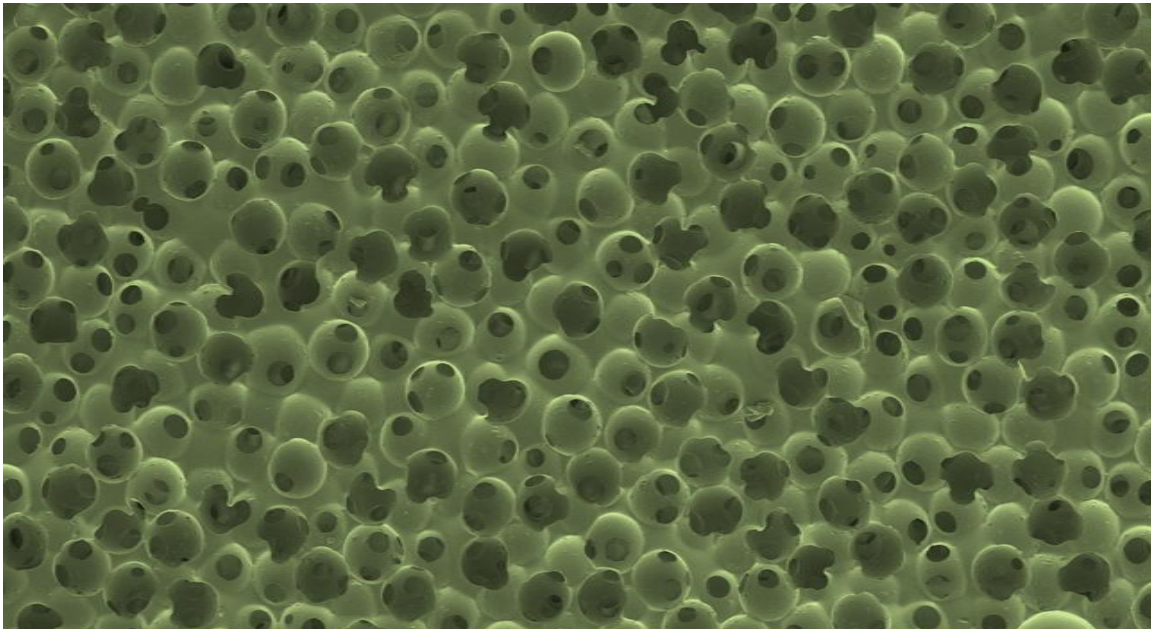


# STAR

## Sphere Templated Angiogenic Regeneration

---

### Technical Summary



Healionics Corporation  
14787 NE 95th St  
Redmond, WA 98052  
USA  
+1 425.818.1987  
info@healionicscorp.com  
[www.healionicscorp.com](http://www.healionicscorp.com)

© Healionics Corporation 2007. All rights reserved. US and Foreign Patents Pending.  
These Products are for Research Use Only. Not intended for animal or human therapeutic or diagnostic use unless otherwise stated.

## STAR: Sphere Templated Angiogenic Regeneration Materials

### Introduction

The fabrication technology we term Sphere Templated Angiogenic Regeneration (STAR) permits the construction of porous biomaterial shapes and scaffolds with precision control of pore structural dimensions. We have shown that important healing processes such as angiogenesis can be powerfully stimulated by carefully controlling the size of the pores and the pore interconnections in implanted biomaterials.

The tight pore size control makes STAR materials more attractive than many other currently available porous biomaterials used in medical device and tissue regeneration applications. STAR can be applied to reduce problems associated with implantable glucose sensors, long-term catheters, and wound healing. The pro-angiogenic nature of STAR materials also makes them appealing candidates for use as tissue engineering scaffolds, where vascularization is critical for tissue growth. STAR technology has yielded promising early-stage experimental results in constructs for the repair of esophagus, cardiac tissue, cartilage, and pelvic prolapse.

### STAR Process

STAR scaffolds are formed by sintering together an array of packed 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. The process steps in fabricating STAR material are summarized in Figure 1 (1).

STAR pore structures have been fabricated from a variety of synthetic and natural polymers, including silicone rubbers, hydrogels, and proteins. We have found that the biological effects of STAR materials are primarily determined by the controlled pore geometry, largely independent of material chemistry.

For applications that require longer term biostable material chemistry, such as catheter cuffs and glucose sensor biointerface layers, silicone rubber is our STAR material of choice. Silicone has soft

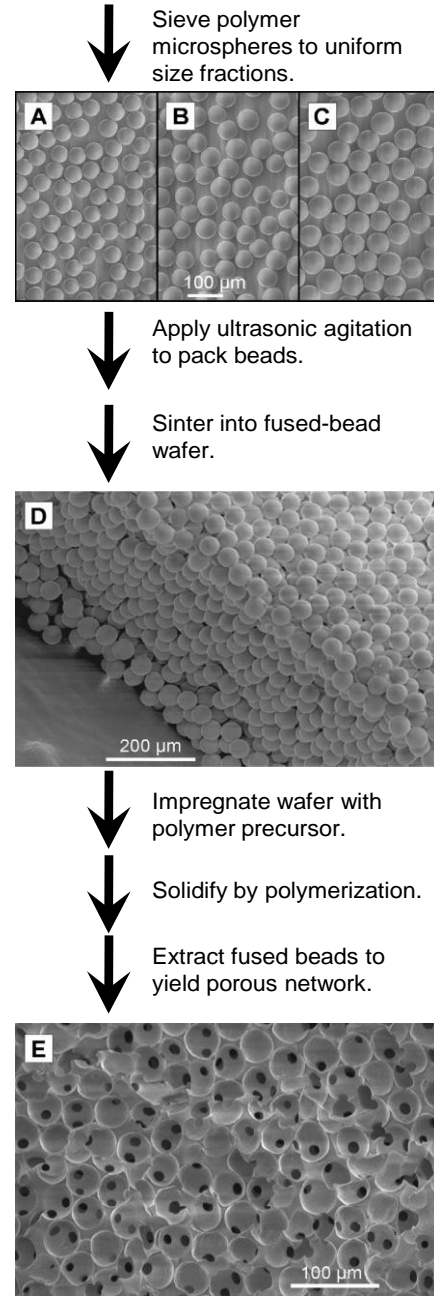


Fig. 1. Process steps for STAR materials. (A) SEM image of 50-µm PMMA spheres. (B) 55-µm PMMA spheres. (C) 60-µm PMMA spheres. (D) Wafer of sintered PMMA microspheres. (E) Cross-section of porous STAR material templated from fused-microsphere wafer.

tissue-like mechanical properties, process-compatible chemistry, and well established biomedical uses. Other materials may be preferred for biodegradable scaffolds or different uses.

Patent protection for the STAR material process and means for promoting angiogenesis is pending (2).

## **Applications**

Two urgent needs – minimizing foreign body encapsulation of implantable glucose sensors, and reducing catheter infection problems – present immediate uses for STAR materials.

### **Need: Implantable Glucose Sensors**

Approximately 21 million people in the US have diabetes, accounting for 7% of the population. The economic and social costs are rapidly escalating (3). Worldwide the number is well in excess of 120 million (4) and growing rapidly.

A stable long-term implantable continuous glucose monitoring system (CGMS) would allow diabetics to continuously monitor their blood glucose levels without painful finger pricks several times a day (5). More importantly, a CGMS would allow prevention of the hyperglycemic excursions that ultimately lead to a number of long-term consequences, including blindness, nerve degeneration, and kidney failure. Plus, it would reduce the risk of hypoglycemia – known as insulin shock – which can result in loss of consciousness (3).

Recently marketed percutaneous CGMS devices from several companies provide only limited duration use (3-7 days) and are relatively unstable, needing calibration against conventional finger prick methods. We see a potential for improved bio-acceptance with STAR materials to improve this functionality for both percutaneous and fully implanted sensors.

### *Problem Summary and the use of STAR Materials*

Implantable devices are susceptible to losing functionality because of the body's natural "foreign body reaction" (FBR) to any implanted object. The FBR ultimately results in encapsulation of the foreign object with a thin layer of dense fibrous tissue, forming an isolating barrier from the surroundings. This response is useful if the object is a bullet, splinter, or other unwanted intruder, but in the case of an implanted biosensor, the capsule eventually prevents the device from communicating chemically or electrically with the surrounding tissue environment.

Application of tightly dimensioned STAR material technology to subcutaneous implants (6) has been shown to stimulate the growth of highly concentrated blood vessels in the capsule tissue, suggesting that STAR offers a significant contribution towards successful long-term implantable sensors.

Designers of biosensors and other medical devices have classically focused on ways to manipulate and control the FBR in hopes of creating a better integrated tissue-implant interface. Numerous studies have shown that pore size is a primary determinant of the extent and nature of the FBR (7-14). Some investigators have achieved encouraging results (8-12); however, these results have been limited by the amorphous pore structures and broad pore size distributions of materials used in these studies. A solution to overcome the FBR by manipulating pore geometry is particularly attractive because it avoids the potentially harmful side effects of growth factor based approaches (5).

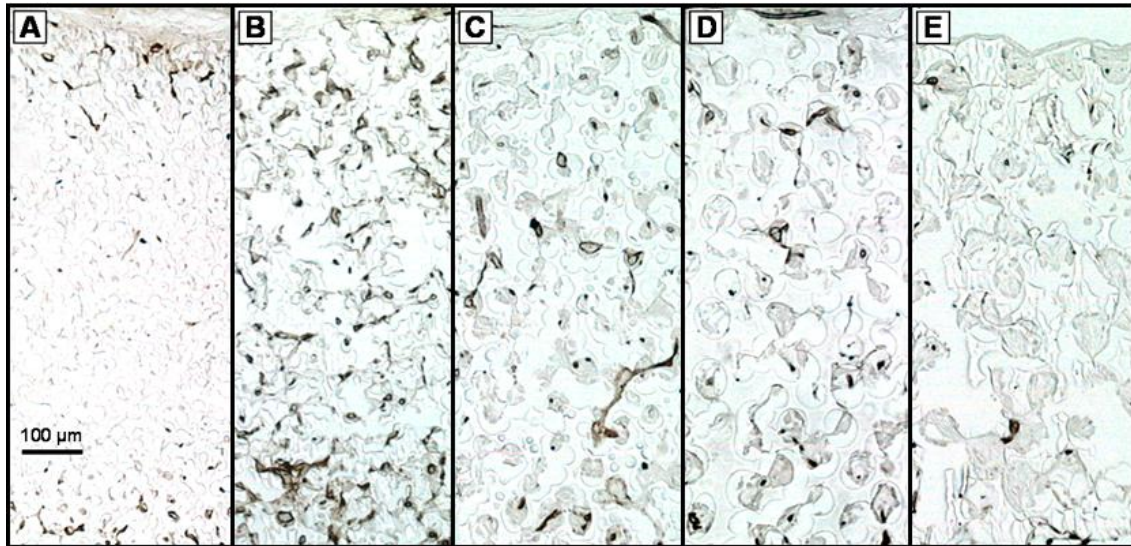


Fig. 2. Blood vessels stained to show angiogenesis in subcutaneously implanted STAR materials of various pore sizes. (A) 20- $\mu\text{m}$  pore diameter. (B) 35- $\mu\text{m}$  pore diameter. (C) 50- $\mu\text{m}$  pore diameter. (D) 70- $\mu\text{m}$  pore diameter. (E) 90- $\mu\text{m}$  pore diameter. Blood vessel density is maximized in the 35- $\mu\text{m}$  pores. The core of the 20- $\mu\text{m}$  pore material is devoid of blood vessels.

### Preliminary Results

STAR technology has allowed the pro-angiogenic effect of porous material geometry to be studied with greater precision. When STAR materials formed from poly(2-hydroxyethyl methacrylate) (polyHEMA) were implanted under the skin of mice for 4 weeks, histological analysis showed that vessel density within the capsule tissue depends on pore size. As Figures 2 and 3 show (6), there is a sharp maximum in the vessel density versus pore size curve at approximately 35- $\mu\text{m}$  pore diameter, both within the pore structure and within the adjacent tissue.

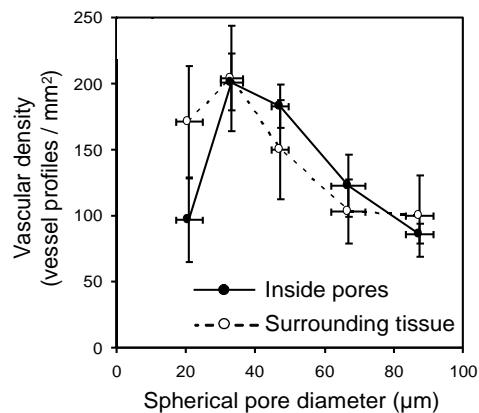


Fig. 3. Intra-pore vascular density in outer 200  $\mu\text{m}$  of subcutaneous polyHEMA implants after 4 weeks compared against vascular density in surrounding tissue within 50  $\mu\text{m}$  of implant.

Figure 4 (15) shows the relative encapsulation effects at a silicone STAR-tissue interface after 8 weeks implantation, compared with a smooth nonporous silicone.

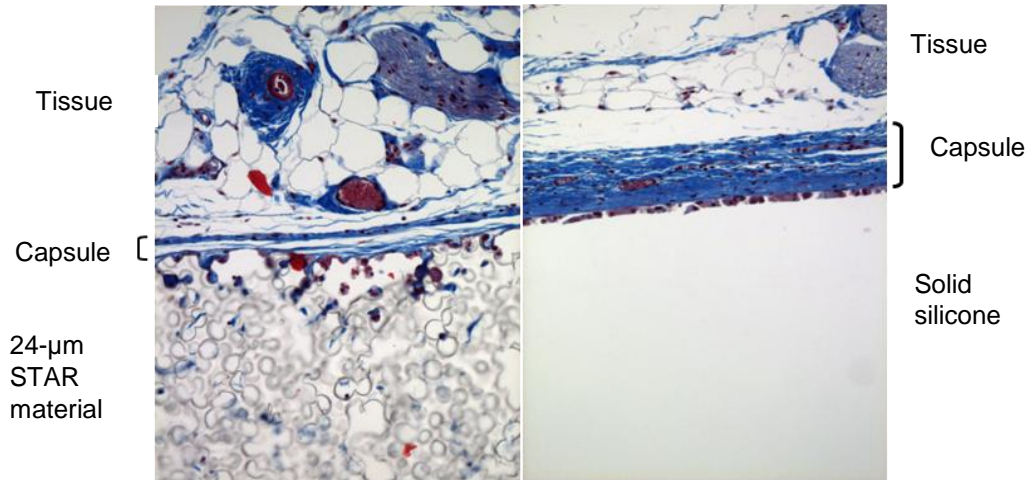


Fig. 4. Silicone STAR implants encapsulation comparison after 8 weeks. Note differences in vascularization, capsule thickness, and capsule density.

#### *Rationale for the STAR Approach*

Results to date suggest that encasing implantable sensors with a sleeve of silicone STAR material will extend long-term functionality. STAR materials have been shown to have a twofold effect on the FBR, both stimulating concentrated growth of blood vessels in close proximity to the sensor and inducing a thinner capsule with a looser structure. These effects facilitate the diffusion of glucose molecules through the pore structure.

#### **Need: Improved Skin-Catheter Sealing**

Approximately 250,000 catheter-related infections occur in U.S. intensive-care units each year, at an estimated cost to the healthcare system of \$25,000 per episode (16). More are associated with nursing homes and other chronic care facilities. Longer term catheterizations are increasing for dialysis treatments, chemotherapy, and life support.

Anti-infective catheter cuffs are currently applied to reduce infection rates and improve catheter retention (17-19). While effective, these treat the outcome of imperfect sealing at the entry site rather than creating a better integration with the surrounding tissue.

STAR technology offers an attractive potential solution to this tunnel path infection problem with longer-term use percutaneous devices. Target applications for STAR technology include peripheral inserted central catheters (PICCs), chronic central venous catheters (CVCs), peritoneal dialysis (PD) catheters, and chronic dialysis catheters.

### *Problem Summary and the Use of STAR Materials*

With percutaneous devices such as catheters, the skin is unable to heal in a way that seals tightly around the device. Gaps at the skin-biomaterial interface provide a gateway for bacterial invasion. Present cuffs are not pore size optimized for skin cell ingrowth and anchorage and still allow infection.

STAR technology offers a distinct approach to the catheter infection problem. Percutaneously implanted STAR materials with optimized pore size (on the order of cell dimensions) have been shown to give remarkable increases in skin cell ingrowth and anchorage (20), and to promote blood vessel development. The improved biointegration of epidermal cells and enhanced pro-angiogenic properties suggest that the STAR approach can be used to greatly reduce infection via the skin-biomaterial interface without needing prolonged antibacterial treatment.

### *Detailed Background*

When a percutaneous device such as a catheter penetrates the skin, basal cells from the adjacent cut edges proliferate and migrate toward the device across the wound bed. When the cells reach the surface of the device, they migrate inward along its side, creating a groove that fills with cellular debris. This groove provides a welcoming environment for harmful bacteria, often leading to infection. Figure 5 illustrates this process (21).

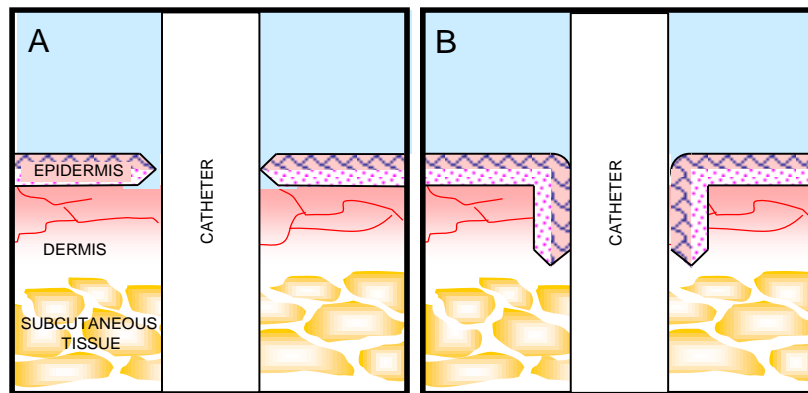


Fig. 5. Schematic of the typical wound response to a percutaneous device. (A) The edges of the epidermis are separated by the device. (B) The epidermis migrates and proliferates inwards along the implant, forming a groove that allows bacterial entry.

Porous catheter cuffs are intended to form a seal by allowing tissue ingrowth. With the materials presently used in these cuffs, fibroblasts (connective tissue-forming cells) from the deeper tissue successfully invade the porous material but often die, leaving behind a region of cell debris, connective tissue, and inflammatory cells. As before, basal cells proliferate and migrate through the debris inward along the side of the implant (permigration). This response compromises the porous material from forming a longer-term functioning barrier or seal against bacteria, again leaving a gap that can lead to infection of the deeper tissue and impaired mechanical integration. Figure 6 illustrates this process of permigration (19).

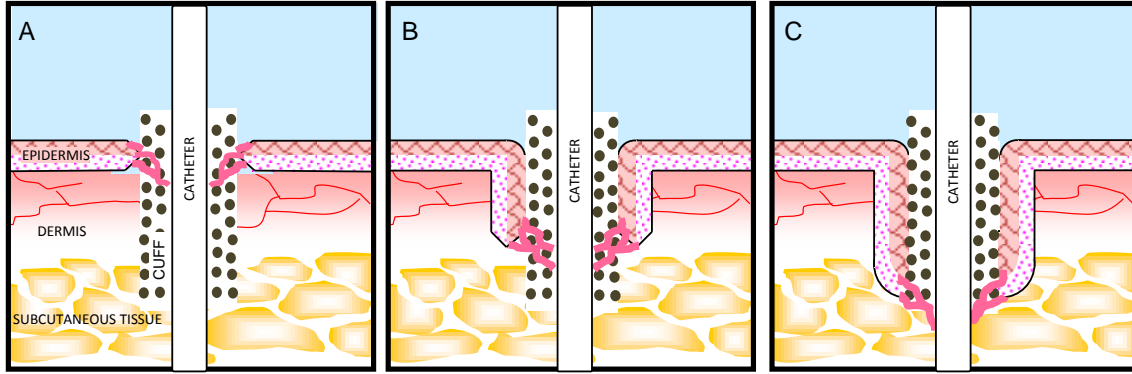


Fig. 6. Schematic of the typical permigration process. (A) Epidermal tissue grows into porous cuff. (B) The leading edges of the epidermis may migrate through the open-pore structure. (C) The epidermis has migrated all the way through the cuff, and the interface is again vulnerable to infection.

### *Organ Culture and In Vivo Results*

First results were demonstrated from a rafted organ culture model (22). Both polyHEMA and silicone STAR implants of various pore sizes were used to study skin cell morphology at the percutaneous interface. When using silicone STAR materials, spherical pore diameters of 40  $\mu\text{m}$  or 60  $\mu\text{m}$  were found to strongly promote the integration of skin cells. Figure 7 shows epidermal keratinocyte (outer layer skin cell) integration into a silicone STAR material. Materials with spherical pore diameters smaller than 20  $\mu\text{m}$  did not show skin cell integration, since the pores were too small to permit access by the cells (23).

STAR materials have been extensively studied in a percutaneous mouse model. Keratinocytes, dermal cells, and basement membrane proteins developed inside the pores. The presence of basement membrane proteins indicates potential for stable anchorage of skin to biomaterial (24).

### *Rationale for the STAR Approach*

A porous interface layer with pores large enough to permit epidermal cell ingrowth yet small enough to provide enough surface area for cell anchorage (thereby preventing permigration) can lead to a quiescent, well-integrated, healed interface that seals out unwanted bacteria.

The desired level of spatial control of wound healing processes cannot be fully realized with other presently available materials. A material where the pore dimensions can be finely tuned with a high level of precision optimizes the wound healing response at the percutaneous interface and achieves the desired result shown in Figure 7 (23).

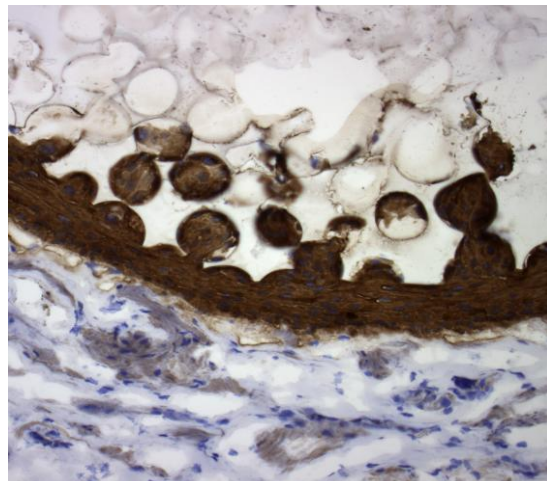


Fig. 7. Integration of skin into silicone STAR material with 60- $\mu\text{m}$  pores (staining for keratinocytes at skin-biomaterial interface after 6 days in organ culture).

## Summary

Precise control of porous biomaterial geometry gives enhanced biological response compared to previous more amorphous compositions. The STAR materials process gives tightly toleranced dimensions optimized for angiogenic effect. Results to date show distinct improvements with great promise for better device performance.

## References

1. Marshall AJ, Irvin CA, Barker T, Sage EH, Hauch KD, Ratner BD. *Polym. Prepr. Am. Chem. Soc., Division of Polymer Chemistry*, 44, 100 (2004).
2. WIPO patent application, WO/2005/032418, Novel Porous Biomaterials.
3. American Diabetes Association, [www.diabetes.org](http://www.diabetes.org).
4. Wild, S. *Diabetes Care*, 27, 1047 (2004).
5. Service RF, *Science*. 297, 5563 (2002).
6. Marshall AJ. "Porous Hydrogels with Well-Defined Pore Structure for Biomaterials Applications." (Ph.D. Dissertation, University of Washington, 2004).
7. Campbell CE, von Recum AF. *J Invest Surgery* 2, 51 (1989).
8. Brauker JH et al. *J Biomed Mater Res*. 29, 1517 (1995).
9. Sharkawy AA, Klitzman B, Truskey GA, Reichert WM. *J Biomed. Mater. Res.* 40, 586 (1998).
10. Sharkawy AA, Klitzman B, Truskey GA, Reichert WM. *J Biomed Mater Res.* 40, 598 (1998).
11. Ward WK, Slobodzian EP, Tiekotter KL, Wood MD. *Biomaterials* 23, 4185 (2002).
12. Rosengren A, Bjursten LM, *J Biomed. Mater. Res.* 67A, 918 (2003).
13. James SJ, Pogribna M, Miller BJ, Bolon B, Muskhelishvili L. *Biomaterials* 18, 667 (1997).
14. Sanders JE et al, *J Biomed Mater Res.* 65A, 462 (2003).
15. Marshall AJ, Irvin CA, Ratner BD. *NAVBO Vascular Matrix Biology and Bioengineering Workshop*, Whistler, BC, March 15-18, 2007.
16. Slaughter SE. *Postgrad Med*, 116, 59 (2004).
17. Jansen JA, Walboomers XF. *J Mater Sci Mater in Med*, 12, 1033 (2001).
18. Seare W, Moncrief J, Popovich R, Moncrief D, Simmons V, Settle S, Simmons E. *Advances in Peritoneal Dialysis*, 11, 197 (1995).
19. Stickler DJ. *Curr Opin Infect Dis* 13, 389 (2000).
20. Tavakkol Z, Usui M, Marshall AJ, Fleckman P, Ratner BD, Olerud JE. *J. Invest Dermatol.*, 127, S37 (2007).
21. von Recum AF." *J Biomed Mater Res.* 18, 323 (1984).
22. Miyashita Y, Usui ML, Underwood RA, Hauch KD, Marshall AJ, Ratner BD, Fleckman P, Olerud JE. *J Invest Dermatol* 124, A33, (2005).
23. Tavakkol Z, Fukano Y, Usui M, Underwood R, Hauch K, Ratner BD, Fleckman P, Olerud JE. *1st International Conference on Wound Healing and Technology*, Seattle, WA, August 28-30, 2006.
24. Tavakkol Z, Miyashita Y, Usui ML, Underwood RA, Marshall AJ, Hauch KD, Fleckman P, Ratner BD, Olerud JE. *Regenerate World Congress on Tissue Engineering and Regenerative Medicine*, Pittsburgh, PA, April 24-27, 2006.