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Reduced foreign body response at nitric oxide-releasing subcutaneous implants

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Abstract

The tissue response to nitric oxide (NO)-releasing subcutaneous implants is presented. Model implants were created by coating silicone elastomer with diazeniumdiolate-modified xerogel polymers capable of releasing NO. The host tissue response to such implants was evaluated at 1, 3, and 6 weeks and compared to that of uncoated silicone elastomer blanks and xerogel-coated controls incapable of releasing NO. Delivery of NO (~375 pmol/cm^2 of implant surface area) reduced the foreign body collagen capsule ("scar tissue") thickness by >50% compared to uncoated silicone elastomer after 3 weeks. The chronic inflammatory response at the tissue/implant interface was also reduced by >30% at NO-releasing implants after 3 and 6 weeks. Additionally, CD-31 immunohistochemical staining revealed ~77% more blood vessels in proximity to NO-releasing implants after 1 week compared to controls. These findings suggest that conferring NO release to subcutaneous implants may promote effective device integration into healthy vascularized tissue, diminish foreign body capsule formation, and improve the performance of indwelling medical devices that require constant mass transport of analytes (e.g., implantable sensors).

Keywords

Foreign body response; Angiogenesis; Inflammatory response; Nitric oxide; Xerogel; Wound healing

1. Introduction

While a great deal of research has focused on the design of more biocompatible tissue-based sensors, their utility and function continue to be impaired by the body's response to foreign materials [1–4]. The foreign body response is a physiological cascade triggered upon implantation that begins with protein adsorption to the implant surface and the recruitment of inflammatory cells [5,6]. Neutrophils modulate the host response initially (minutes to hours) whereas macrophages respond over a longer period (days) [6,7]. While macrophages efficiently rid the wound site of microscopic matter such as bacteria and dead cells, they are unable to digest macroscopic implants, leading to chronic inflammation and macrophage fusion into foreign body giant cells (FBGC) that can perpetually remain at the tissue/implant interface

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[6,7]. Still unsuccessful in their attempt to digest the implant, FBGC secrete cytokines that trigger fibroblasts to deposit a dense avascular layer of collagen (termed a 'capsule') around the implant to permanently sequester it from the surrounding tissue [5,6].

All aspects of the foreign body response conspire to impede the performance of implanted sensors. For example, macrophages and other inflammatory cells recruited to the implant are known to consume oxygen and glucose and produce reactive oxygen species, all of which may influence sensor response [1]. Both adsorbed proteins and the hypovascular collagen capsule isolate the implant and act as substantial barriers to analyte diffusion from blood capillaries to the sensor. To circumvent such problems, a great deal of research has focused on mitigating the body's response to foreign materials. Strategies to reduce the inflammatory response include altering the microarchitecture of the implant surface [8–10] and applying compounds such as osteopontin that are known to inhibit FBGC formation [11]. Likewise, administering pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF) [12,13] and inhibitors of type I collagen synthesis such as halofuginone [14] have been proposed as methods to increase angiogenesis and diminish capsule formation, respectively. While several treatments have shown promise at addressing certain facets of the foreign body response, few approaches deal collectively with the entire cascade.

Recently, polymers that slowly release the biological mediator nitric oxide (NO), an endogenously-produced free radical, have proven useful in the design of more biocompatible sensors [3,15,16]. For example, NO-releasing polymer coatings have been shown to drastically improve the function of intravascular gas sensors [17] and prevent implant-associated infection in vivo [18] by reducing both platelet [19] and bacterial [20] adhesion to surfaces. Nitric oxide also plays a vital role in multiple processes of the wound healing cascade [21], and promotes angiogenesis by modulating VEGF production [22,23]. Amadeu et al. reported that exogenous application of a NO donor to a wound site decreased recruitment of inflammatory cells and accelerated re-epithelialization [24]. In related work, Gifford et al. reported that the inflammatory response to implanted NO-releasing sensors was reduced compared controls [25]. Nitric oxide also plays a critical role in collagen deposition by fibroblasts [26], and studies have demonstrated that collagen synthesis at a wound site was decreased in a dose-dependent manner with the application of an exogenous NO donor [27]. Taken together, these studies suggest that NO administration at the site of a subcutaneous implant may limit the foreign body response by promoting angiogenesis, diminishing the inflammatory response, and reducing collagen capsule formation.

Herein, we report the effect of NO on the foreign body response to subcutaneous implants in a rat model. Nitric oxide release was conferred to medical-grade silicone elastomer implants via coating with a well-characterized NO-releasing xerogel polymer [18,28]. Capsule formation, angiogenesis, and the inflammatory response were monitored at 1, 3, and 6 weeks via histological examination of explanted tissue samples. The results indicate that NO release is an attractive strategy to promote wound healing and improve the tissue integration properties of subcutaneous implants.

2. Methods and materials

N-(6-aminohexyl)aminopropyltrimethoxysilane (AHAP3) and isobutyltrimethoxysilane (BTMOS) were purchased from Gelest (Morrisville, PA) and stored under nitrogen. Ethanol (absolute) and hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA) and used as received. Distilled water was purified with a Millipore Milli-Q Gradient A-10 water purification system (Bedford, MA) to a final resistivity of 18.2 MΩ·cm and a total organic content of <6 ppb. Nitric oxide and argon were purchased from National Welders (Raleigh,

NC). Class VI medical-grade silicone elastomer was purchased from McMaster-Carr (Atlanta, GA).

2.1. NO-releasing xerogel-coated implants

Nitric oxide-releasing xerogel coatings were applied to medical-grade silicone elastomer as described by Nablo et al. [18]. Briefly, xerogel solutions were prepared by mixing ethanol (1.2 mL), water (640 μL), and 0.5 M HCl (110 μL) followed by dropwise addition of BTMOS (1.28 mL). The solution was mixed for 18 h followed by addition of AHAP3 (860 μL) and additional mixing for 30 h. Class VI medical grade silicone elastomer was cut into square sections 8 × 8 × 2 mm^3 and cleaned by sonicating in ethanol, water, and ethanol again for 30 min each. The silicone squares were sterilized in a steam autoclave at 121 °C for 25 min, and then coated with sol via a dip-coating procedure. The initial sol coating was allowed to solidify into a xerogel prior to the application of a second coating of sol. To ensure even coating, the squares were spun at ~1 rev/s for 3 d while drying. The xerogel-coated silicone squares (i.e., implants) were then placed in a 55 °C oven for 1 d followed by storage in a desiccator.

Half of the xerogel-coated implants were modified with diazeniumdiolate NO-donors, while the others were left unmodified to serve as controls. To facilitate diazeniumdiolate synthesis, xerogel-coated implants were placed in an in-house NO reaction vessel that was subsequently sealed and flushed with Ar to remove atmospheric O_2. The vessel was then pressurized to 5 atm NO for 2.5 d, and then flushed copiously with Ar [18]. The diazeniumdiolate-modified implants were removed and stored at −20 °C until use.

2.2. Nitric oxide release measurements

Nitric oxide release from implants coated with diazeniumdiolate-modified xerogel was monitored with a Sievers 280 chemiluminescent NO analyzer (Boulder, CO). The instrument was calibrated with an atmospheric sample that had been passed through a NO zero filter and a 24.1 ppm NO gas standard (balance N_2). Xerogel-coated implants were immersed in deoxygenated phosphate buffered saline (PBS; 10 mM, pH 7.4) at 37 °C. The NO released was carried from the buffer to the analyzer by a stream of N_2 bubbled into the solution at a flow rate of 80 mL/min. In the instrument, NO was detected via its formation of a chemiluminescent byproduct upon reaction with ozone. Discrete NO-release measurements were taken over 6 weeks. Between measurements, implants were stored in sealed vials of PBS at 37 °C.

2.3. In vivo studies to examine the foreign body response

The effect of NO release on the foreign body response was evaluated in adult male Sprague-Dawley rats (250 – 300 g; Charles River Laboratories, Raleigh, NC). The animal protocol was approved by the Institutional Animal Care and Use Committee at Duke University. Prior to implantation, all implants were sterilized by exposure to germicidal UV light in a sterile biosafety cabinet [18]. After sterilization, all implants were stored in sterile petri dishes on dry ice.

Rats were anesthetized with 2.5% isoflurane (v/v in O_2), administered a subcutaneous injection of flunixin (2.5 mg/kg), and their backs were shaved. Betadine was applied to the shaved region and the rats were placed on a heating pad in a sterile operating field. Six transverse 1 cm incisions were made approximately 1 cm from the dorsal midline along both sides of the animal. Using blunt dissection, subcutaneous pockets were created at the site of each incision. Each subcutaneous pocket received one of the following implants: NO-releasing xerogel-coated silicone, xerogel-coated silicone not capable of NO release (control), or uncoated bare silicone (blank). Each rat received two of each type of implant, and position was controlled for with 72 implants over 12 total rats. The wounds were closed with non-absorbable sutures and cleaned with hydrogen peroxide. Care was taken to ensure that each rat recovered, flunixin was

administered every 12 h for 2 d (2.5 mg/kg), and each rat was given ad libitum access to food and water.

At 1, 3, and 6 weeks, 4 of the rats were anesthetized with 2.5% isoflurane (v/v in O_2) and shaved, and the implants were removed with surrounding tissue. Tissue samples were placed in 10% buffered formalin for 24 h, embedded in paraffin, and sectioned into 5 μm-thick slices. Tissue samples for histological analysis were stained with Gomori's trichrome, hematoxylin & eosin (H&E), and CD-31 immunohistochemical stain. Images of the trichrome and H&E samples were collected using 10× and 20× objectives with an Olympus optical microscope (Melville, NY) equipped with a SPOT RT KE Slider digital color camera (Diagnostic Instruments; Sterling Heights, MI). Tissue samples treated with the CD-31 immunohistochemical stain were examined with a Zeiss Axiovert 200 inverted microscope equipped with a Syto 9 filter set. Images were captured with a 20× objective with a Zeiss Axiocam digital camera (Chester, VA).

2.4. Histological evaluation and data analysis

Capsule thickness data were obtained from trichrome-stained tissue samples. The foreign body capsule was defined as the region of dense collagen oriented parallel to the implant. Regardless of implant type, characteristic foreign body capsules were not observed at 1 week. Tissue samples collected at 3 and 6 weeks exhibited developed capsules in contrast to the loosely-deposited randomly-oriented collagen farther away from the implant. Capsule thickness was determined by direct comparison with the scale bar on each image. For all implant types, 3 images were analyzed from each of 4 rats with 3 capsule thickness measurements per image (36 total measurements). Collagen density was calculated by applying a digital threshold with Photoshop (Adobe Software; San Jose, CA) to images of tissue in regions within 100 μm or 200 μm of the implant surface. The threshold was applied such that all pixels resulting from tissue were converted to black pixels to allow differentiation from the white background. The number of black pixels was then normalized to the total number of pixels in the image and reported as the collagen density index (CDI). At each time point, the CDI was calculated from 3 images analyzed from each of 4 rats (12 total measurements). The number of blood vessels in proximity to each implant was determined by capturing images of the CD-31-stained samples at pre-determined locations around each implant. Each image was situated such that tissue/implant interface comprised one edge of the field and the tissue section imaged extended one visual field (~330 μm) into the tissue away from the implant. A blinded observer counted the number of CD-31-stained blood vessels per field for each image. The average number of blood vessels was calculated from 6 images collected from 4 rats at each time point (24 total measurements per implant type per time point). The inflammatory response was analyzed from the H&E-stained tissue samples. Images were cropped to display tissue within 50 μm of the implant surface. By applying a digital filter with Photoshop, pixels corresponding to the nuclei of inflammatory cells were selected based on their unique purple color imparted by the H&E stain. The number of pixels corresponding to inflammatory cells was then digitally counted and normalized to the total number of pixels in the image, and reported as the inflammatory response factor (IRF). Average IRF values were calculated from a minimum of 16 images taken from 4 rats. Data are expressed as mean values ± standard error of the mean, and were analyzed for significance ($p < 0.05$) with a nonparametric Kruskal-Wallis *H*-test [29].

3. Results and discussion

3.1. Nitric oxide releasing xerogel coatings

Diazeniumdiolate-modified xerogels have been studied previously as coatings to reduce both platelet and bacterial adhesion via NO release [28]. Herein, the tissue and wound healing properties of NO-releasing xerogel coatings were evaluated in a subcutaneous implant rat

model. Nitric oxide release from an optimized 40% AHAP3 (v/v balance BTMOS) xerogel coating is shown in Figure 1. In total, approximately 375 pmol of NO was delivered per cm^2 of surface area from the xerogel-coated silicone implants. The release of NO from diazeniumdiolate-modified xerogels is initiated upon immersion in aqueous solution and continues as water diffuses deeper within the xerogel matrix to further decompose diazeniumdiolates [28]. Notably, ~50% of the total NO was released within the first 5 h for the materials used in these studies and >99% of the release was complete after 72 h. Small fluxes of NO (< 1 pmol cm^{-2} s^{-1}) were detectable up to 1 week (data not shown), after which no signal was observed above the baseline response of the chemiluminescent NO analyzer. This NO release profile is similar to that of other controlled release systems including polymers designed to elute antibiotics [30] or therapeutic antibodies [31], where the majority of the active compound is released within several hours of immersion in aqueous solution. The flux of NO from diazeniumdiolate-modified xerogels is tunable based on the amount of aminosilane NO-donor precursor (i.e., AHAP3) used to prepare the coatings [28]. While it may be possible to achieve higher NO fluxes and longer sustained release by further increasing the volume percentage of AHAP3, stability testing has shown that inclusion of aminosilanes at concentrations greater than 40% (v/v with alkylsilane) results in poor material stability [28].

3.2. Effect of NO release on foreign body capsule formation and collagen deposition

The decrease in sensitivity of subcutaneous sensors has been attributed to both biofouling (i.e., surface-adsorbed proteins) and tissue encapsulation [4,32]. While both responses (i.e., biofouling and encapsulation) are detrimental to sensor performance, the primary impediment to analyte transport through implanted sensor membranes has been shown to be due to tissue and not the adsorbed biofouling layer [32]. Indeed, tissue effects were found to contribute 3–5 times more resistance to analyte transport than protein biofouling. Wisniewski and coworkers suggest that efforts to develop more biocompatible sensor membranes should focus on altering the tissue response as opposed to protein biofouling [32]. In light of these findings, we examined both capsule thickness and collagen density over a period of 6 weeks at NO-releasing, control, and blank (uncoated silicone elastomer) implants. The foreign body capsule was identified by its characteristic densely-packed collagen oriented parallel to the implant surface (Figure 2). Since capsule formation generally begins 2–3 weeks after implantation [6], it was not observed for any implants until the 3 week time point. Qualitatively, the capsules surrounding NO-releasing implants (Fig. 2C) appeared thinner than those surrounding blank and control implants (Fig. 2A, B). These observations were confirmed by calculating the average capsule thickness from 12 images taken across 4 rats for each implant type at each time point. As shown in Figure 3, capsule thickness at NO-releasing implants was significantly less ($p < 0.05$) than at xerogel-coated controls and bare silicone elastomer implants at both 3 and 6 weeks. The reduced capsule thickness observed between xerogel-coated controls (i.e., xerogel-coated implants not capable of NO release) and NO-releasing samples indicates that the decrease in capsule thickness is attributable to NO release. The reason that capsule thickness at xerogel-coated controls was reduced compared to bare silicone elastomer implants is not entirely understood. However, it is likely due to differences in surface chemistry such as charge and hydrophobicity. Previous studies have shown that protein adsorption is greatly influenced by surface properties [33,34], and that initial protein adsorption to an implanted biomaterial may partially dictate the ensuing foreign body response [5,6]. Thus, altered protein adsorption may account for the observed reduction in capsule thickness between xerogel-coated controls and bare silicone elastomer implants. Previous work from our laboratory has shown reduced bacterial and platelet adhesion at control xerogel surfaces [28].

Figure 3 shows that the capsule thickness at bare silicone elastomer implants did not change significantly between 3 and 6 weeks (67 ± 3 μm vs. 63 ± 4 μm, respectively). As such, capsule formation at uncoated implants was essentially complete after 3 weeks. In contrast, the capsules

at xerogel-coated control and NO-releasing implants continued to develop between 3 and 6 weeks, indicating that capsule formation at those implants was delayed compared to bare silicone elastomer implants. Future experiments will be conducted to determine if the decrease in capsule thickness at NO-releasing implants is temporary (i.e., observed only at time points ≤ 6 weeks) or if short-term NO-release permanently reduces capsule thickness.

In addition to directly measuring capsule thickness, the density of collagen near the implant surfaces was also evaluated. To obtain quantitative data, a method of digital thresholding was used to differentiate tissue from the image background. The number of pixels resulting from tissue (versus background) was normalized to the total number of pixels in the image. Since the primary tissue component within the analysis area was collagen, the resulting quotient was reported as the "collagen density index" (CDI), with a CDI value of 100 indicating that every pixel within the selected image area represented tissue. As shown in Figure 4, NO-releasing implants were characterized by significantly reduced collagen density at distances up to both 100 and 200 μm from the implant surface at 1 week compared to bare silicone elastomer and xerogel-coated control implants. The CDI within 100 μm remained significantly lower compared to bare silicone elastomer implants at both 3 and 6 weeks, due in part to the reduction in capsule thickness as noted above.

The exact mechanism by which NO-release decreases capsule thickness and collagen density at the tissue interface remains unclear. Previous work has shown that NO's modulation of cytokines involved in the wound healing response alters collagen deposition [26]. The cytokine most often associated with collagen production is transforming growth factor-β (TGF-β) [26]. TGF-β has been shown to upregulate collagen production by both human dermal fibroblasts [35] and rat mesangial cells [36]. In the same studies, application of exogenous NO donors decreased TGF-β secretion and collagen deposition [35,36]. Other studies have also shown that inhibitors of TGF-β signaling decrease collagen content in foreign body capsules in vivo [14]. Thus, NO may act directly to modulate cytokines such as TGF-β to decrease collagen deposition.

3.3. Effect of NO release on angiogenesis

Over the course of the foreign body response, an implanted biomaterial becomes sequestered within a dense foreign body capsule that is void of blood capillaries [6]. Analogous to the dense collagen layer, the lack of capillaries also presents negative consequences for tissue-based sensors, which require constant transport of analytes from the blood to ensure accurate and consistent function [1]. Sufficient vascularization is also critical for effective wound healing [37,38] and tissue regeneration [39] to sustain cellular proliferation and vitality. Thus, polymers that enhance new blood vessel formation (i.e., angiogenesis) are desirable as coatings for indwelling medical devices. Notably, NO has been shown to play a key role in angiogenesis by promoting the expression of vascular endothelial growth factor (VEGF), a potent pro-angiogenic cytokine [23]. In the present study, angiogenesis was monitored by treating explanted tissue samples with a CD-31 immunohistochemical staining technique, where blood vessels are fluorescently labeled. Representative images of tissue samples explanted after 1 week and treated with CD-31 are shown in Figure 5. Tissue adjacent to bare silicone elastomer and xerogel-coated controls (Fig. 5A, B) exhibited reduced vascularization compared to tissue adjacent to NO-releasing implants (Fig. 5C). Evaluation of 24 images per implant type per time point allowed for quantitative determination of the number of blood vessels in tissue adjacent to each implant. As shown in Figure 6, ~77% more blood vessels were observed within ~340 μm of the NO-releasing implants compared to xerogel-coated control implants after 1 week. Likewise, NO release resulted in significantly greater angiogenesis compared to controls after 3 weeks as well.

While a greater number of blood vessels were also observed at NO-releasing implants at 6 weeks compared to both control and bare silicone elastomer implants, the difference in blood vessel density was not significant due to variability across samples. Significantly enhanced angiogenesis was thus observed at early time points (i.e., 1 and 3 weeks) for NO-releasing implants but not at later periods. Such data is consistent with the temporal efficacy of other angiogenic therapies [40]. Silva and Mooney have reported that VEGF delivered as an injected bolus was quickly cleared from the site of administration and led to lower levels of angiogenesis at 6 weeks than VEGF slowly released from an implanted hydrogel [41]. The xerogel implant coatings described in our study deliver NO as a bolus (Figure 1), with the vast majority of NO released over the initial 10 h post-implantation. Such delivery stimulates angiogenesis at early time points (i.e., 1 and 3 weeks). By 6 weeks, however, the NO release is complete and the coatings no longer promote angiogenesis. Methods to deliver NO for longer periods are currently being explored in our laboratory to evaluate the effect of extended NO release on angiogenesis.

3.4. Effect of NO release on the inflammatory response

Implantation of a subcutaneous biomaterial creates a wound that inevitably triggers the host inflammatory response and recruitment of phagocytic cells such as monocytes, neutrophils, and macrophages [6]. While such a response is necessary for effective wound healing, the presence of phagocytic cells negatively impacts implanted sensors via their surface adhesion and the release of interfering species. To improve the analytical performance of such devices, it is desirable to mitigate the inflammatory response in tissue adjacent to the sensor. Gifford et al. have reported that a needle-type glucose sensor capable of releasing NO reduced the recruitment of inflammatory cells after 24 h in subcutaneous tissue [25], thereby demonstrating the feasibility of NO release as a means to control the inflammatory response. However, implanted sensors would ideally function accurately for periods beyond 24 h and we thus monitored the inflammatory response at NO-releasing subcutaneous implants over 6 weeks. Representative H&E-stained tissue samples are shown in Figure 7, with inflammatory cells clearly identifiable by the characteristic purple color imparted to their nuclei by the H&E stain. The tissue/implant interface of bare silicone elastomer and xerogel-coated control implants was characterized by an abundance of inflammatory cells. In contrast, the tissue adjacent to the NO-releasing implants exhibited a markedly lower inflammatory response as evidenced by fewer cells. To quantitatively assess the progression of the inflammatory response at each type of implant, an inflammatory response factor (IRF) was determined at each time point. Figure 8 shows that while NO-releasing implants did not significantly reduce the inflammatory response at 1 week (Fig. 8A), a significant decrease was observed at both 3 and 6 weeks compared to both bare silicone elastomer and xerogel-coated control implants (Fig. 8B and C). Indeed, the IRF at NO-releasing implants was reduced by >30% relative to bare and control implants at both 3 and 6 weeks. The IRF for each implant type did not change significantly between 3 and 6 weeks, indicating that a reduced chronic inflammatory response due to NO release may be long-lasting. Of note, the analgesic provided to the rats during the experiment (flunixin) belongs to a class of compounds known as non-steroidal anti-inflammatory drugs (NSAIDs) that derive their efficacy by modulating the inflammatory response [42,43]. All rats were treated with the same dose of flunixin throughout the experiment. As such, differences in the IRF can be attributed solely to differences in implant chemistry and not the analgesic itself. Similar experiments conducted with non-NSAID analgesics may result in different inflammatory responses to the subcutaneous implants.

A further question that remains is understanding the mechanism by which short-term (≤ 1 week) NO release modulates the chronic inflammatory response at extended periods (i.e., 3 and 6 weeks) after NO release has subsided. The fact that the inflammatory response at all three implant types was not statistically different at 1 week may be expected since it has been

suggested that the initial inflammatory response to implanted materials is primarily the result of surgical trauma and not the implant itself [10]. The significant decrease in inflammatory response at 3 and 6 weeks at NO-releasing implants may be due to NO's ability to downregulate pro-inflammatory cytokines such as macrophage chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) [26]. It has also been suggested that a reduction in the inflammatory response may be mediated by nitrosated proteins [44]. Such modified proteins may form near the implant during the early period of high NO release. The ability of NO to regulate other inflammatory modulators may translate short-term NO release into longer-term anti-inflammatory activity. Studies to determine the effect of xerogel-derived NO on cytokine regulation and protein nitrosation are currently planned. Also of interest is the fact that some inflammatory cells such as macrophages and neutrophils are capable of generating NO themselves to battle microbial infection and orchestrate wound healing [26]. Previous studies have shown that NO production by macrophages may be a self-regulating pathway [44]. The application of an exogenous NO donor to murine macrophages exerted biphasic regulation of the expression of inducible nitric oxide synthase (iNOS), the enzyme responsible for high-output NO production [45]. Likewise, it has been suggested that NO may also regulate iNOS expression in neutrophils via modulation of the cytokine IL-8 [26]. While it is clear that NO production by inflammatory cells is regulated in part by NO itself, it is not yet certain what dose of NO and timing of administration are necessary for optimal tissue integration for subcutaneous implants. Such studies are currently planned in our laboratory.

4. Conclusions

Nitric oxide-releasing polymer coatings applied to subcutaneous implants were shown to influence multiple aspects of the foreign body response in a rat model. Delivery of ~375 pmol NO per cm^2 of implant surface area resulted in a drastic reduction in both capsule thickness and collagen density at the tissue/implant interface. The NO release was also shown to increase angiogenesis and reduce the chronic inflammatory response. Decreased tissue resistance and enhanced angiogenesis may lead to improved transport of analytes to an implanted sensor, while a reduced inflammatory response would likely enhance sensor function. Thus, NO-releasing sensor membranes may represent a new paradigm for improving the analytical performance of implantable subcutaneous sensors. The potential application of NO-releasing coatings extends well beyond sensors to include other biomedical implants that would benefit from improved tissue integration. For example, drug delivery devices may function more reliably when encapsulation by dense collagen is avoided, while pain and unsightly scarring may be minimized by reducing inflammation and undesirable collagen deposition at artificial prostheses and cosmetic implants.

The mechanisms by which short-term NO release influences the longer-term course of the foreign body response are not entirely understood. It is likely that in addition to any direct physiological effects, NO's role as a key signaling molecule in the wound-healing process may facilitate its long-term effects. The initial modulation of inflammatory and wound-healing cytokines by implant-released NO may lead to the observed differences in the inflammatory and foreign body responses at 3 and 6 weeks. It is possible that in addition to directly participating in certain aspects of the foreign body response (e.g., collagen deposition), NO's influence on one facet may indirectly influence others. For example, the reduced inflammatory response observed at NO-releasing implants may play a critical role in altering capsule formation since fewer macrophages may lead to lower levels of cytokines that trigger collagen deposition. Likewise, thinner capsules may allow blood vessels to form closer to the implants, thereby leading to enhanced vascularization in tissue proximal to the implant. Studies are planned to evaluate the effect of NO on cytokine production, as well as to understand the effect of extended NO release durations on the foreign body response.

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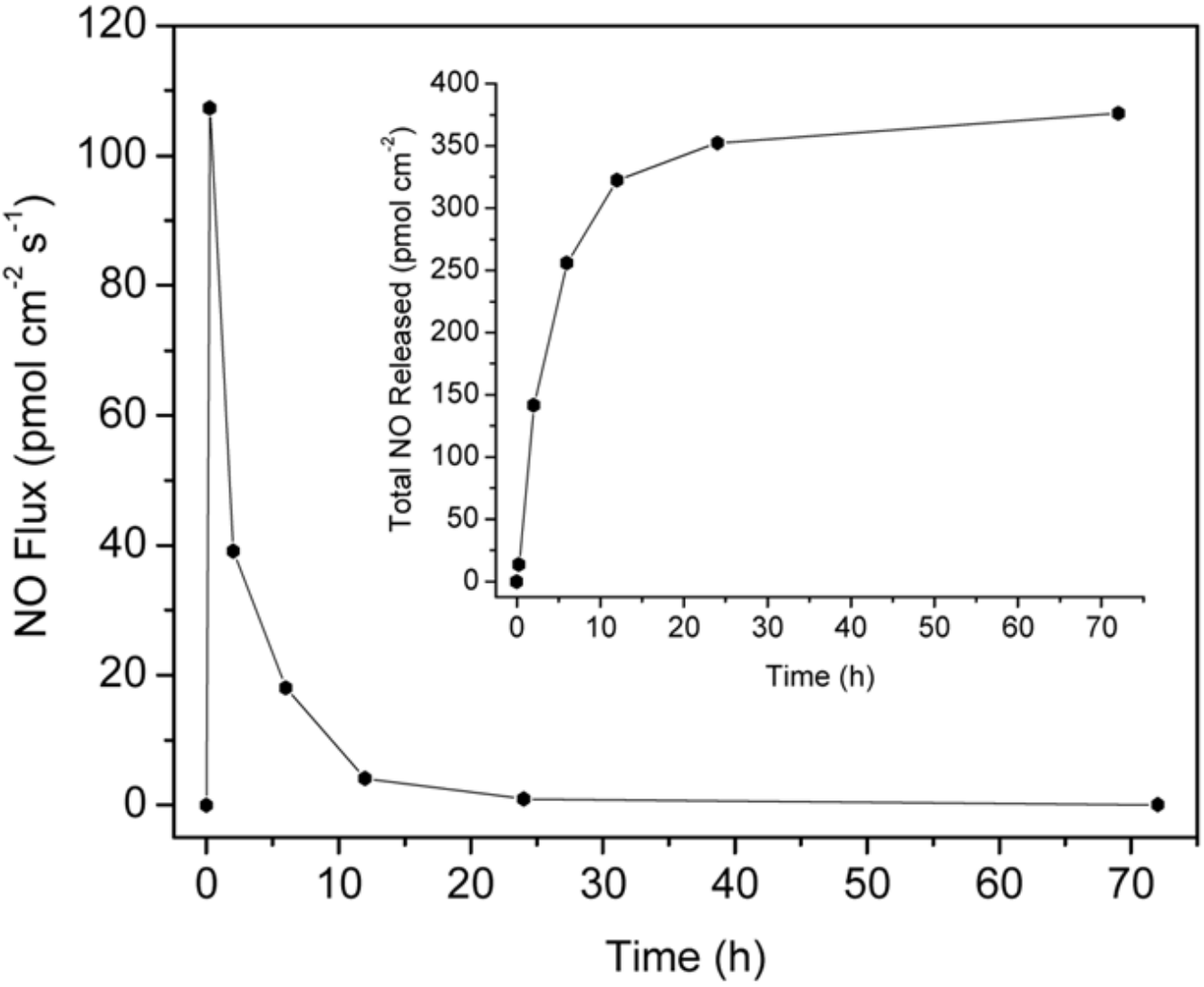

Figure 1.
Nitric oxide release from silicone elastomer implants coated with diazeniumdiolate-modified 40% AHAP3/BTMOS (v/v) xerogels. Inset: Total NO release.

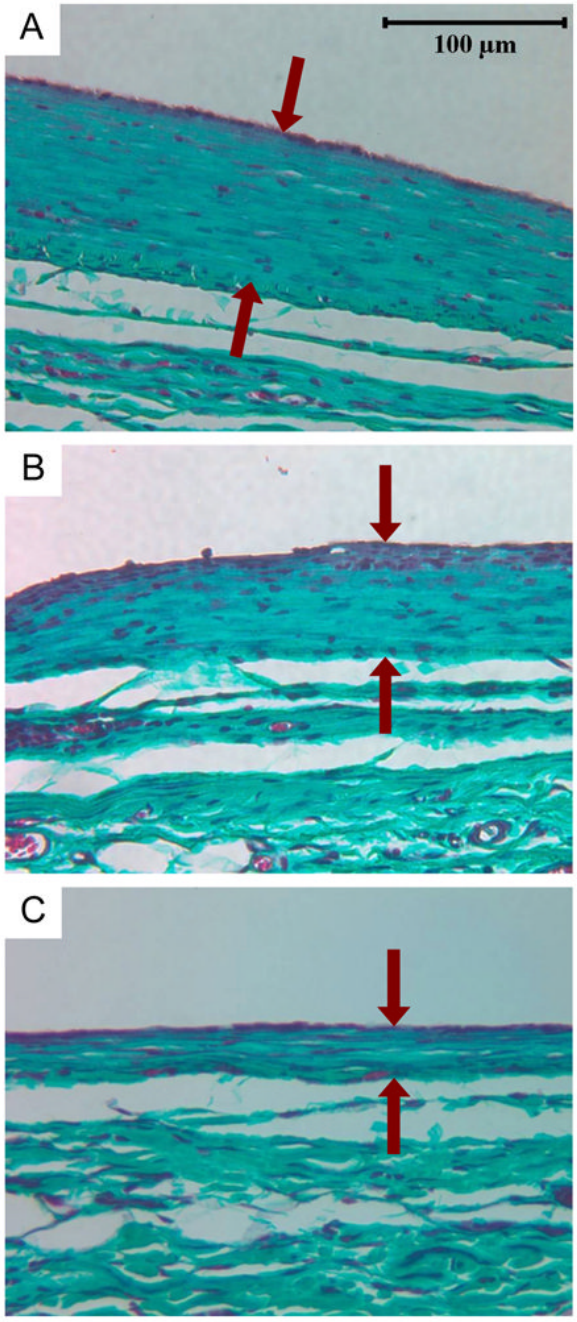

Figure 2.
Optical micrographs showing foreign body capsule formation after 6 weeks at (A) bare silicone elastomer; (B) xerogel-coated control; and, (C) NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Scale is the same in each image and arrows denote the foreign body capsule. The implant was located in the upper white region of each image.

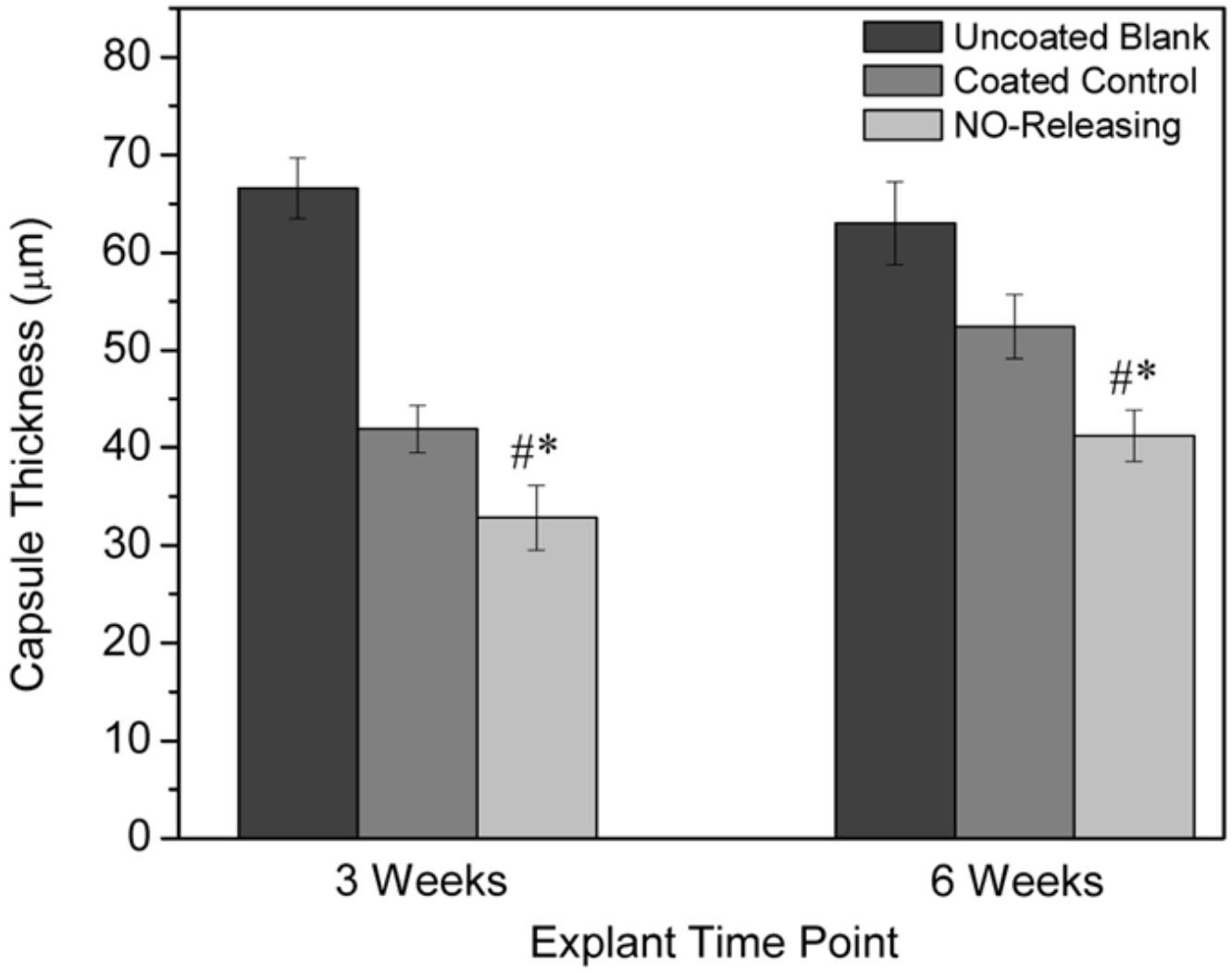

Figure 3.
Foreign body capsule thickness at uncoated blank, xerogel-coated control, and NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Significant differences ($p < 0.05$) between NO-release implants and blanks (#), and NO-release and controls (*) are indicated.

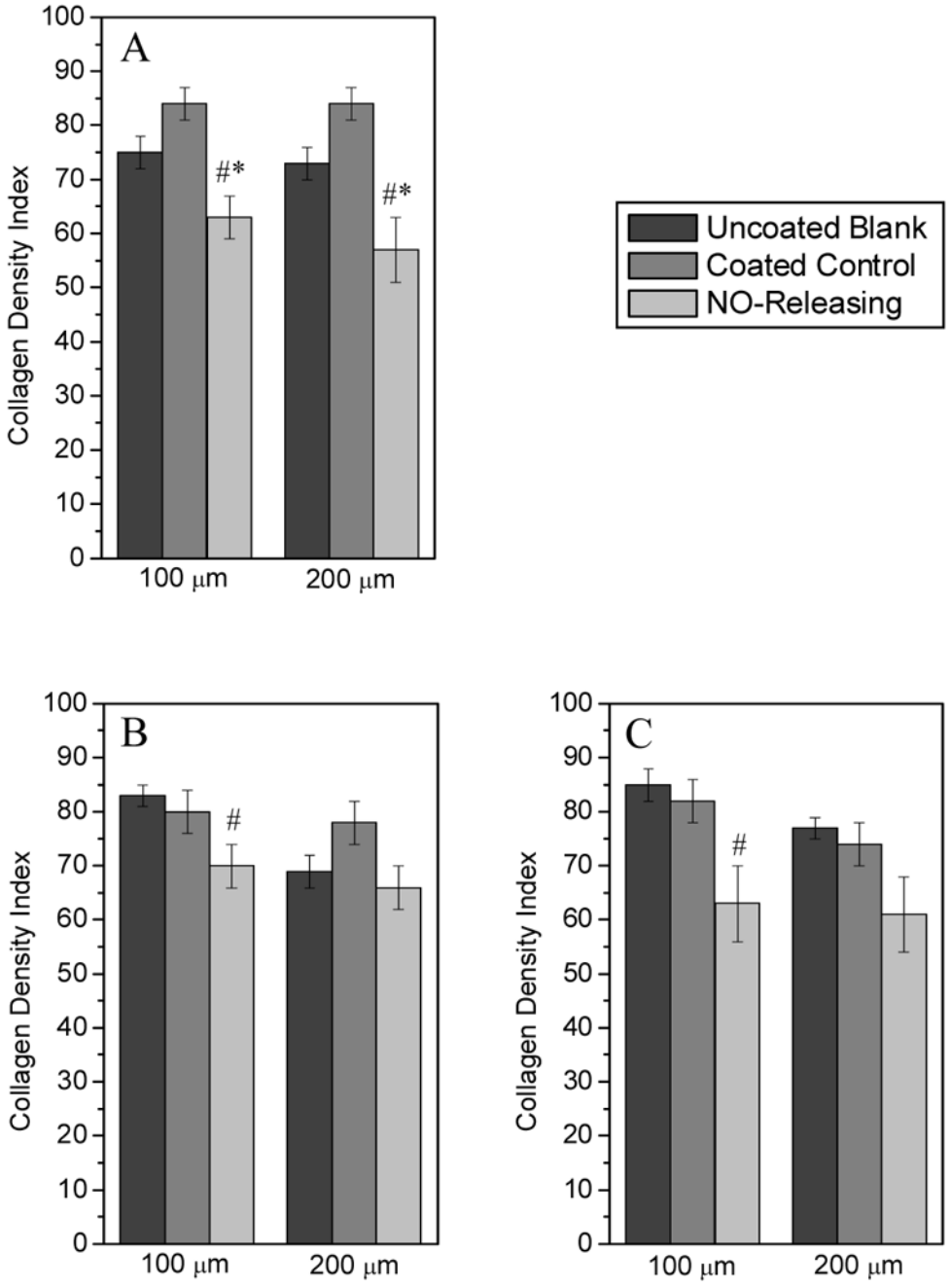

Figure 4.

Collagen density indexes observed at (A) 1 week; (B) 3 weeks; and, (C) 6 weeks at uncoated blank, xerogel-coated control, and NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Significant differences ($p < 0.05$) between NO-release implants and blanks (#), and NO-release and controls (*) are indicated.

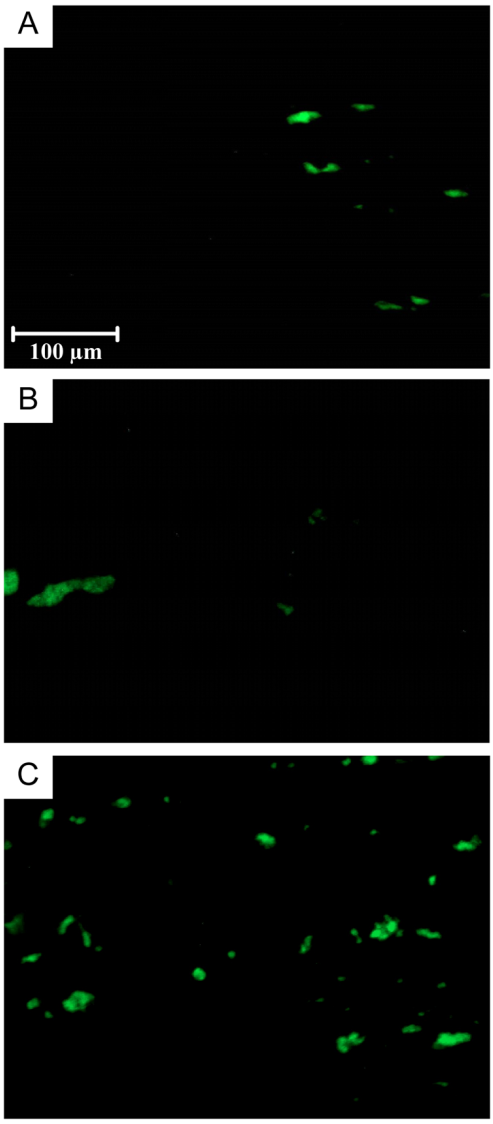

Figure 5.
Fluorescence micrographs of CD-31-stained tissue samples adjacent to (A) bare silicone elastomer; (B) xerogel-coated control; and, (C) NO-releasing xerogel-coated subcutaneous implants after 1 week. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). The green fluorescence represents blood vessel presence via positive labeling of endothelial cells with CD-31 immunohistochemical stain. Scale is the same in each image. Each image shows tissue within approximately 340 μm of the implant, which was located at the bottom of each image.

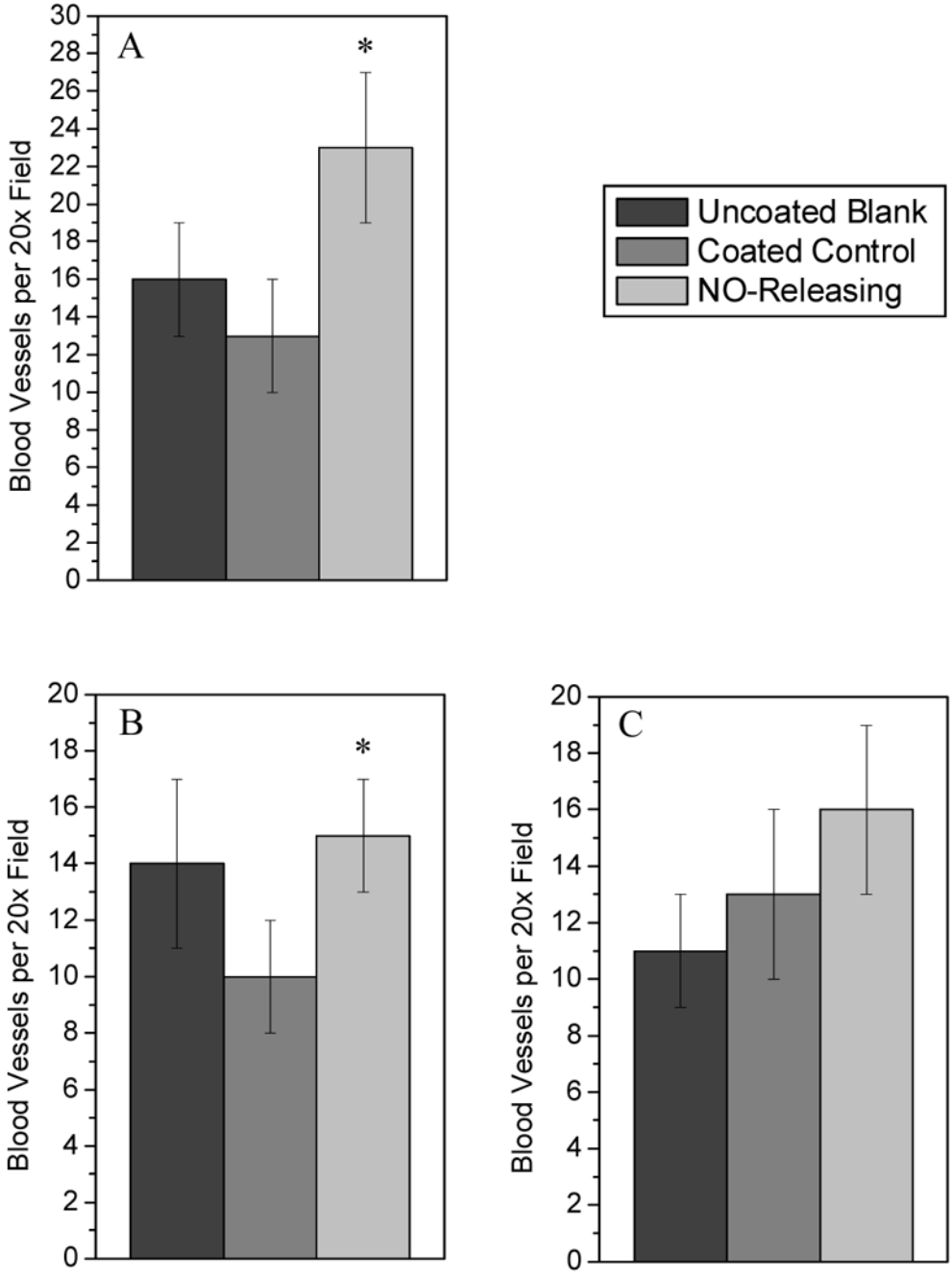

Figure 6.

Blood vessels observed per 20× field at (A) 1 week; (B) 3 weeks; and, (C) 6 weeks at uncoated blank, xerogel-coated control, and NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Significant differences ($p < 0.05$) between NO-release implants and controls (*) are indicated.

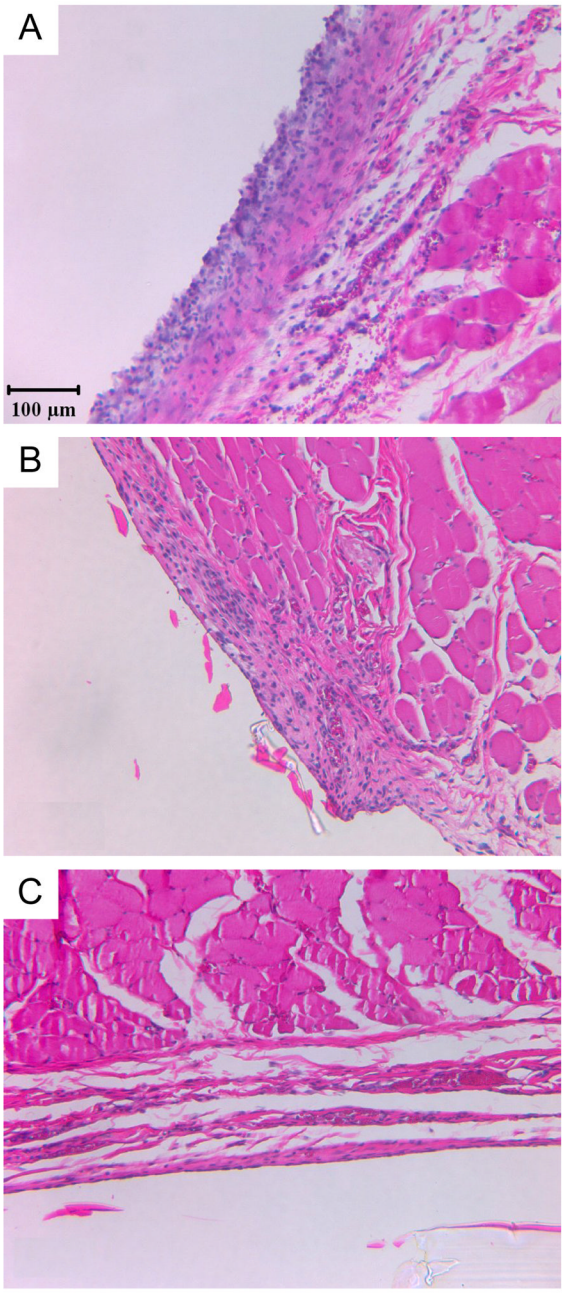

Figure 7.
Optical micrographs of hematoxylin & eosin (H&E)-stained tissue samples showing the inflammatory response after 3 weeks at (A) bare silicone elastomer; (B) xerogel-coated control; and, (C) NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Nuclei of inflammatory cells stain purple while collagen appears pink with H&E stain. Scale is the same in each image. The implants were located in the white region at the left (A, B) or bottom (C) of each image.

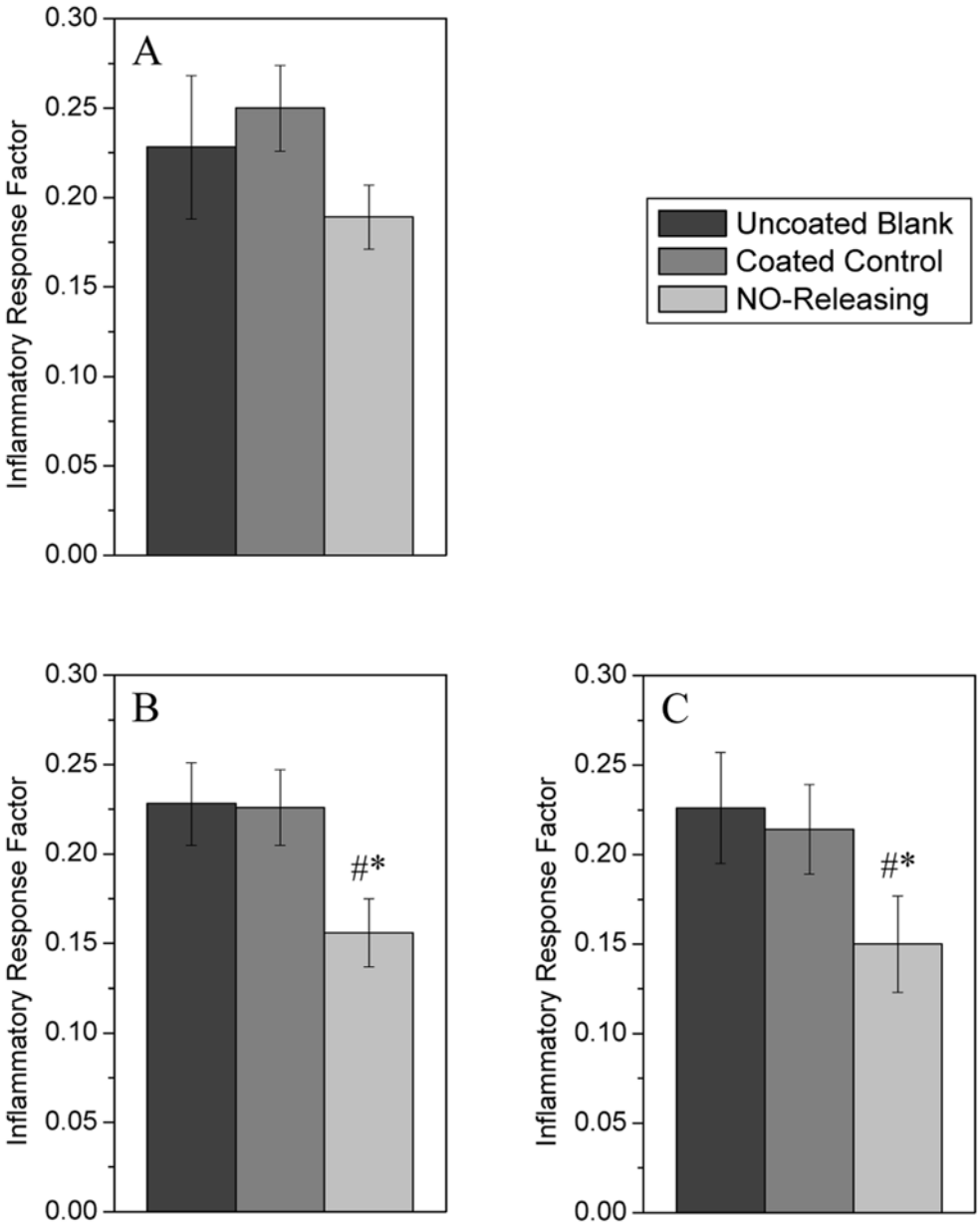

Figure 8.

Inflammatory response factors observed at (A) 1 week; (B) 3 weeks; and, (C) 6 weeks at uncoated blank, xerogel-coated control, and NO-releasing xerogel-coated subcutaneous implants. Xerogel polymer coating was 40% AHAP3/BTMOS (v/v). Significant differences ($p < 0.05$) between NO-release implants and blanks (#) and NO-release implants and controls (*) are indicated.