Autologous Fat Grafting for Treatment of Breast Implant Capsular Contracture: A Study in Pigs

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Abstract

Background: Capsular contracture (CC) is a common complication after breast augmentation. Autologous fat grafting may be effective for restoring tissue vascularization and function.

Objective: The authors evaluated the efficacy of autologous fat grafting in a porcine model as a treatment for CC after breast augmentation.

Methods: This prospective study was performed in 20 female 30-day-old pigs. Each animal was implanted with three 30-cc textured silicone implants (stage 1 of the experiment). Group A served as the untreated control group. To induce CC, 2 mL of autologous fibrin glue was applied to the pericapsular space in group B and C animals at implantation. Three months after implantation (stage 2), the CCs of all groups were assessed by Baker classification and applanation tonometry (AT). Liposuction was performed in group B to harvest fat for these animals. Three months after group B underwent fat grafting, all 3 groups were reevaluated. Reassessments included Baker classification, AT, histologic analysis, and tensiometry (stage 3).

Results: The deposition of mature and immature collagen was similar for the 3 groups. The amount of fat remaining around the implanted capsules did not differ significantly between the groups. At stage 3, group B exhibited significantly larger tonometry areas than did group C. The CCs in groups B and C were significantly thicker than those of group A, but the difference between groups B and C was not significant. Capsule rupture forces did not differ significantly between groups A and B but were significantly higher in group C compared with the other groups.

Conclusions: Results in this animal model indicate that pericapsular lipoinjection may be a promising treatment for CC in humans.

Keywords
autologous fat grafting, breast augmentation, capsular contracture, silicone breast implants, applanation tonometry

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Capsular contracture may develop slowly or rapidly and usually occurs within the first year of implantation. However, some patients experience CC after the first year. Despite numerous theories and anecdotal suggestions, the etiology of CC remains unclear and is likely multifactorial. Proposed contributors include filler material, implant placement technique, surface texture, presence of foreign bodies (such as glove talc), and subclinical infections near the area of implantation. Capsular contracture is usually evaluated by Baker classification. A less subjective method is applanation tonometry (AT), initially described by Moore (in 1979) and further developed by Gylbert.

Current treatments for CC are either surgical or pharmacologic. Surgical options include capsulotomy or capsulectomy and replacement of the implant. Pharmacologic treatment usually involves intracapsular injection of steroids and antibiotics. Another option to prevent or treat CC is zafirlukast, a leukotriene receptor antagonist. Moreover, a growing body of clinical evidence strongly supports the therapeutic potential of mesenchymal stem cells to revascularize ischemic tissue and restore function. Studies of fat grafting also indicate its effectiveness as a long-term filler and a treatment for CC, among other conditions.

Grafted fat exhibits many qualities of an ideal filler: it is autologous, completely biocompatible, available in sufficient quantities in most patients, naturally integrated into host tissue, removable if necessary, and a potentially permanent treatment. Thus, fat grafting has become very popular in aesthetic and reconstructive surgery, as a primary procedure and an adjunct to other procedures. Its success is highly dependent on the technique of administration.

Fat grafting the breast is currently utilized in the following situations: to improve contour irregularities in breast reconstruction, to correct defects after lumpectomy or partial injuries, to cosmestically enhance or enlarge breasts, to camouflage implants after augmentation, and to reconstruct breasts after mastectomy (via fat infiltration only). To our knowledge, there have been no studies of fat grafting as a treatment for CC. We used a porcine model to study autologous fat grafting as a treatment for CC after breast augmentation with textured silicone gel implants.

**METHODS**

This 6-month, 3-stage, prospective experiment was conducted in 30-day-old Duroc Gilt pigs (N = 20). The study design and methods were approved by the Committee for the Ethical Use of Animals at Pontifical Catholic University of Paraná, Brazil. Each pig received 3 implants: A, B, and C. In stage 1, each pig was anesthetized and then implanted with three 30-cc Silimed textured silicone implants (Rio de Janeiro, Brazil) (Figure 1); the implants were placed in the ventral subglandular space (Figure 2). Group A served as the study control. To induce CC in groups B and C, autologous fibrin glue (2 mL) was applied to the implant surface and into the pericapsular space during implantation (Figures 3 and 4). The glue was prepared with 4 mL of pig plasma, 500 IU of 10% CaCl (Sigma-Tau Pharmaceuticals, Gaithersburg, Maryland), and 1000 IU of thrombin (Monarch Pharmaceuticals, Bristol, Tennessee). Three months after implantation (stage 2), the degree of CC was assessed in each animal according to Baker classification and AT (Figure 5). After these assessments, group B animals underwent autologous fat harvesting via liposuction, and the autologous fat was refined and injected (Figures 6 and 7).

**Tonometry**

Tonometry was performed according to the method described by Moore and further developed by Gylbert, who applied a known force to the breast, then measured
the flattened area and calculated the intramammary pressure. After trichotomy, the animal’s skin was colored with green gouache paint at each implant site. A glass disk (8-cm diameter, 10-mm thickness) was applied over the painted skin. To calculate the area flattened by the disk, a sheet of paper (same size as the glass disk) was placed between the glass and the painted skin surface. After compression, the image marked on the paper, which was either round or elliptical, was digitized with a scanner and measured with ImageJ software (Oracle, Redwood City, CA, USA).
Larger areas indicated a lower degree of CC surrounding the implant.

Fat Harvesting (Group B)

Fat was harvested from the groin of each animal in group B. Only autologous fat was injected. The technique applied in our study has been described in detail elsewhere. Briefly, a blunt-tipped, 2-hole Coleman harvesting cannula (Mentor, Minneapolis, Minnesota) was attached to a 10-mL Luer-Lok syringe (Becton Dickinson, Franklin Lakes, New Jersey). Three-millimeter incisions were made, and the cannula was advanced through the groin of each animal while the surgeon applied digital manipulation to retract the syringe’s plunger, thus creating a gentle negative pressure. This slight negative pressure, combined with the cannula’s curetting action through tissues, allowed fat parcels to move through the cannula and Luer-Lok aperture into the syringe barrel. When full, the syringe was disconnected from the cannula and replaced with a plug that sealed the lock. The plunger was removed from the syringe before its contents were centrifuged for fat refinement.

Fat Refinement and Transfer (Group B)

The fat was centrifuged to separate its components into distinct layers. To ensure sterile conditions, we opted for a smaller centrifuge with a central rotor and sleeves, which can be sterilized. The harvested fat was processed at 3000 rpm for 3 minutes. The upper level (the least dense) consisted primarily of oil. The middle portion was primarily fatty tissue, and the bottom layer comprised blood, water, and any aqueous elements. After the oil layer was decanted, the plug of the syringe was removed to release the liquid layer (the layer of greatest density). Any remaining oil was wicked away with absorbent material. The refined fat was then transferred into a 3-mL Luer-Lok syringe for injection.

Lipoinjection Technique (Group B)

The 2-mm cannulae used for lipoinjection are much smaller than those used for harvesting, and each distal end contains a single hole. Similar to the harvesting cannulae, the proximal ends of these cannulae had a hub that fit into the Luer-Lok syringe. The blunt cannulae allowed for immediate pericapsular placement of the autograft parcels within the subdermal plane and were more stable and less traumatic than other injection techniques. Through retro-injections, 5 mL of autologous fat was inserted. (After withdrawal of the cannula, the fatty-tissue parcels “fell” into the natural tissue planes as the host tissue collapsed around them.)

Reassessments

Three months after lipoinjection of group B animals (stage 3), the following assessments were performed in every animal (all groups): Baker classification, AT, histologic analysis of the capsule (hematoxylin-eosin [H&E] staining and Picrosirius Red staining [Polysciences, Warrington, Pennsylvania]), immunohistochemical analysis with adiponectin (rabbit polyclonal antibody adiponectin; ABBiotec, San Diego, California), and tensiometry (Figures 8-10). The same surgeon (G.B.R.) performed all explantations, and no capsules or implants ruptured during this procedure.

Histologic Analysis of Capsules

After AT, the animals were euthanized. A sample of surrounding skin and pericapsular tissue from each implantation area was embedded in paraffin. Sections of the samples were stained with H&E and Picrosirius Red. The polarized Picrosirius demarcated areas of the capsules rich in collagen from areas that lacked collagen. The total thickness of the pericapsular tissue and the thickness of the collagen and noncollagen layers were determined from an average of 10 measurements obtained from slides of each specimen.

Rupture forces of all capsules were assessed via tensiometry with the EMIC DL 500 elastic testing machine (Emic, São Paulo, Brazil). The binomial test was utilized to compare the groups regarding the likelihood of normal contracture. Repeated-measures analysis of variance was chosen to compare the stages (1, 2, and 3) and groups A, B, and C with regard to quantitative variables. The least significant difference test was used to compare stages and groups two by two. P values less than .05 indicated statistical significance.
Results
The deposition of collagen, both mature (type I) and immature (type III), was similar for the 3 groups. When the amount of fat remaining around the implant capsule was analyzed via immunohistochemistry, no significant difference was found between the 3 groups. At stage 3, the tonometry area of group B was significantly larger than that of group C ($P < .001$), likely because group B capsules softened after lipoinjection. The capsules of groups B and C (in which CC was induced with fibrin glue) were significantly thicker than those of group A ($P < .001$). However, there was no significant difference in capsule thickness between groups B and C ($P = .342$). With respect to rupture forces, there was no significant difference between groups A and B ($P = .671$). However, group C had significantly higher rupture force than group A ($P = .0017$) or group B ($P = .006$). A difference in capsule softness, favoring the fat-grafted animals (group B), was observed via AT.

Discussion
Capsular contracture is the most common complication after aesthetic and reconstructive breast surgery; the incidence ranges from 0.6% to 50%.$^9$ It is attributed to the gradual and progressive retraction of fibrous scar tissue around the prosthesis. The periprosthetic fibrous capsule is similar to the dense fibrotic collagenous capsules of other fibrotic conditions; it is composed of dense connective tissue containing tightly packed collagen fibers, reticular fibers, and an inner surface of fibrocytes and histiocytes in a single layer that forms an epithelium-like structure. The most common feature of these capsules is dense fibro-collagenous or fibrovascular connective tissue containing foreign-body giant cells or granulomas, which is consistent with an inflammatory or local immune response.

Most studies of CC have focused on the effects of a particular therapy on normal capsule formation. Several animal models, primarily involving rats and rabbits, have been developed to investigate CC.$^5,10$ Adams et al$^{11}$ placed saline implants in rabbits to examine the relationship between capsule thickness and the degree of CC. Ravin et al$^{10}$ placed silicone implants in rats and induced CC with radiation. Only a few studies of CC have been conducted in pigs. Minami et al$^{12}$ utilized AT and histologic examination to analyze the composition and behavior of capsules around smooth and textured breast implants in 33 pigs, but CC was not induced in their study.

We studied CC in Duroc Gilt pigs to determine whether our findings would parallel those of clinical studies of CC. According to Clugston et al,$^{13}$ the epidermal structure of pigs resembles that of humans in certain respects: the

Figure 8. Left: The surface of an implant deformed by capsular contracture (after explantation). Right: Appearance of the device before implantation. Implants are 30-cc textured silicone devices (Silimed, Rio de Janeiro, Brazil).

Figure 9. Image shows the thickness of the capsule (0.77 mm) and its adherence to the surface of the implant. Implants are 30-cc textured silicone devices (Silimed, Rio de Janeiro, Brazil).

Figure 10. Tensiometry was performed on all capsules to measure rupture force.
dermis is thick and rich in elastic fibers. Pigs proved to be an appropriate model in our study of CC. We induced CC with autologous fibrin glue, which increased capsular thickness around the textured implant. Fibrin glue appears to be more physiologically appropriate than methods used in previous studies. Although Shah et al. induced pathologic capsules with bacteria, the reproducibility and control of their model have not been validated. Our model is the first to induce CC in pigs via fibrin glue. This is important clinically because fibrin glue facilitates conditions already known to produce capsule formation in humans, such as hematoxylin and infection.

The therapeutic potential of mesenchymal stem cells for revascularization of ischemic tissue and restoration of function appears to be based on the release of angiogenic and antiapoptotic growth factors, in turn facilitating the recruitment of endothelial progenitor cells into newly sprouting vessels. Recent studies have shown that the stromal-vascular cell portion of adipose tissue contains a rich reservoir of regenerative precursor cells with proangiogenic capabilities comparable to bone marrow–derived stem cells. Given the capacity of stem cells for angiogenesis, Coleman et al. used liposuction and adipose fat injections in an effort to regenerate and repair damaged subcutaneous tissue. Cardiologists who have researched preadipocytes for treatment of myocardial infarction have shown a positive correlation between stem cells and revascularization.

Several studies have demonstrated the value of fat grafting in patients who undergo breast reconstruction procedures that include expanders and prostheses. Fat grafting improves aesthetic results and tissue quality, adds volume, and may prevent CC. However, to our knowledge, there have been no studies of fat grafting as the sole treatment for CC in aesthetic breast augmentation. We analyzed the degree of CC via assessments described previously, including Baker classification, AT, histologic analyses of the capsule (H&E and Picrosirius Red staining), immunohistochemical analysis, and tonometry. However, it can be difficult to determine Baker classification in small animals such as rats and rabbits. Moreover, because the criteria for Baker classification are examiner dependent, this method is less than ideal—even for pigs. Despite this, we found no significant differences in Baker classification between group B (fat-grafted) and group C (not fat-grafted).

Several researchers have evaluated CC with AT, a more objective method. To reduce the number of factors that could influence results, all implants used in our study were textured. Tonometry showed a reduction in mean applanation area after CC was induced with fibrin glue in groups B and C. At the end of the 6-month study, significant enlargement of the mean applanation area was observed in fat-grafted animals (group B); this did not occur in untreated animals (group C). Therefore, fat grafting may reduce the hardness of the capsule and surrounding tissue.

Results of histologic staining showed no significant difference in collagen deposition among the study groups. As in other studies, immature collagen deposition increased initially; by the end of our study, more mature collagen was observed. It is noteworthy that, despite the use of autologous fibrin glue to induce CC, collagen deposition did not differ between the study groups. The only significant difference was in capsule thickness. The capsules that received fibrin glue (groups B and C) were significantly thicker than control capsules (group A). There was no difference in capsule thickness between group B (fat-graft treated) and group C. Therefore, it is plausible that fat grafting does not alter collagen deposition or capsule thickness. However, the capsules did soften as the grafted fat created new vascularization in adjacent tissue.

As observed in other studies, all capsules in our experiment had the same basic 3-layer histologic structure. The internal area abutting the silicone surface appeared to be either a single layer or multilayered, containing macrophages and fibroblasts. In some cases, a pseudoepithelial cellular layer was observed at the implant-capsule interface (synovia-like metaplasia). The middle layer consisted of loosely arranged connective tissue, including internal vasculature. The outer layer consisted of dense connective tissue with an external vascular supply.

In addition to histologic analysis, we examined the quantity of fat that remained in surrounding tissue to ascertain whether the graft persisted around the capsule throughout the study. Immunohistochemical analysis with adiponectin was employed. Although a literature search yielded no other studies of adiponectin for this purpose, our pathologist (L.N.) has had considerable experience with adiponectin and had already tested it in pigs, with excellent results. As expected, more adipocytes were present in the surrounding tissue of fat-grafted animals (group B), but statistical analysis revealed no significant difference in fat deposition among the 3 groups. It is likely that many of the extremely sensitive adipocytes did not survive the histologic study.

As noted by Coleman et al. and Rigotti et al., the presence of stem cells in fat grafts may explain the apparent healing and the improvement in tissue quality. However, the mechanisms of these effects have not been clarified. Some investigators have suggested an interaction between the grafted fat and neighboring tissue, which may promote repair of surrounding tissues, directly or through angiogenesis or vasculogenesis. Others have cited the plasticity between preadipocytes and macrophages, such that some or all of the healing effect may be secondary to an enhanced immune response, leading to permanent tissue remodeling.

Although our study was in animals, fat-grafting applications have been well established in humans. It is noteworthy that, despite the lack of difference in Baker classification after lipoinjection, the capsules of treated animals were
softer than those of untreated animals—according to AT and subjective evaluation by palpation.

CONCLUSIONS
Pericapsular lipoinjection may be a promising treatment for CC. We plan to continue this research by applying the technique in humans who experience mild to moderate CC after breast augmentation. If patients perceive a greater degree of capsule softness, the need for implant replacement may be eliminated or delayed.

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REFERENCES