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Silicon induces minimal thromboinflammatory response during 28-day intravascular implant testing

Melissa E. Melvin, M.D., William H Fissell, M.D., Shuvo Roy, Ph.D., and David L Brown, M.D.

Abstract

Microelectromechanical systems (MEMS) are used to machine miniaturized implantable medical devices. Our group has used MEMS technology to develop hemofiltration membranes for use in renal replacement therapy which possess enhanced selectivity and permeability. The use of silicon in blood-contacting environments may be limited, however, due to contact activation of the coagulation cascade by silicon which form the surface oxides in atmospheric conditions. As well, reports of long-term biocompatibility of blood-contacting silicon devices are lacking. The aims of this pilot study were: 1) to develop a model for investigating the effects of intravascular implants and 2) to characterize the degree of thrombosis and tissue inflammation incited by prolonged implantation of silicon materials. Silicon implants with and without polyethylene glycol (PEG) coatings were surgically implanted transluminally through rat femoral veins. Gore-Tex and stainless steel implants served as controls. Implants were left *in vivo* for four weeks. All femoral veins remained patent. Veins associated with silicon implants exhibited rare thrombi and occasional mild perivascular inflammation. In contrast, Gore-Tex and stainless steel controls caused moderate vein thrombosis and provoked a moderate to marked cellular infiltrate. Under scanning electron microscopy, bare silicon implants were found to have significant adherent microthrombi, while PEG-treated implants showed no evidence of thrombi. PEG-treated silicon appears to be biocompatible and holds potential as an excellent material with which to construct an implantable, miniaturized hemofiltration membrane.

Keywords

biocompatibility; biofouling; nanotechnology; MEMS component materials; silicon; artificial kidney; hemofilter

Introduction

End stage renal disease affects over 425,000 Americans and continues to increase in prevalence at 8% annually.^{1,2} Renal transplantation (the gold standard) is hindered by a shortage of donor organs, while dialysis is expensive, inconvenient, and confers significant morbidity and mortality. Miniaturized, implantable, or wearable renal replacement systems could improve the implementation of newer dialysis prescriptions, such as prolonged and daily therapies at home.^{3–5} Tissue engineering may provide the solution for vascular-filtration interface issues.⁶

Our group has focused on developing a functional hemofiltration membrane as part of perfecting the miniaturization of renal replacement therapy.⁷ Wide variation in pore size within conventional polymer membranes leads to imprecise filtration characteristics. As the mean pore size is reduced, solutes above the desired molecular weight cutoff of the membrane are effectively retained, but the hydraulic permeability of the membrane is reduced.^{8,9} Engineering narrower pore size distribution maximizes the mean pore size of the membrane and allows for sharper transitions from passage to retention.^{7–12}

MEMS (microelectromechanical systems) technology-associated silicon micromachining techniques provide an attractive toolkit for the development of miniaturized implantable medical devices. MEMS technology offers nanometer-scale control of device features using an industrially-mature, high-volume, low-unit-cost manufacturing process.¹³ Our group developed a novel ultrafiltration membrane constructed from silicon and related MEMS materials. This next-generation hemofilter has well-controlled pore sizes and shapes to improve hydraulic permeability and membrane selectivity, and will be miniaturized for use in wearable or implantable renal replacement.⁷

A potential significant downside to the use of silicon as a hemofiltration membrane is the native layer of silicon dioxide (silica, SiO₂) that forms spontaneously under atmospheric conditions. Silica is used to stimulate clotting in clinical assays, and blood contact with the hydroxyl groups of oxidized silicon has the potential to initiate blood coagulation by contact activation. Another coagulation pathway involves protein adsorption and conformation change on artificial surfaces, which can initiate clot formation by promoting platelet attachment and degranulation.^{14,15} *In vitro* experiments have shown that platelet adhesion on bare silicon and its derivatives was significantly greater than platelet adhesion on polyurethane.¹⁶ Despite the advantages of MEMS technology in manufacturing miniaturized devices, chemical or biological surface modification of surface oxides may be necessary for deployment of silicon-based medical devices in blood. Nevertheless, the native oxide does have certain advantages as an initial coating. First, its formation is spontaneous upon exposure of silicon to air and self-limiting in thickness to 1–2 nm. Consequently, the need for sophisticated furnaces, which is used for growth of thicker films, is eliminated. In contrast, the thickness of Si₃N₄, which has also been used to coat MEMS devices, is typically in the 100–2000 nm range, and requires specialized vapor deposition equipment. Second, the oxide can readily provide a high density of silanol (Si-OH) groups for chemical modification of the silicon surface via self assembly techniques.

Surface modification of silicon with polyethylene glycol (PEG) films has been attempted to reduce protein adsorption.^{17–20} While PEG-coated silicon has shown evidence of biocompatibility, much of this evidence centers around soft tissue implants, and intravascular placement has not been specifically evaluated.^{21,22}

We developed an *in vivo* technique to assess the thrombosis and inflammation characteristics of materials that may be used in the construction of intra- or trans-vascular implants. Small, thin samples of implant materials are inserted transversely through rat femoral veins so that different segments of the implant are simultaneously exposed to adventitial, soft tissue, and blood environments. The implants and vein segments are

retrieved for analysis after days to months *in vivo*. This permits simultaneous assessment of tissue-material interactions in three tissue types without threatening the survival of the animal should clot or inflammation occur. We tested bare and surface-modified silicon implants using this assay.

Materials and Methods

Approval for animal use was granted by the University Committee for the Use and Care of Animals (UCUCA) in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, 1986).

Preparation of silicon implants

The silicon implants were a gift of H-Cubed, Inc. (Olmsted Falls, OH), who performed the micromachining of silicon wafers into miniature “darts”. These implants consisted of a tapered shaft 500 μm wide at the base, 60 μm thick, and 2–4 mm in length. The silicon surface of some implants was covalently modified with polyethylene glycol (PEG) using a previously reported solution phase method, modified to omit all sonication steps and continuing the PEG deposition for 12 hours.²³ The technique for PEG attachment was a single-step mechanism to covalently couple silicon surface silanol groups (Si-OH) to a PEG polymer through a trimethoxysilane group, thereby forming a Si-O-Si-PEG sequence by a methanol dehydration reaction. PEG coatings added <5 nm to the overall thickness as determined by ellipsometry. All surfaces were generally hydrophilic with water contact angles 10–20° and ~35° on bare and PEG-coated darts, respectively. Five silicon darts were surface-modified with polyethylene glycol (PEG), while six had no coating applied (termed “bare silicon”).

Transvascular Model

Eleven silicon “darts” and eleven controls were implanted in adult, male Fisher F344 rats weighing approximately 300g. Rats were anesthetized by intraperitoneal injection of ketamine (75 mg/kg, Sigma-Aldrich, St. Louis, MI). The inguinal area was shaved, prepped, and draped sterilely. A 2-cm longitudinal incision was made overlying the femoral vessels, and the femoral artery and vein were exposed using an operating microscope. A “dart” was then inserted through the diameter of the femoral vein transversely such that blood flow was not restricted (Figure 1A). One implant was placed per groin. Following transvascular placement, the implants were loosely covered with a portion of the inguinal fat, and the incision was closed in two layers. All implants remained *in vivo* for four weeks. Animals were allowed to eat and drink *ad libitum*.

Gore-Tex sutures and stainless steel controls

Polytetrafluoroethylene (Gore-Tex) sutures (CV-6, TTc-9 taper needle, Gore-Tex, Inc., Elkton, MD) and stainless steel needles (C-1 taper needle, Ethicon, Inc., Cornelia, GA) were implanted as controls. Stainless steel and Gore-Tex were selected as control materials because each is commonly used in medical implants and is considered to induce minimal tissue inflammation. Each Gore-Tex suture was placed through the diameter of the femoral vein similar to the silicon dart implantation. After the suture was placed, the needle was

removed and the suture was trimmed to leave lengths of suture protruding from each side of the vein (Figure 1B). Each stainless steel needle was placed through the vein, and the sharp needle tip was cut off, leaving the body of the needle in place through the vein wall (Figure 1C).

Explantation of implants

After four weeks *in vivo*, all implants and their associated vein segments were explanted. The points at which each implant traversed the vein were marked for later identification, and the implants were removed from the veins.

Histologic analysis of femoral vein and surrounding tissues

Femoral vein segments were placed into a 4% paraformaldehyde solution for two hours, and then into 70% ethanol until embedding. Each fixed femoral vein was paraffin-embedded, serially cross-sectioned in five micrometer sections, and stained with hematoxylin and eosin. The degree of thrombus within the vein lumen and perivascular inflammation was assessed by a single, blinded viewer. Each vein was assigned subjective grades based on the overall degree of thrombus and tissue inflammation seen. [Thrombus: “–“ none, “+” small (<25% of vein lumen), “++” moderate (25–50% of vein lumen), “+++” marked (50–100% of vein lumen); Inflammation: “–“ none, “+” mild, “++” moderate, “+++” marked].

Analysis of silicon implants

Each silicon implant was explanted, gently rinsed with phosphate-buffered saline, incubated in 4% paraformaldehyde for one hour, and then held for processing in 70% ethanol. Each explant was examined by scanning electron microscopy (SEM). As our study was intended to provide a preliminary assessment of silicon biocompatibility, the small sample sizes and qualitative nature of the observations are not suited to statistical significance testing.

Results

All rats survived to explantation. Upon exploration, some implants were found to have migrated out of their vein lumens. Such veins were not evaluated. Fully evaluated implants and vein segments were as follows: bare silicon, n=4; PEG-coated silicon, n=4; Gore-Tex, n=2; stainless steel, n=3. At explantation, all veins were found to be patent by strip test. On gross examination, there was no inflammation and very few fibrous adhesions surrounding the implants (Figure 1, panels D–F).

Light Microscopy

The points of entry of all implants were determined histologically by identifying the mild disturbances in the vein walls and by the ink markings placed during explantation. Cross-sections through the veins at these locations were chosen for grading.

One of four vein segments associated with a bare silicon implant developed a small thrombus (<5% of cross-sectional area). None of the four vein segments implanted with PEG-coated silicon developed thrombi (Table 1; Figure 2, A and B). All three vein segments associated with the stainless steel controls developed moderate (25–50% of cross-sectional

area) thrombi (Table 1, Figure 2C). The two vein segments implanted with the Gore-Tex controls also developed moderate thrombi (Table 1, Figure 2D).

There was no inflammation seen around any of the four vein segments associated with the bare silicon implants (Figure 2A). A mild perivascular inflammatory response was noted around each of the four vein segments associated with the PEG-coated silicon implants (Table 1; Figure 2B). By contrast, all three of the veins implanted with the stainless steel controls developed moderate perivascular inflammation, and each of the two veins associated with the Gore-Tex controls developed marked inflammation (Table 1; Figure 2, C and D). The inflammatory response was focal - concentrated in the soft tissue immediately surrounding the vein wall near the point of implant entry. Examination revealed a recruitment of macrophages and lymphocytes to the soft tissues surrounding the control materials.

Scanning Electron Microscopy

Surface analysis of the silicon implants by SEM revealed that the bare silicon material appeared to have significant adherent thrombi on the smooth surfaces of the intravascular portions of the implants (Figure 3, panels A,C), characterized by red blood cells, platelets, and fibrous debris. In contrast, PEG-treated silicon implants showed occasional adherent platelets and red blood cells, but no evidence of organizing platelet-red cell-fibrin thrombi (Figure 3, panels B,D). We did not perform specific tests on explanted darts beyond gross inspection and SEM examination to investigate changes in physico-chemical properties. There was no observable degradation in the thickness of the explanted silicon darts. The 28-day stability of the PEG coating was excellent as evidenced by the absence of adherent thrombi, and presence of only a few platelet and red blood cells on the darts.

Similarly, silicon implants that had migrated outside of the vessels, (and extravascular segments of those that remained in place), had adherent material (not shown).

Discussion

In the design of an implantable bioartificial kidney, silicon nanoporous hemofilters are desirable due to their narrow pore size range and enhanced membrane selectivity and permeability.²⁴ However, thrombosis continues to plague conventional hemodialysis patients, and will threaten any implantable hemofilter. A significant disadvantage of silicon is its potential to initiate the clotting cascade.

In this study, silicon showed excellent blood and soft tissue biocompatibility. Only one small thrombus was witnessed within a vein associated with a bare silicon implant. It is possible that without PEG coating, the bare silicon induced the thrombus. A confounding variable is the minor intimal damage caused during implantation, an inherent limitation of the transluminal model. PEG is well known for its properties of low protein adsorption and cell adhesion. The ordered arrangement of water molecules surrounding each PEG chain provides a hydrated shell that limits protein adsorption to the silicon surface, and thus, recognition by platelets.

Electron microscopic surface analysis revealed that PEG surface modification significantly reduced the degree of thrombosis on the intraluminal silicon surfaces. This finding supports the theory that the thrombus seen in the vein associated with the bare silicon implant developed by extension from the surface of the untreated silicon. In contrast, moderate thrombi were seen in every vein in which a control (Gore-Tex suture and stainless steel) was implanted. The results of this study provide reasonable evidence that silicon, as a structural material for nanoporous membranes in an implantable hemofilter, is less thrombogenic than other common vascularly-implanted materials.

Induction of inflammation is another potential undesirable effect of an implant material, and can lead to the eventual failure or need for explantation of an implanted device. Other studies have demonstrated the biocompatibility of silicon for use in implantable medical devices.^{25–28} It was demonstrated that repeated electrical activation of an *in vivo* MEMS drug delivery device did not induce a significant inflammatory response.²⁹ Furthermore, leukocytes were rarely present with long-term, *in vivo* silicon implants.³⁰ In the current study, both bare silicon and PEG-coated implants induced less perivascular tissue inflammation than did either of the control materials. A marked inflammatory reaction was observed in the tissues surrounding Gore-Tex sutures, and a moderate response was seen in the perivascular tissues around stainless steel controls.

The very mild pro-inflammatory effect of PEG-coated darts is likely due to free PEG moieties, which resulted from the hydrolysis of the silane linkers attached to the silicon surface. The *in vivo* model used in this study allowed us to collect simultaneous data on the interactions of implants with blood, vein wall, and perivascular soft tissues. A key advantage of this model is that the blood contact characteristics of any implant material of this relative size can be assessed by direct transluminal implantation, while allowing for continued vascular patency. Many conventional methods of evaluating tissue-material interactions of implants rely on *in vitro* assays to examine thrombosis and inflammation. While valuable information can be gained to provide preliminary guidance on implant material choices, the *in vitro* assays do not fully capture the interactions between the *in vivo* milieu and implant material. Traditionally, long-duration *in vivo* biocompatibility investigations require use of large animals and placement of the implant materials in customized packages. In contrast, the method presented in the paper is generic to materials that can be machined into a thin slivers or dart shapes and implemented in rodents. The insertion of the material through the vascular wall and flowing blood allows simultaneous testing of material-tissue interactions at two sites. Therefore, our method serves as a relatively low-cost intermediate step between *in vitro* assays and large animal biocompatibility investigations. A technical challenge of this study was that a number of samples migrated out of their original position through the vein lumens into the subcutaneous tissues. Neither the Gore-Tex nor the stainless steel controls had been fixed to surrounding tissues upon placement through the veins. Technical refinements, such as tying the ends of the suture together, and bending the needles into a circular configuration should be considered in future studies using this transluminal model.

Conclusion

Bare and PEG-coated silicon implants provoked minimal thrombosis and no tissue inflammation compared to control materials commonly used in vascular applications. Additionally, the PEG coating provided resistance to biofouling of the silicon surfaces in contact with active blood flow. The transluminal model presented herein proved to be a useful tool to evaluate the biocompatibility and tissue-material interactions of implantable materials. These results are promising evidence for the use of silicon nanopore membranes in the development of an implantable hemofilter. The eventual realization of a bioartificial kidney could lead to a bridge-to-transplant or destination therapy for end-stage renal disease.

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Figure 1.

Appearance of implants upon implantation (A–C) and explantation after 4 weeks *in vivo* (D–F). A, D: Bare silicon “dart”. B,E: Gore-Tex suture control. C,F: Stainless steel control.

Figure 2.

Hematoxylin and eosin stained 10× sections of vessels after explantation. Sections were taken through areas of implant presence; implants removed. A: Bare silicon implant – no thrombus or perivascular inflammation. B: PEG-coated silicon implant–no thrombus; mild inflammation (arrows). C: Stainless steel needle – moderate intraluminal thrombus (thick arrow) and moderate inflammation (thin arrows). D: Gore-Tex suture – moderate intraluminal thrombus (arrow) and marked inflammation (oval).

Figure 3. Scanning electron micrograph images of the intravascular portions of bare silicon implant surfaces (A,C) and PEG-coated implant surfaces (B,D), following four-week implantation.

Table 1

Histologic results of light microscopy. Thrombosis: “–” none, “+” small (<25% of vein lumen), “++” moderate (25–50% of vein lumen), “+++” marked (50–100% of vein lumen). Inflammation: “–” none, “+” mild, “++” moderate, “+++” marked.

	Thrombosis	Inflammation
Bare Silicon	+	–
PEG-Coated Silicon	–	+
Gore-Tex Control	++	+++
Stainless Steel Control	++	++