Evaluation of a Sphere-Templated Polymeric Scaffold as a Subcutaneous Implant

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Objective: To evaluate the performance of a sphere-templated poly(2-hydroxyethyl methacrylate) (poly-[HEMA]) tissue scaffold as a subcutaneous implant by comparing it with widely used high-density porous polyethylene (HDPPE) implant material.

Design: We implanted sphere-templated porous poly-(HEMA) and HDPPE disks into the dorsal subcutis of C57BL/6 mice for 4 and 9 weeks. Excisional biopsy specimens of the implants and surrounding tissue were assessed for host inflammatory response, tissue ingrowth, and neovascularization using trichrome, picrosirius red, and anti–endothelial cell antibody staining.

Results: The poly(HEMA) and HDPPE implants showed resistance to extrusion and elicited a minimal inflammatory response. Both implants supported cellular and collagen ingrowth, but ingrowth within the HDPPE implant was thicker owing to the larger porous structure (>100 μm) of HDPPE, whereas the poly(HEMA) implant had much thinner collagen fibrils within much smaller (40-μm) pores, suggestive of less scar-type reaction. Neovascularization was supported by both implants. Blood vessels were identified within the fibrous ingrowth of the HDPPE and within individual pores of the poly(HEMA).

Conclusions: Sphere-templated poly(HEMA) implanted as a subcutaneous tissue scaffold stimulates a minimal inflammatory response and supports cellular infiltration, collagen formation, and neovascularization. Because of its tightly controlled porous structure, poly(HEMA) appears to induce less scar-type ingrowth compared with HDPPE.


Facial soft-tissue volume can change because of trauma, tumor removal, and aging. Current techniques to enhance facial soft-tissue volume include the injection of biofillers, placement of facial implants, and transfer of tissue into the face. Injectable tissue biofillers have the advantage of being minimally invasive and of having a decreased postinjection recovery time compared with surgical procedures aimed at restoring facial volume, but biofillers are used for subtle volume enhancement only. More significant defects in facial volume require the placement of a facial implant, transfer of autologous tissue into the face, or a combination of both. The ideal facial implant is inert, noncarcinogenic, noninflammatory, and nonallergenic and integrates into the surrounding tissue. High-density porous polyethylene (HDPPE) (MEDPOR; Stryker) is a reliable commonly used facial implant material that has been shown to exhibit rapid tissue ingrowth in its pores, which are larger than 100 μm.1 Disadvantages of HDPPE include a risk of infection and a subsequent risk of extrusion, although incidence of these complications is infrequent. Resistance to infection and decreased incidence of extrusion of porous implants should be minimized by vascularization and ingrowth of tissue, providing mechanical stability, and by access to blood vessels for phagocytic cells, allowing a greater potential to eradicate bacteria by means of normal immune surveillance.2,3

The HDPPE implant is especially vulnerable in fields with decreased vascularity, such as previously irradiated wound beds.4 Our group has developed a porous scaffold consisting of poly(2-hydroxyethyl methacrylate) (poly[HEMA]) prepared by a unique sphere-templating technique that in previous studies has been shown to induce reconstruction of normal tissue when implanted percutaneously and to resist extrusion.5 The sphere-templating technique permits the construction of porous biomaterial scaffolds with precise control of the structural dimensions of interconnected pores and identical pore size. If the pores are 30 to 40 μm in diameter,
The material can induce the reconstruction of vascularized nonfibrotic tissue. The HDPPE implants are produced with a broad range of pore sizes and shapes, with most pores larger than 100 μm and a pore volume of 50%. The goal of our study was to assess the poly(HEMA) scaffold's ability to support tissue ingrowth, angiogenesis, and resistance to extrusion when implanted in a subcutaneous model compared with HDPPE.

**METHODS**

**IMPLANT MATERIALS**

We prepared cross-linked, porous poly(HEMA) hydrogels as previously described. Each hydrogel had a thickness of 1.3 mm and pore size of 40 μm with interconnecting pore throats approximately 16 μm in diameter. We acquired sheets of HDPPE, 1.5 mm thick, with a manufacturer-specified pore size larger than 100 μm and a pore volume of 50%. Each material was fashioned into 4-mm disks.

**SUBCUTANEOUS IMPLANTATION AND RETRIEVAL**

Animal studies were conducted with University of Washington Institutional Animal Care and Use Committee approval in compliance with the 1985 National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twelve 8-week-old male C57BL/6 mice (Charles River Laboratories) were used for the study. Each mouse underwent implant with a poly(HEMA) and a HDPPE disk in the dorsal subcutis. Mice were anesthetized with an intraperitoneal injection of ketamine hydrochloride (150 mg/mL) and xylazine hydrochloride (10 mg/mL, at 0.02 mL/g of weight) (Phoenix Pharmaceuticals Inc). The dorsal skin was shaved and cleansed with 10% povidone-iodine solution. One full-thickness incision approximately 2 cm in length was made in the dorsal midline. Subcutaneous dissection below the panniculus carnosus was performed, creating a pocket on each side of the midline into which each implant was placed. The animals were transferred back to their housing and observed closely to inspect for excessive reaction to the implanted materials. The implants were harvested at 4 and 9 weeks after implantation. The animals were humanely killed via carbon dioxide inhalation, and excisional biopsy specimens of the implanted sites with a margin of normal soft tissue were obtained.

**HISTOLOGICAL EXAMINATION OF IMPLANTS**

On excisional biopsy, the specimens were fixed in 10% neutral-buffered formalin or zinc fixative (2mM zinc acetate, 37mM zinc chloride, and 3mM calcium acetate) and then processed for paraffin embedding. Masson trichrome stain (Sigma-Aldrich Corp) was used to assess the general cell morphology and collagen fibers. We used picrosirius red (Direct Red 80; Sigma-Aldrich) staining to differentiate the thickness and organization of collagen bundles to distinguish scar-type collagen formation vs normal collagen formation around and within the implants. When examined through cross-polarized microscopy, normal-appearing collagen, including reticular fibers, are yellow-green, and more disorganized fibers associated with scar formation and fibrosis appear bright yellow or red-orange.

To further assess angiogenesis into the implants, we performed immunocytochemistry using MECA-32 antibody, a mouse panendothelial cell antigen-specific antibody (1:10; BD Bioscience/Pharmingen). Rat IgG (1:10; BD Bioscience/Pharmingen) served as a negative control. The secondary antibody consisted of a biotinylated goat IgG (1:10; BD Bioscience/Pharmingen). The streptavidin-biotin immunoperoxidase detection system (ABC; Vector Laboratories) was used with 3,3'-diaminobenzidine (SigmaFast; Sigma-Aldrich Corp) as the peroxidase substrate. Methyl green was used as a nuclear counterstain.

At 4 and 9 weeks after implantation, the poly(HEMA) and HDPPE implants demonstrated no evidence of infection or extrusion. All implants were firmly anchored within the subcutaneous tissue. Gross examination revealed the poly(HEMA) implants surrounded by increased vascularity compared with the HDPPE implants. Qualitative histological examination after Masson trichrome staining (Figure 1) demonstrated a normal inflammatory response and a thin foreign body capsule around each implant, with a slightly thicker capsule surrounding the HDPPE. No severe neutrophil or macrophage invasion surrounded or infiltrated the implants. Host fibroblasts associated with collagen fibers had incorporated themselves into both implants, greater initially at the periphery, with increased cellularity and collagen within the central portion of the implant at 9 weeks. The 40-μm poly(HEMA) pores were well visualized within each implant, with fibers and cells weaving through the interconnecting pore throats (Figure 1). Uniform pores did not exist within the HDPPE implant; rather, the implant consisted of large porous spaces (macropores) between the solid material through which tissue infiltrated (Figure 1). The 40-μm pore poly(HEMA) implants permitted a thinner distribution and lower density of collagen fibrils within each pore compared with the gross nature of collagen infiltration into the large spaces within the HDPPE. Partly because of the larger pore space between implant material, the HDPPE implants had thicker and higher-density collagen fibers within the implant compared with the poly(HEMA) implants. The characteristics were demonstrated by the trichrome-stained sections (Figure 1) and the greater amount of bright yellow and orange appearance of the collagen fibers under cross-polarized microscopy of the picrosirius red–stained sections (Figure 2). Furthermore, the ratio of green to orange collagen fibers within the poly(HEMA) implants appeared to be more consistent with surrounding native tissue, with more green fibrils present compared with the HDPPE (Figure 2).

Both implants supported the formation of new internal blood vessels as evidenced by the trichrome and endothelial cell antibody staining. Because of the absence of true pores within the HDPPE implant, the vessels were contained within the substance of the larger fibrous ingrowth within the implant (Figure 3). Within the poly(HEMA) implant, individual blood vessels were visible within individual pores and were seen traversing between pores (Figure 3).
We proposed to evaluate a porous sphere-templated poly(HEMA) tissue scaffold’s ability to perform as a subcutaneously placed implant and compared it with the commonly used and reliable HDPPE implant. Our specific goals included the assessment of biocompatibility and the ability to support tissue ingrowth and neovascularization. Tissue-scaffold spatial properties, including pore size and interconnectivity, affect cellular penetration, adhesion, and the ability to support angiogenesis—all important characteristics in the design of any implant aimed to facilitate tissue regeneration.

The poly(HEMA) material is a hydrogel commonly used in soft contact lenses and has been shown to be a useful biomaterial that can be modified for porosity and surface chemistry. Our laboratory has developed a sphere-templated technique of fabricating tissue scaffolds that allows for tight control of pore structural dimensions.10 A large surface area favors cell attachment and growth, and large pore volumes and increased porosity are favorable for easy diffusion of nutrients and growth factors and for egress of waste from the scaffold, which all constitute requirements for regeneration of tissue.11 Many earlier studies have shown that porosity affects the nature of the healing reaction and that certain pore sizes are more conducive to vascularized fibrotic healing than others.11,12 The continuity of pores within a synthetic matrix is important because mass transport and cell migration will be inhibited if the pores are not interconnected, even if porosity is high. Marshall et al demonstrated that the sphere-templated poly(HEMA) implant, with all pores identical in size and interconnected, showed a peak in vascular density with 35- to 40-µm pores. We used poly(HEMA) with 40-µm pores for this study and compared its performance with that of HDPPE with manufacturer-specified pores of 100 to 250 µm.

Both implants demonstrated acceptable tissue reaction without implant extrusion or chronic inflammatory response, as would be suggested by a massive neutrophil or macrophage invasion. As expected, both implants generated a minimal foreign-body inflammatory response. The surrounding response resulted in a very thin capsule around the poly(HEMA) implants and a slightly thicker capsule around the HDPPE implants, as visualized microscopically. Under gross examination, these capsules were not palpable through the skin, and their microscopic difference in apparent thickness was unlikely to be functionally significant.

The poly(HEMA) material also supported tissue ingrowth within the implant, but the tissue formation within the poly(HEMA) implant was different from the exuberant fibrous ingrowth seen within the HDPPE implant. Because of the presence of much smaller uniform pores, tissue infiltration into the poly(HEMA) implants was finer, with a distinct gradient of penetration of collagen fibrils, fibroblasts, and individual blood vessels from the periphery into the center of the implant as time progressed. Significant penetration of biological material in both implants that could lead to reduced localized movement was apparent. Although smaller collagen fibers were present within each pore of the poly(HEMA) implant, the overall density of collagen within the implant was satisfactory for implant anchorage. The smaller collagen fiber penetration within the poly(HEMA) implant also appeared to be consistent with less scar-type healing response compared with the response within the HDPPE implant. Although scar-type collagen fibrils existed within the poly(HEMA) implant, the ratio of thicker scar-type fibrils to finer, organized collagen fibrils was less than that of the large fibrous ingrowth seen within the HDPPE implant, suggesting less of a scar-type healing response with the poly(HEMA) implant.8,9 This advantage could possibly be exploited with a biodegradable poly(HEMA) tissue scaffold that our laboratory has developed. A trocar-injectable biodegradable form of the sphere-templated poly(HEMA) tissue scaffold is currently being evaluated and compared with hyaluronic acid facial fillers. We aim to determine whether the sphere-templated poly(HEMA) can lead to more permanent volume augmentation by induced native tissue formation within the biodegradable tissue scaffold that, once completely degraded, results in a healed tissue mass closely resem-
bling the surrounding tissue. Although the degradation products of biodegradable tissue scaffolds might induce an inflammatory response, the biodegradable form of poly(HEMA) will likely not induce such a response given its hydrogel nature and results from preliminary toxicology studies.\textsuperscript{13,14}

Both implants supported neovascularization. Vessels within the HDPPE implants were present within the biological ingrowth into the macropores, whereas in the poly(HEMA) implants, individual blood vessels were frequently present within individual pores of the implant. Although in this study the HDPPE and poly(HEMA) implants did not demonstrate evidence of extrusion, 1 possible advantage of neovascularization directly within the pores of the poly(HEMA) implant could be the potential for a greater resistance to infection in a compromised vascular bed (ie, irradiated field) owing to an increased surface area of the implant directly in contact

Figure 2. Picrosirius red–stained cross-sections of implant materials (original magnification $\times 4$). A, Poly(2-hydroxyethyl methacrylate) (poly[HEMA]) implant. B, High-density porous polyethylene (HDPPE) implant. Images were acquired with cross-polarized microscopy. The white line indicates the boundary between the native host subcutaneous tissue (asterisk, panniculus carnosus) and the tissue capsule surrounding the implant (double arrows). Thicker, disorganized collagen fibrils appear bright yellow or red-orange; thinner, organized fibers are more green-yellow. The poly(HEMA) implant demonstrates more green fibers within and surrounding the implant, similar to native tissue, whereas the HDPPE implant has a higher density of bright yellow and orange fibers.

Figure 3. Endothelial cell antibody–stained cross-sections demonstrating examples of blood vessels filled with red blood cells present within surrounding host tissue (double arrows) and infiltrating the implants (single arrows). A, Poly(2-hydroxyethyl methacrylate) (poly[HEMA]) implant. B, High-density porous polyethylene (HDPPE) implant.
with the vascular system and the phagocytic cells it can deliver. Furthermore, vascularity within individual pores of the scaffold could also be useful in nourishing cells that might be preseeded in vitro onto the poly(HEMA) tissue scaffold, facilitating in vivo tissue regeneration at implantation.\(^5\)

In conclusion, this study serves as a foundation demonstrating that, as a subcutaneous implant, the sphere-templated poly(HEMA) tissue scaffold exhibits good biocompatibility and supports cellular infiltration, collagen formation, and neovascularization. Because of its tightly controlled porous structure, the sphere-templated poly-(HEMA) implant also may induce less scar-type healing response than the HDPPE implant.

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Author Contributions: Dr Bhrany had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Bhrany, Irvin, and Ratner. Acquisition of data: Bhrany, Irvin, Fujitani, and Liu. Analysis and interpretation of data: Bhrany, Irvin, Fujitani, Liu, and Ratner. Drafting of the manuscript: Bhrany, Irvin, Fujitani, and Liu. Critical revision of the manuscript for important intellectual content: Bhrany and Ratner. Statistical analysis: Fujitani and Liu. Administrative, technical, and material support: Irvin. Study supervision: Bhrany and Irvin.

Conflict of Interest Disclosures: Dr Ratner is a founder and stockholder of Healionics, Inc, a company that has licensed the sphere-templated scaffold technology from the University of Washington.

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REFERENCES