-free tape, a polypropylene sling, although it has still been associated with a 5%-19% failure rate.1,2

According to Rovner and Goudelocke,3 “the ideal periurethral injectable agent has not yet been identified although many of the currently used agents have acceptable efficacy in selected populations.” Various injectable bulking agents have been developed and used to treat sphincter insufficiency since 1938,4-7 but none has been proved optimal for a number of reasons, including early absorption, disintegration, migration, and protrusion.1,8 Polytetrafluoroethylene (Polytef, Coloplast, Minneapolis, MN) has been abandoned because of the “migration” of its particles.9 Collagen with glutaraldehyde-crosslinked bonds (Contigen, C.R. Bard Inc., Covington, GA)5 begins to lose effectiveness within 6-9 months after implantation. Particulate siloxane (Macroplastique, Uroplasty Inc., Minnetonka, MN) remains relatively stable at the injection site (two thirds of patients were dry at 24 months10) but has caused excessive foreign body reactions in some patients.11

The injection of heavy carbon-coated zirconium beads of 220-500 μm in diameter (DuraspHERE, Carbon

This study was sponsored by Artes Medical, San Diego, CA; at the time of the experiments, Drs. Gottfried and Lemperle were employees of Artes Medical; Drs. Lemperle and Lappin and Mr. Stone have no financial interest in any of the tested or mentioned products.

From the Surgical Animal Laboratory, University of California, San Diego, California; and Sierra Biomedical, Division of Charles River Laboratories, San Diego, California

Reprint requests: Gottfried Lemperle, M.D., Ph.D., Division of Plastic Surgery, University of California, San Diego, School of Medicine, 302 Prospect Street, La Jolla, CA 92037. E-mail: glempere@uid.com

Submitted: November 3, 2010, accepted (with revisions): December 13, 2010

© 2011 Elsevier Inc.
All Rights Reserved

Medical Technologies Inc., St. Paul, MN) has been discontinued because of repeated dislocation. Coaptite, a suspension of absorbable calcium phosphate microspheres, 75-125 μm, suspended in carboxymethylcellulose is safe and biocompatible but has been observed to be associated with urethral prolapse owing to the lack of tissue ingrowth. Dextranomer beads and hyaluronic acid (Zuidex, Q-Med, Uppsala, Sweden), used for tissue bulking, have been associated with granuloma and sterile abscess formation. The optimal bulking agent for stress urinary incontinence must demonstrate long-term tissue biocompatibility and safety, tissue persistence, and ease of use.

Injectable dermal fillers have been increasingly used for the correction of facial wrinkles and soft tissue defects. The safety record of injectable dermal fillers has been established since the early 1980s through the millions of injections administered. ArteFill (Suneva Medical Inc, San Diego, CA) is a suspension of 40-μm in diameter, round and smooth polymethylmethacrylate (PMMA) microspheres suspended in bovine collagen solution. ArteFill was approved by the Food and Drug Administration in 2006 as the first and only permanent solution. ArteFill was composed of 30-50-μm diameter nonabsorbable PMMA microspheres (approximately 6 million/mL) suspended in 3.5% bovine collagen solution. The collagen carrier consisted of a telocollagen and therefore was nonallergenic. Of the 4 pigs from each of groups 1-4, 3 received 4 anatomically separated 0.5-mL injections of U40, and the fourth received similar injections of bovine collagen alone (control pigs).

Test Agent U40
U40 was composed of 30-50-μm diameter nonabsorbable PMMA microspheres (approximately 6 million/mL) suspended in the same 3.5% bovine collagen solution. Each of the 6 pigs in group 5 acted as their own internal control and received 2 anatomically separated 0.5-mL injections of U40, and the fourth received similar injections of bovine collagen alone (control pigs).

Test Agent U125
U125 was composed of 125-μm diameter PMMA microspheres (approximately 100 000/mL) suspended in the same 3.5% bovine collagen carrier. Each of the 6 pigs in group 5 acted as their own internal control and received 2 anatomically separated 0.5-mL injections of U40 and U125 beads.

An end of the study of group 5 on day 30 appeared justified because “migration” of PMMA-microspheres has never been detected after day 1, when the lymph vessels and venules have been disrupted during injection and would be open for possible washing away of smaller microspheres. Furthermore, the carrier collagen, which is fluid under pressure at the moment of injection (tixotropy), returns to the gel state immediately, and keeps the microspheres in its gel for ≥3 weeks. This gel will prevent transportation through open vessels at a later state. On day 3, the implant as a whole was covered by fibrin and locked in place.

Implantation Procedure
The sedated pigs were intubated and subsequently anesthetized using isoflurane in 100% oxygen. The ideal anatomic site to inject urinary bulking agents is between the subepithelial vascular layer and the muscular layer (Fig. 1). An anatomic study of the human female urethra showed the absence of a well-defined sphincteric structure in the bladder neck region, although most thick rhabdomyosphincter fibers could be detected in the middle and caudal thirds of the urethra. Therefore, the injections were done in the midurethra of the pigs.

In groups 1-4, a single-channel cystoscope (Olympus CF140, Olympus America Inc., Center Valley, PA) was used for the intraurethral injections. In group 5, a newly developed urethra

### MATERIAL AND METHODS

#### Pigs
A total of 22 female Yucatan miniature swine, 6-9 months old and weighing 30-40 kg, were obtained from S&S Farms (Ranchita, CA). All the pigs were maintained in accordance with the U.S. Department of Agriculture Animal Welfare Act and the Guide for Care and Use of Laboratory Animals of 1996 for ≤6 months.

### Table 1. Study design

<table>
<thead>
<tr>
<th>Group</th>
<th>Pigs (n)</th>
<th>Control Group</th>
<th>U40</th>
<th>U125</th>
<th>Death (Days After Implantation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>NA</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>NA</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>NA</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>NA</td>
<td>182</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0</td>
<td>6*</td>
<td>6*</td>
<td>30</td>
</tr>
</tbody>
</table>

U40, 40-μm microspheres; U125, 125-μm microspheres; NA, not applicable.

* All pigs in group 5 received both U40 and U125 microspheres.
injection device (UroScope, Paragon Medsystems, San Diego, CA; patent no. 6432045) for introduction into the female urethra under direct vision, using 10-cm-long 23-gauge needles, and a product-filled injection catheter (U.S. patent no. 6926699) for injection inside the bladder were tested.

The U40 and 3.5% bovine collagen solution were injected submucosally in the midurethra through a 10-cm-long, 25-gauge needle; the U125 was injected through a 23-gauge needle, in 4 different blebs of approximately 0.5 mL each, assisted by the UroScope or a catheter. All injections resulted in moderate immediate bulking of the urethral mucosa. The degree of bulking per site, the individual volumes injected per site, and the urethral position of the blebs were recorded on videotape using a cystoscope.

Euthanasia
At 8 days (group 1), 1 month (group 2), 3 months (group 3), and 6 months (group 4) after U40 or bovine collagen periurethral implantation, all 4 pigs in each group were killed using telazol/xylazine followed by an approved euthanasia “cocktail.” The preterminal examination of the anesthetized pigs from groups 1 through 4 consisted of a second endoscopy and written and video recordings of the gross appearance of the injection sites. The 6 pigs from group 5 were killed without the preterminal evaluation at 1 month after implantation using similar procedures.

Necropsy and Histologic Examination
Complete gross necropsy was conducted of all pigs by qualified SBi personnel, and 3 portions from the periphery of both lungs, the liver, and the spleen were fixed in neutral-buffered 10% formalin. For direct histomorphologic examination, the urethral injection sites and portions of the liver, lung, spleen, regional (eg, inguinal, pelvic, and iliac) lymph nodes were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. An unaffiliated board-certified veterinary pathologist (P.B.L.) evaluated all the slides.

Tissue Dissolution
At necropsy, 50 g of lung and liver were placed in separate conical digestion flasks. All local lymph nodes were harvested to a total weight of 7-15 g. Each flask containing tissue was filled with 150 mL of 1 M potassium hydroxide (KOH) solution and placed in an oven at 40°C to digest (KOH has no effect on PMMA microsphere integrity). After 1 week of digestion and then centrifugation, the solid material accumulated in the tip of the conical tube was harvested and evenly distributed on a tissue culture plate.

Microsphere Recovery for Control Samples
A sample of 50 g of calf liver was placed in 150 mL of 1 M KOH solution and digested for 3 days at 40°C. The sample was spiked with 500 of the U125 beads and 500 of the U40 beads, and 28 of the U125 were recovered from a 20-mL aliquot of the original 1:1 with water-diluted digest. According to the number of beads spiked and additional dilution factors, this equated to approximately an 85% recovery factor for the U125 beads.

Microsphere Counts
The tissue digests of the test pigs and spiked controls were viewed in 3 low power (10× objective) fields, and the average microsphere numbers/field are listed in Table 2. They reflect the total number of microspheres in a 50-g organ sample.

RESULTS
Implant Site Observations
At gross necropsy, no abnormalities related to test substance administration were observed in any tissues other than the urethra. The urethral submucosal blebs corresponding to the PMMA microsphere injection sites were visible by day 8 through day 182. The tissues surrounding the implant sites were without signs of irritation, ulceration, erythema, edema, or inflammation.

Histomorphologic Findings
At day 8, microscopic lesions in the urethra of all 4 pigs treated with U40 showed mild foreign body inflammation and the presence of clusters of uniformly sized empty circular spaces consistent with the PMMA microspheres (Fig. 1). The foreign body reaction consisted of macrophages and multinucleated giant cells that surrounded and infiltrated each lesion to a depth of approximately 100 μm. All microsphere voids were located within the implantation site, and no microspheres were found free in the tissue.

At day 30, ingrowth of the granulation tissue between the U40 microspheres was evident. Macrophages surrounded most U40 and U125 microspheres, with multiple fibroblasts evident in the spaces between some microsphere clusters (Fig. 2). The implantation site in some...
pigs was surrounded by a thin fibrous capsule and few vessels with a diameter <20 μm.

At day 92, the periurethral implant-associated lesions appeared to be similar to those noted at day 29, with a well-defined fibrous capsule surrounding the granulation tissue. Fibrous connective tissue was identified in the center of the microsphere implants. The number of reactive cells was reduced compared with the day 29 appearance, suggesting that the U40 microsphere immunogenic response had decreased over time.

At 182 days, fully integrated microsphere aggregates with few surrounding blood vessels were demonstrated (Fig. 3). Single beads were found only along the presumed needle track in the submucosa of pig 13. The mucosal epithelium was intact even in the areas extending over large microsphere lesions. In the lung and lymph nodes of pigs 14 and 15, some PMMA U40 clusters were evident; the presence of PMMA microspheres in noninjection sites did not appear to result in occluded lung vessels, infarctions, or granulomas.

The test substances U40 and U125 induced, as expected, a low-grade foreign body reaction to the presence of PMMA microspheres, which did not damage the surrounding tissue. During this evaluation (6 months), the microsphere aggregates remained as discrete, well-circumscribed “living” foci in the soft tissue. These findings were similar to the reported human histologic findings 10 years after the injection of PMMA microspheres, in which scattered macrophages, fibroblasts, and mature collagen fibers appeared to encapsulate the microspheres.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Terminal Day</th>
<th>U40 Found (n)</th>
<th>U125 in Any Organ (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lymph Nodes</td>
<td>Lung</td>
</tr>
<tr>
<td>Group 1 (U40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1.3</td>
<td>91.7</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 (control)</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2 (U40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>1.7</td>
<td>52.3</td>
</tr>
<tr>
<td>8 (control)</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 3 (U40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>92</td>
<td>0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>10</td>
<td>92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 (control)</td>
<td>92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 4 (U40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>182</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>182</td>
<td>3.3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>15</td>
<td>182</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>16 (control)</td>
<td>182</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 5 (U40 and U125)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>28</td>
<td>121</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Figure 2. At 1 month after implantation, U125 PMMA microspheres shown surrounded by macrophages and fibroblasts. Bleb contained both U40 and U125 microspheres for comparison. Hematoxylin-eosin stain, original magnification ×40.
Transportation of PMMA Microspheres Away From Implantation Site

Organ dissolution and microscopic examination revealed U40 PMMA microspheres in the lung tissue and local lymph nodes, consistent with transportation from the injection site by the blood and/or lymphatic vessels (Table 2). In contrast, no U125 microspheres were detected in the dissolved tissues of the group 5 pigs. The apparent absence of U125 microspheres in the distant organs strongly suggested a low risk of peri-implantation, migration, or translocation of the U125 PMMA microspheres.

Because approximately 6 million 40-μm spheres are contained in 1 mL of U40 and approximately 100 000 of 125-μm spheres in 1 mL of U125, the relatively small numbers of detected 40-μm microspheres in the tissues distant to the injection site were most likely associated with transport through ruptured veins and lymph vessels during the first minute after injection.18 From the results in group 5, it can be stated that the risk of migration of 125-μm PMMA spheres carried in a 3.5% collagen solution, when injected submucosally into the urethra, is very low.

Statistical Analysis

To compare U40 and U125 in all groups, the Fisher exact test was applied because of the unpaired number of pigs with microspheres in distant organs. For transport to both lungs and lymph nodes, 11 of 18 animals with U40 injections versus 0 of 6 with U125 injections resulted in 2-tailed P value of .016 (Table 3).

This P value predicted the superior safety of 125-μm spheres compared with the 40-μm microspheres. The U125 spheres had a 3-times greater diameter and 27-times larger volume than the U40 spheres. The microspheres of either size appeared to be safe and associated with a persistent urethral bulking effect in miniswine.

COMMENT

The previously reported detection of an 80-μm Teflon particle in the lung of a dog after urethral sphincter injection5 and careful study of the human urinary sphincter anatomy prompted us to consider the larger size of 125-μm PMMA microspheres for urethral bulking. The female urethra is wrapped by a tiny equivalent to the corpus spongiosum of the male urethra, which is the periurethral venous plexus.21,22 The diameter of its veins can reach ≤80 μm compared with the subcutaneous plexus of the skin of the collecting venules with a diameter of only 30-50 μm.23

The U40 microspheres identified in the lung and lymph nodes (Table 2) might have entered the open venules and lymph vessels either by direct injection or because the collagen carrier was in a fluid state and could be washed away by the negative pressure in the venules. Experiments in mice18 have shown that macrophages one half the size of the 40-μm microspheres can phagocytose them but cannot move or migrate with the indigested particles thereafter.18

Theoretical Transport of Microspheres to Other Organs

Microspheres or particles injected into the submucosal tissue can be transported to lymph nodes or downstream organs only if the veins or lymph vessels have been ruptured during injection. This phenomenon would likely occur within seconds to minutes after the injection. Augsburger et al23 performed impressive studies of the vascularity of the urethral venous plexus in female dogs, which have diameters of 35-60 μm. Considering the histologic sections of human urethras, their veins appear to be of a similar diameter.21

The diameter of the single branches of the submucosal venous plexus and the extramuscular urethral veins in the level of the sphincter plays a crucial role in the determination of a safe diameter of the PMMA microspheres. Both drain their contents into the caval vein and consequently into the lungs. According to our histologic slides from the normal pigs, the diameter of the branches of the submucosal venous plexus varied between 20 and 80 μm (Fig. 2). It appears almost impossible that 125-μm beads would be washed away through the open vessels of the urethral venous plexus.

CONCLUSIONS

The PMMA microspheres were extremely biocompatible and have a >2-decade-long history of safety and efficacy

Table 3. Microsphere migration

<table>
<thead>
<tr>
<th>Organ</th>
<th>U40 Found</th>
<th>U125 Found</th>
<th>2-Tailed P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>10/18</td>
<td>0/6</td>
<td>.017</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>5/18</td>
<td>0/6</td>
<td>.280</td>
</tr>
<tr>
<td>Both organs</td>
<td>11/18</td>
<td>0/6</td>
<td>.016</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Figure 3. At 6 months after implantation, collagen of U40 implant replaced totally by mature granulation tissue and small vessels (ie, it has become a “living implant”). Hematoxylin-eosin stain, original magnification ×40.
as a permanent injectable soft tissue filler material in human skin.\textsuperscript{17} In 2006, the Food and Drug Administration approved PMMA microsphere-based ArteFill injectable wrinkle filler as the first and only permanent injectable substance for the treatment of smile lines.\textsuperscript{17} The present preclinical safety and efficacy study in the urethra of miniswine revealed that PMMA microspheres of 125 $\mu$m, rather than 40 $\mu$m, should be used for injections into the urethra’s submucosal tissue to avoid potential transportation of the particles away from the implantation site. An additional advantage of the U125 microspheres is their long-lasting fixation in the injected location.

The example of Durasphere illustrates the importance of the chemical surface structure of the injected microspheres.\textsuperscript{11} The carbon coating apparently prevents fibroblasts from encapsulating the Duraspheres with collagen and makes them prone to the dislocation caused by gravity.\textsuperscript{11} The PMMA microspheres have been shown to remain stationary and unchanged for many decades in the human body,\textsuperscript{17} constantly stimulating granulation tissue for their own encapsulation and thus creating a “living, vascularized implant.”

The U125 has met all 8 main characteristics of an “ideal” injectable soft tissue bulking agent.

1. Biologically inert at implantation site (noninflammatory, noncarcinogenic, nonimmunogenic)
2. Low viscosity (easy to inject through a 23-gauge needle)
3. Particles large enough to prevent transportation through venules and lymph vessels
4. High persistence at implantation site (encapsulation prevents dislocation by gravity)
5. Capable of resisting mechanical strain (favorable elasticity prevents erosion over time)
6. No adverse events on adjacent musculature
7. Easily removable\textsuperscript{19}
8. Cost effective

Compared with the currently available urethral bulking agents, U125 would have the following advantages, if successfully tested in clinical trials:

Compared with collagen injections (ie, Contigen), U125 is a permanent implant and will not be absorbed within 6-12 months\textsuperscript{5}

Compared with silicone particles (ie, Macroplastique), U125 causes little foreign body reaction and no granulomas\textsuperscript{10}

Compared with the heavier carbon-coated zirconium oxide beads (ie, Durasphere), U125 will be fixated at the location at which it was injected and will not migrate owing to gravity.\textsuperscript{13}

Compared with calcium phosphate microspheres (ie, Coaptite), U125 stimulates fibrous encapsulation and will not be absorbed over time\textsuperscript{14}

Compared with dextran microspheres in hyaluronic acid (ie, Deflux, Zuidex), U125 will not cause sterile abscess, granulomas, or prolapse\textsuperscript{15,16}

Compared with experimental hydrogel microspheres of 1-750 $\mu$m from polyacrylonitrile, it will not cause granulomas,\textsuperscript{24} which are predictable for this wide range of particles\textsuperscript{5}

Our experiments have demonstrated that the injection of both U40 and U125 caused persistent mucosal blebs in the urethra of pigs. The increased sphere size of 125 $\mu$m improved the safety profile of these PMMA microsphere-based bulking agents, as revealed by our organ dissolution studies. No migration of the 125-$\mu$m PMMA microspheres could be detected compared with the 40-$\mu$m spheres. The positive results of our study should encourage additional investigation of U125 as a safe, effective, and long-lasing injectable urinary bulking agent for the treatment of female stress urinary incontinence. In a clinical study, 2 blebs must be injected submucosally in opposing sites in the midurethra to allow symmetric compression.

Acknowledgment. To David Lipsitz, M.D., Director of Northeast Urology Research (Concord, NC) for clinical expertise and performing the injections; William Wustenberg, D.V.M. (Farmington, MN), Mark Young, D.V.M., Sierra Biomedical (San Diego, CA), Andrew Perry, M.D., Ph.D., and Perry Scientific (San Diego, CA) for technical support; and Paul Clapton, M.S., University of California, San Diego (San Diego, CA) for statistical analysis.

References