Biocompatibility Comparison of Novel Soft Tissue Implants vs Commonly Used Biomaterials in a Pig Model

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Abstract

Objective. To develop a model to evaluate biocompatibility, integration, and substrate independence of novel porous bioscaffolds for maxillofacial and plastic reconstruction using sphere-templated angiogenic regeneration technology compared with currently available synthetic and biologic soft tissue implants.

Study Design. A prospective pilot study using animals.

Setting. Military medical center.

Subjects and Methods. Five pigs underwent dorsal subcutaneous implantation of a polypropylene-based material coated with precision pore silicone granules (sphere-templated scaffold), expanded polytetrafluoroethylene, human dermis, and porcine dermis. Sham and undissected sites were also used as controls. Specimens were harvested 7, 21, 90, and 180 days after surgery and evaluated histologically for inflammation, neovascularization, and collagen deposition.

Results. All materials and sham sites induced a mild to moderate inflammation that decreased over time, except for human dermis, which elicited a moderate to severe inflammatory response. The responses were varied and measurable using subjective scoring methods. The sphere-templated scaffold demonstrated numerous foreign body giant cells adjacent to the silicone granules, which were not seen in any of the other specimens.

Conclusion. Subjective scoring of pathology slides and measurement of capsule thickness appeared to show differences between the materials, but these differences require a larger number of subjects and proper statistical analysis to assess. The robust foreign body reaction elicited by the polypropylene/silicone-based scaffold argues against the use of this material in future studies. The authors advocate using inert biodegradable substances for future bioscaffold constructs.

Keywords

soft tissue implants, augmentation, bioscaffold, expanded polytetrafluoroethylene, human dermis, porcine dermis

Soft tissue implantation has been used for decades to replace lost volume and correct cosmetic deformities. Current treatment to replace soft tissue deficiencies often consists of autologous tissue transfer from adjacent or distal parts of the body.[1-4] Autologous grafting procedures, however, are frequently limited by the availability of tissues, and flap harvesting results in further morbidity at the donor site as well as increased operative time.[1-4] Consequently, the use of biologic, synthetic, and semisynthetic biomaterials has been introduced as alternatives to autologous grafts for soft tissue augmentation in the maxillofacial region.[5-15] Appropriate reconstruction requires that the corrective "filling" material possesses similar mechanical properties as human soft tissue and skin. It should also be biocompatible with the surrounding tissue to produce a minimal host inflammatory response.

The currently available synthetic polymers for facial reconstruction include silicone, high-density polyethylene (MEDPOR; Porex Surgical, Newnan, Georgia), polydimethylsiloxane (Silastic; Dow Corning, Midland, Michigan), and expanded porous polytetrafluoroethylene (ePTFE; Gore-Tex, W. L. Gore & Associates, Flagstaff, Arizona). These materials are an improvement from other described alloplastic and autologous materials because they are relatively inert, are nontoxic, and have a relatively low complication rate. More recently, the use of ePTFE has gained popularity and is considered the gold standard for isolated soft tissue augmentation and replacement.[12-13,16] Although useful, ePTFE remains as a permanent, nonvascularized implant for the lifetime of the patient. Immediate or long-term complications from its use may include infection, extrusion, capsular formation, migration, and unnatural texture and appearance.[16,17]

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Biologic grafts such as cadaveric acellular dermis and porcine dermis have also been proposed for use as viable alternatives for soft tissue augmentation in facial reconstruction. However, risks associated with their use include inflammation, abscess formation, skin contractures, and loss of volume of the original implant.[7,8] Injectable biomaterials such as collagen dermal fillers and hyaluronic acid derivatives are also commonly used for facial soft tissue augmentation.[5,14,15,18-20] Injectable hyaluronic acid has an excellent safety profile because of its wide distribution throughout native connective and epithelial tissues but has the significant disadvantage of poor longevity (6-12 months).[5,14,15,18-20] The ideal injectable biomaterial does not exist currently because most of the effects are temporary. Only alloplastic implants provide a long-term solution for facial soft tissue augmentation.

An ideal graft material depends to some degree on which tissue is being replaced but in general includes the following characteristics: readily available, inexpensive, biocompatible with the surrounding host tissue to produce a minimal host inflammatory response, durable, safe, noncarcinogenic, easily shaped, reversible, not associated with donor site morbidity, resistant to infection, ability to maintain volume, and ability to restore the integrity of the grafted structure.[5,6] At the present time, no surgical graft material has been shown to be ideal, and research is needed to develop and test the safety and efficacy of novel implant materials that have potential use in facial soft tissue reconstruction.

A better alternative to existing implants may be available with novel materials fabricated by Sphere Templated Angiogenic Regeneration (STAR) technology (Healionics Corporation, Redmond, Washington), which permits the construction of porous biomaterial scaffolds that enable angiogenesis and healing while reducing the foreign body response.[21] STAR scaffolds are a 3-dimensional scaffold formed by tightly packing an array of spherical beads of controlled size, casting a polymer into the interstitial space between the beads, and dissolving away the beads to yield a pore network of interconnected spherical voids (see **Figure 1**).

STAR scaffolds have shown promise in preliminary studies with remarkable vascularization (critical for tissue growth, healing, and successful implantation) and integration with surrounding host tissues.[21] STAR scaffolds have been produced from a variety of medical-grade materials that range from nondegradable to fully biodegradable, including silicone, poly(2-hydroxyethyl methacrylate), and hyaluronic acid.

The manufacturer claims that the improved biointegration, reduced foreign body response, increased angiogenesis, and tissue regeneration with reduced scarring observed with STAR scaffolds result from the sphere template pore geometry and not the implant material itself. An implantable STAR scaffold made from hyaluronic acid may combine the proven safety and performance of this material demonstrated in the injectable form with improved longevity through replacement of the scaffold with collagen ingrowth. Given the cost associated with manufacture of a hyaluronic acid–based implant, a model for experimentation was
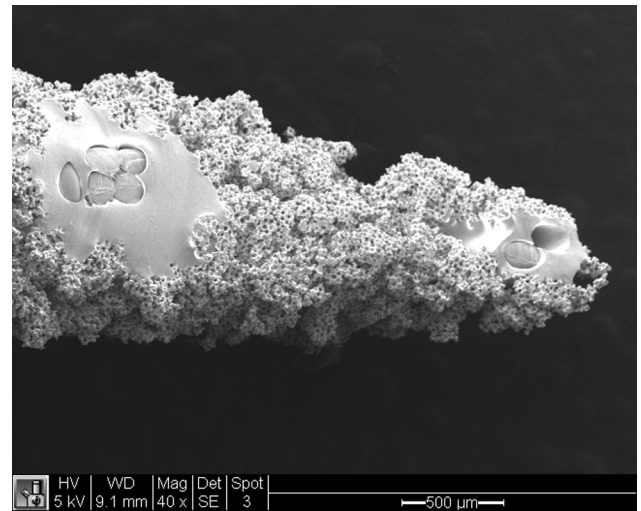

Figure 1. Electron micrograph of Sphere Templated Angiogenic Regeneration (STAR) technology coated mesh.

necessary. This study seeks to assess the feasibility of the model to demonstrate substrate independence of the material with a more readily available and inexpensive polypropylene biomaterial in an animal model.

Methods

Materials. Four types of implant materials were used in this study. These include a novel STAR polypropylene-based scaffold biomaterial coated with precision pore silicone granules (Healionics Corporation), ePTFE (Gore-Tex; W. L. Gore & Associates), freeze-dried acellular human dermis (AlloDerm; LifeCell Corp, Branchburg, New Jersey), and cross-linked acellular porcine dermis (Permacol; Covidien, Mansfield, Massachusetts). Sheets of implant materials were cut into uniform blocks by using a precut plastic sterile template at the time of surgery measuring 3.0×1.0 cm each. Each graft measured approximately 0.5 mm in depth.

Animal model. The use of pigs provides the most suitable animal model available for comparison with human skin in the evaluation of novel soft tissue implants. With the exception of the lack of apocrine sweat glands, porcine skin is similar to human skin in epidermal thickness, composition, vascularization, and healing.[22-24]

Five Yorkshire pigs (*Sus scrofa*) weighing approximately 50 kg at approximately 4 months of age were obtained from Oshiro Farms (Waianae, Hawaii). The pigs gained up to 25 kg by 180 days with a modest increase in length. The study protocol was approved by an institutional animal care and use committee. Investigators complied with the policies as prescribed in the US Department of Agriculture (USDA) Animal Welfare Act and the National Research Council's "Guide for the Care and Use of Laboratory Animals." Ceftiofur 5 mg/kg intramuscularly (IM) was administered 1 day preoperatively.

Surgery and tissue collection. The study was conducted in the Department of Clinical Investigation at Tripler Army
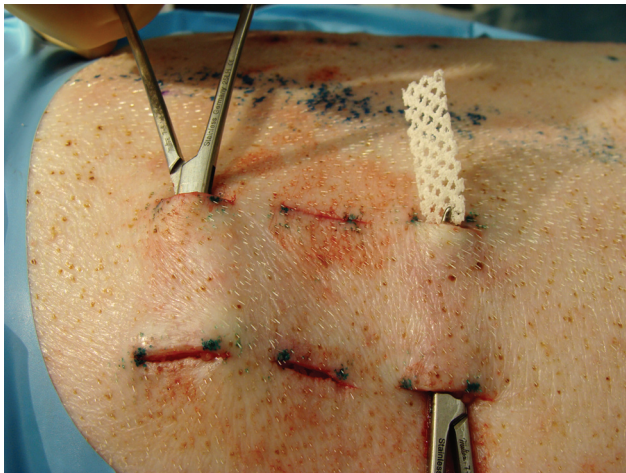
Figure 2. Subcutaneous pocket for 1 × 3-cm grafts.

Figure 3. Inflammation scores (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe). ePTFE, expanded porous polytetra-fluoroethylene; STAR, Sphere Templated Angiogenic Regeneration.

Medical Center. Under general anesthesia, the dorsum was shaved and tattooed to mark the planned graft sites. The animal was then prepared with chlorhexidine scrub and draped sterilely. In each pig, 3 strips of each graft material were implanted for a total of 12 grafts. Sham sites (dissection without implantation) were also created in each pig. The placement of grafts, sham surgical sites, and nonsurgical sites was randomized prior to each surgery to minimize cross-reactivity or interference between different materials. A pocket was created between two 1-cm incisions by blunt dissection to implant the grafts in the subcutaneous tissue (see **Figure 2**). Each similar material was separated by 1 cm, and each implant type was separated by 5 cm. Results from published studies suggest that a distance of 5 cm should be sufficient to minimize the possibility of cross-reactivity or interference between materials.[25-29] Each strip was secured with 4-0 polydioxanone sutures in a single interrupted subcuticular fashion.

One pig each was sacrificed at 7, 21, and 90 days with the remaining 2 pigs sacrificed at 180 days after surgery. The sham sites, surgical implants, and surrounding host tissue were harvested en bloc. A separate nonsurgical site was harvested to compare with normal tissue without dissection.

Histology. Specimens were fixed in 10% neutral buffered formalin for at least 24 hours after harvesting and embedded in paraffin, and serial sections (5 μm thick) were stained with hematoxylin-eosin, Masson trichrome, and elastin/van Gieson.[30-33] Digital images were captured using PictureFrame software (Optronics, Goleta, California) and an Olympus IX71 microscope (Olympus America, Center Valley, Pennsylvania). Inflammation and neovascularization were scored by a pathologist on a scale of 0 to 4 (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe).[30-32] The qualitative measurements were scored by evaluating collagen deposition both superficial and deep to the graft site. Quantitative measurements were provided examining the width of each capsule
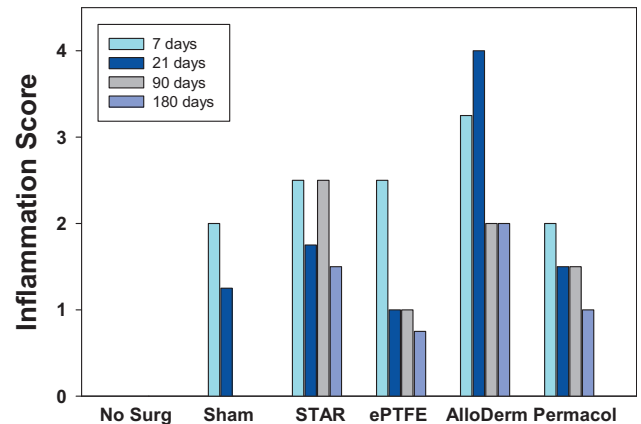
on the superficial and deep sides of the graft. Three to 4 measurements were taken from each side of the graft per slide. These measurements were averaged to create a mean thickness of each implant from 8 to 12 measurements. The thickness of the capsule was measured at the deep and superficial margins of the graft using the PictureFrame application calibrated with 50-μm glass beads.

Results

The animals tolerated the study well, and all animals survived until the planned sacrifice dates. There were no extrusions of implant materials or gross infections noted during the study, although one of the 180-day STAR implants did display mild skin erythema on postoperative day 115. No treatment was required, and the erythema resolved without intervention.

At 7 days, all implant sites showed mild to moderate levels of inflammation with the exception of AlloDerm, which showed a more robust inflammatory response both grossly and microscopically (see **Figure 3**). The response persisted and the AlloDerm grafts achieved maximal inflammation at 21 days, with formation of granulomas and areas of necrosis noted within the graft. The sham site exhibited a mild inflammatory response at 7 days. By 90 days, the sham site was nearly identical to the nonsurgical sites with minimal inflammation, neovascularization, and collagen deposition. Neovascularization was not noted in any of the grafts until 90 days after surgery (see **Figure 4**).

The STAR graft sites were notable for the surrounding foreign body reaction associated with the silicone scaffold, which persisted up to 180 days after implantation (see **Figure 5**). Foreign body giant cells were clustered around silicone particles of the scaffold without apparent invasion of other portions of the surrounding tissue. This finding suggests that the foreign body reaction was a result of the silicone material itself.

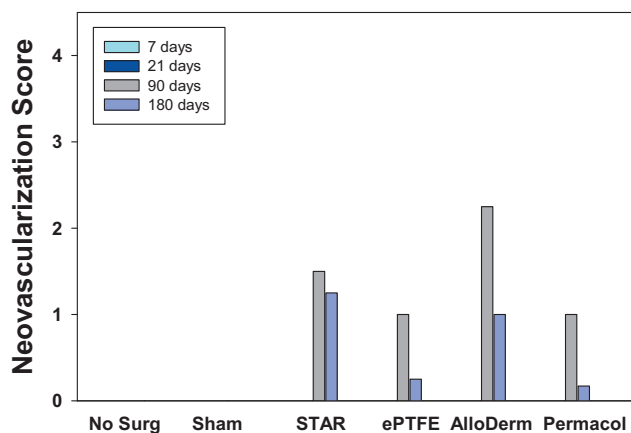All materials formed capsules surrounding each implant, which were measurable using the method described above

Figure 4. Neovascularization scores (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe). ePTFE, expanded porous polytetrafluoroethylene; STAR, Sphere Templated Angiogenic Regeneration.

and relatively consistent within each type of material. Despite the foreign body reaction, the STAR implant formed a consistently thin capsule (see **Figure 6**). The fibroblastic proliferation scores paralleled these measurements.

Discussion

Characteristics of the ideal biomaterial include availability, cost-effectiveness, biocompatibility, durability, safety, malleability, resistance to infection, and, most important, the ability to restore the integrity of the grafted structure.[5,6] AlloDerm and Permacol have been used successfully in facial soft tissue augmentation with excellent vascular ingrowth but have a tendency to resorb in an unpredictable manner.[33] Although ePTFE implants have a proven record of safety and reliability in other parts of the body, their success in facial soft tissue augmentation has fallen short due to capsule formation, extrusion, inadequate tissue integration, avascularity, and unnatural texture and appearance.[16,17] The STAR material seeks to remedy several of these shortcomings through a scaffold with

improved integration, increased angiogenesis, and reduced inflammation and scar tissue formation. Given the versatile design of the STAR material, several different polymers can be cast into the interstitial voids created by the microspheres. A variety of synthetic substrates have been developed using the STAR technology to include silicone, hyaluronic acid, and poly(2-hydroxyethyl methacrylate) or polyHEMA.

The normal physiological response to implanted biomaterials is termed the *foreign body response or reaction*.[34,35] Graft materials produce an initial acute inflammatory response that is followed by a chronic fibroproliferative response.[34,35] Biocompatibility is determined by the intensity of these responses and the ability to resolve the injury to the tissues during implantation.

All materials and sham sites induced a mild to moderate inflammatory response that decreased over time, except for human dermis (AlloDerm), which elicited a moderate to severe inflammatory response. The observed inflammation was likely due to a xenogenic response, which may have been avoided by using acellular dermal matrix from pig rather than human skin.[36]

The STAR scaffold demonstrated a vigorous foreign body response, with giant cells located adjacent to the silicone granules. The robust foreign body reaction argues against the concept of substrate independence of the implant material, highlighting the importance of using inert biodegradable substances for bioscaffold constructs in future studies. Ideally, the use of hyaluronic acid–based or other inert substances for the scaffold may prove to be superior to current implants. In addition, porcine acellular dermal matrix would be a desirable replacement for AlloDerm to reduce the xenogenic response in future studies. Given the small sample size used in this pilot study, it was not possible to garner information on statistical significance of the results or to make comparisons between the materials. Use of 28 subjects (7 pigs per time point) would be necessary to obtain statistically significant conclusions in future studies; however, the results suggest that previous studies[21] demonstrating the improved vascularization and decreased capsular
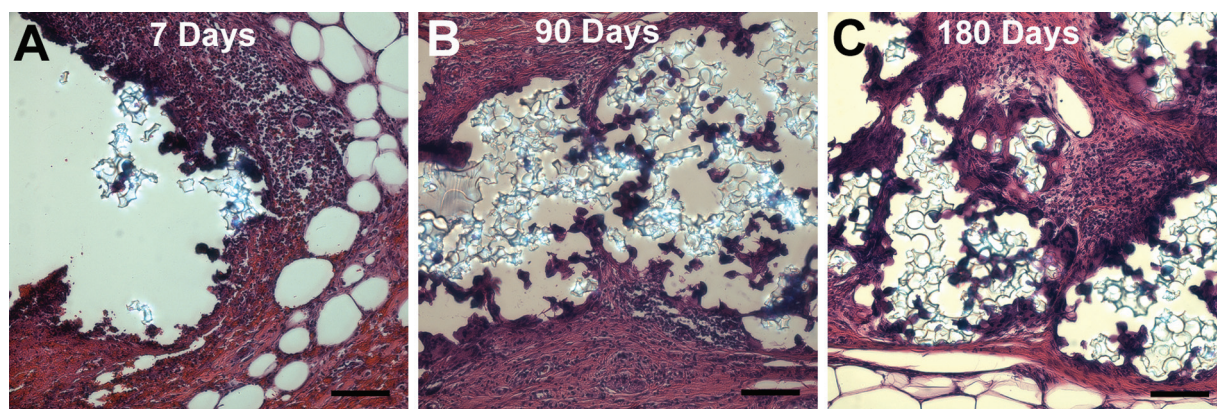

Figure 5. Sphere Templated Angiogenic Regeneration (STAR) scaffold foreign body reaction at (A) 7 days, (B) 90 days, and (C) 180 days (hematoxylin and eosin, ×200, bar = 100 μm).
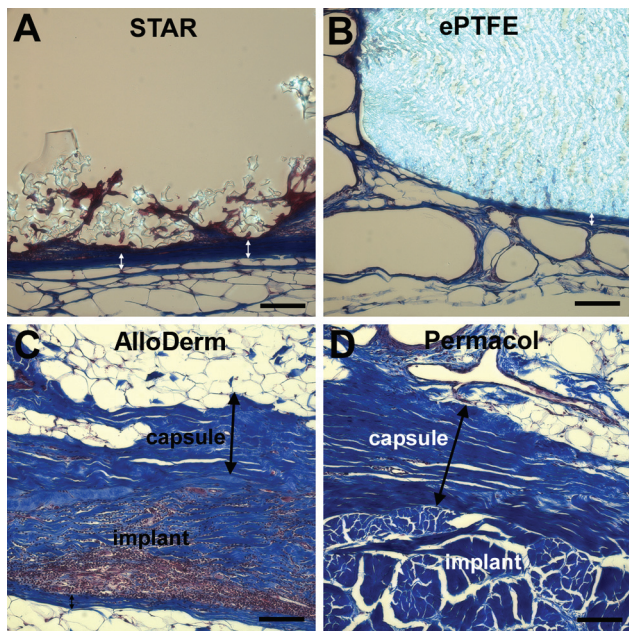
Figure 6. Encapsulation of implant materials at 180 days. (A) Sphere Templated Angiogenic Regeneration (STAR) material, (B) expanded porous polytetrafluoroethylene (ePTFE), (C) AlloDerm, and (D) Permacol (Masson trichrome, ×200, bar = 100 μm).

formation of the STAR scaffold compared with other biomaterials may hold true in facial soft tissue augmentation.

In conclusion, the in vivo pig model using histologic analysis and objective capsule measurements is a suitable means of comparing these biomaterials. We recommend against the use of human cadaveric–derived AlloDerm for future studies in pig models given its propensity to cause a xenogenic response[36] and recommend substitution of this material with a porcine acellular dermal matrix or leaving it out altogether. Despite the scaffold concept of the material, the silicone caused chronic inflammation, and we would advocate using inert biomaterials in future scaffold design. The utility of the model will require a future study adequately powered to show statistically significant differences.

Disclaimer

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US government.

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Author Contributions

Caroline M. Kolb, study design, data acquisition, analysis and interpretation of data, drafting article, revising article, final approval of the version to be published; **Lisa M. Pierce**, study design, data acquisition, analysis and interpretation of data, revising article, final approval of the version to be published; **Scott B. Roofe**, conception and design, analysis and interpretation of data, revising article, final approval of the version to be published.

Disclosures

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