Copolymeric Nanofilm Platform for Controlled and Localized Therapeutic Delivery

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ABSTRACT Nanomaterials such as block copolymeric membranes provide a platform for both cellular interrogation and biological mimicry. Their biomimetic properties are based upon the innate possession of hydrophilic and hydrophobic units that enable their integration with a broad range of therapeutic materials. As such, they can be engineered for specific applications in nanomedicine, including controlled/localized drug delivery. Here we describe a method for the functionalization of the polymethyloxazoline—polydimethylsiloxane—polymethyloxazoline (PMOXA—PDMS—PMOXA) block copolymer with anti-inflammatory molecules to develop copolymer—therapeutic hybrids, effectively conferring biological functionality to a versatile synthetic nanomembrane matrix and creating a platform for an anti-inflammatory drug delivery system. Utilizing self-assembly and Langmuir–Blodgett deposition methods, we mixed copolymers with dexamethasone (Dex), an anti-inflammatory glucocorticoid receptor agonist. The successful mixing of the copolymer with the drug was confirmed by surface pressure isotherms and fluorescence microscopy. Furthermore, at 4 nm thick per layer, orders of magnitude thinner than conventional drug delivery coatings, these dexamethasone—copolymer mixtures (PolyDex) suppressed *in vitro* expression of the inflammatory cytokines/signaling elements interleukin 6 (IL-6), interleukin 12 (IL-12), tumor necrosis factor alpha (TNFα), inducible nitric oxide synthase (iNOS), and interferon gamma inducible protein (IP-10). Finally, PolyDex maintained its anti-inflammatory properties *in vivo* confirmed through punch biopsies with tissue imagery via hematoxylin/eosin and macrophage specific staining using CD11b. Thus, we demonstrated that PolyDex may be utilized as a localized, highly efficient drug—copolymer composite for active therapeutic delivery to confer anti-inflammatory protection or as a platform material for broad drug elution capabilities.

KEYWORDS: nanomedicine · drug delivery · bionanotechnology · block copolymer · inflammation · nanomaterials

Nanomaterials such as block copolymers have been shown to be effective matrices to support protein function for the mimicry of key natural biological processes such as energy conversion, as well as voltage-gated ion transport.[1–10] While conventional lipid-based systems have enabled single protein characterization and mechanisms of functionality to be elucidated, block copolymers represent a highly versatile approach toward tailored biology, whereby specific properties can be engineered into the material to accommodate specific protein geometries, desired block lengths, compositions, and charge properties. The addition of end groups, such as acrylate, can be made to enable UV or chemically induced polymeric cross-linking to enhance material stability for increased device robustness. Furthermore, copolymer-based thin film technology possesses increased robustness over conventional lipid systems in addition to architectural versatility. For example, ultraviolet light that may be harmful to lipid membranes by inducing material degradation can be used for free-radical induced polymerization/cross-linking of polymeric end groups to further increase material stability. Block copolymers, such as polymethyloxazoline—polydimethylsiloxane—polymethyloxazoline (PMOXA—PDMS—PMOXA) materials, can also possess amphiphilic properties by being composed of alternating hydrophilic and hydrophobic groups, which in turn enable air–water interface integration of therapeutic systems that possess hydrophilic or hydrophobic properties or both. This allows for subsequent membrane or drug deposition onto solid substrates bearing a spectrum of characteristics that can be integrated with either block using the Langmuir–Blodgett or self-assembly methods. This technique can also be used to deposit large area, uniform thin films to coat solid-state devices for fundamental cellular interrogation studies by serving as active substrates to study membrane-based signal transduction events and internal cytoregulatory phenomena. In a translational scenario, these nanofilms can serve as highly efficient and robust protective barriers against biological fouling events for medical implants because these noninvasive nanoscale materials can provide potent resistance against

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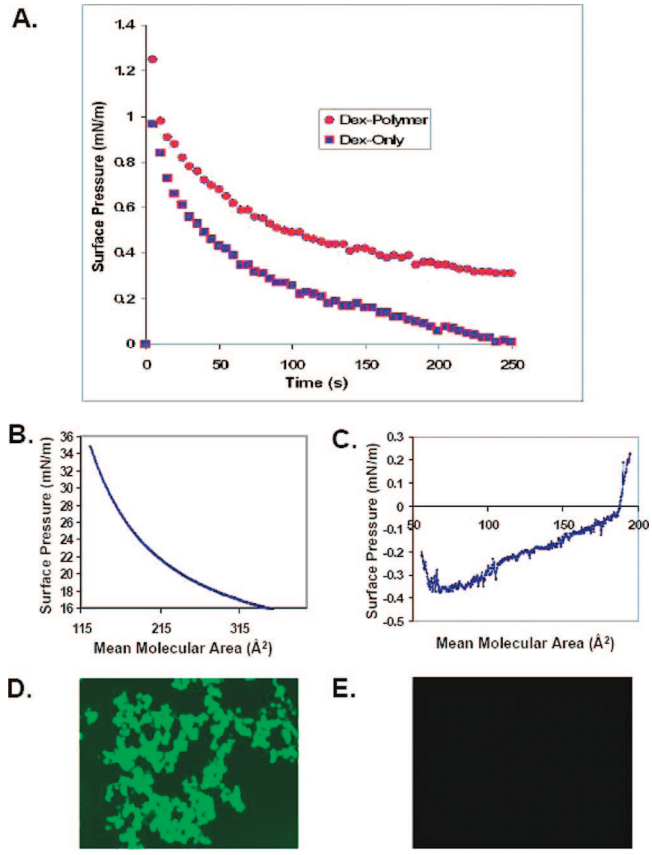

Figure 1. Langmuir film measurement of copolymer-mediated Dex tethering. (A) Observations of surface pressure changes due to the presence of Dex at the air–water interface with or without copolymer. Dex deposited by itself without the copolymer resulted in rapid integration of the Dex with the subphase. Incubation of Dex with the copolymer resulted in a sustained surface pressure. (B) The Langmuir film of codeposition of Dex and copolymer (5 mN/m) is shown with a steady increase in surface pressure and transition from the liquid to solid phase. (C) The deposition of Dex without the copolymer generated a Langmuir isotherm elucidating the preclusion of stable film formation. (D) Fluorescent microscopy of a Langmuir–Blodgett film of FITC-conjugated Dex and copolymer nanofilm is shown. (E) Interfacial preservation of FITC-Dex deposited at the air–water interface without the copolymer is fluorescently imaged with results shown.

innate immune reactions that generate tissue fibrosis and imminent device fouling.

A key element of this study is based upon the functional copolymer-mediated preservation of the tissue−device interface in both a quantitative and macroscale qualitative context. We demonstrated that the nanoscale copolymer was capable of *cloaking* the implant that it coated *in vivo* while producing no dimensional penalties on the implant itself that may be observed with thicker coatings, which can in turn generate inflammatory responses as well. With respects to examining the medical applicability of these drug−nanomaterial complexes, the question of whether the bare material itself is harmful to the body post-drug elution was also addressed. In addition to *in vivo* studies, we have expounded beyond conventional examination of cellular proliferation/morphological analysis and performed *in vitro* studies to examine the internal cellular processes that result from nanomaterial−biology interfacing. As such, this study provided insight into and confirmation of the innately compatible properties of the nonfunctionalized (no drug) copolymeric molecules via comprehensive gene expression studies and the lack of up-regulation in pro-inflammatory cytokine production.

Deposition of the amphiphilic molecules was carried out via interfacial addition of the polymer to a subphase of water. Polymeric solubilization into the subphase was then precluded because of the alternating hydrophobic−hydrophilic nature of the amphiphile. Compression of the material then enabled stable film formation for subsequent interfacial functionalization of the copolymer with the therapeutic systems. These characteristics allow for a number of versatile uses for block copolymers as a drug delivery system and generated platforms for quantitative as well as animal model studies of composite film efficacy in suppressing inflammation at the interface of the protected implant and surrounding tissue.

RESULTS AND DISCUSSION

Copolymeric Nanofilm Functionalization and Characterization. In this study, we analyzed the effectiveness of these block copolymers as a drug delivery system, focusing on the development of an anti-inflammatory drug−copolymer composite. We demonstrated the deposition of PMOXA−PDMS−PMOXA copolymers at the air–water interface followed by the interlacing of the copolymers with dexamethasone (Dex), an anti-inflammatory molecule, for subsequent composite film transfer to the substrate. Dex integration with the copolymeric amphiphiles and subsequent transfer were confirmed via interfacial monitoring of surface pressure changes and Langmuir compression isotherms associated with drug tethering via the copolymer molecules. Initially, surface pressure monitoring of Dex deposition without the copolymer revealed an expected rapid increase in surface pressure. Subsequent interfacial incubation revealed a rapid decrease to a surface pressure of 0 mN/m, which was attributed to the complete integration of the Dex into the subphase. To demonstrate copolymer-mediated Dex tethering and integration, the copolymer was deposited to a starting surface pressure of 5 mN/m, and a 10 min period was taken to enable chloroform evaporation. Following pressure zeroing, subsequent Dex deposition revealed its preservation at the air–water interface as a steady surface pressure increase of ~0.4 mN/m was observed, indicating a contribution of the copolymer in Dex tethering (Figure 1A). Langmuir surface pressure isotherms possessing steady pressure increases were indicative of orderly film compression toward the liquid/solid phase (Figure 1B). Furthermore, Langmuir surface pressure isotherms indicated an inability for film production and surface pressure increase by Dex without the co-

polymer (Figure 1C). The deposition of fluorescein isothiocyanate (FITC)-conjugated Dex/copolymer and Langmuir–Blodgett film transfer was also analyzed by fluorescent microscopy to confirm integration of Dex with the copolymer nanofilm (Figure 1D). Additionally, fluorescent microscopy analysis of the deposition of FITC-conjugated Dex without the copolymer confirmed the necessity of using the copolymer as a tethering agent to preserve the interfacial presence of Dex and its ability for subsequent film transfer (Figure 1E). As such, the initial deposition of the copolymer was shown to be an integral component of the interfacial hybrid nanofilm fabrication process.

The triblock copolymer film was fabricated to a thickness of approximately 4 nm to mimic naturally occurring biomembranes such that the thin film technology could possess the dimensions to allow for therapeutic protein elution as well. Membrane thicknesses were determined using both irreversible rupture tests of suspended membranes and Langmuir–Blodgett deposition of copolymers on gold slides, which also acted as electrodes for electrical property measurements for dimensional characterization.[11] In these studies, the biomimetic polymer film capacitance generated while suspended across a hydrophobic septum (irreversible rupture) or solid-supported membranes (deposited via Langmuir–Blodgett) was used to calculate film thickness as given by

$$C = \frac{\epsilon_0 \epsilon_1 A}{d} \quad (1)$$

where C is the membrane capacitance, ϵ_0 is the permittivity of free space (8.9×10^{-19} F/m), ϵ_1 is the relative dielectric constant of the PDMS hydrophobic block, A is the measured area of the annulus in the septum (250 μm), and d is the thickness of the membrane. To test for enhanced membrane durability over conventional lipid systems, membrane rupture tests were conducted utilizing dual Ag/AgCl electrodes on both sides of the suspended polymer membrane. Transmembrane voltages of increasing magnitude were applied to observe electric field induced irreversible rupture associated with termination of the membrane. For suspended membranes, it was observed that the UV-cross-linked polymer membranes could withstand transmembrane voltages as high as 1.5 V (un-cross-linked = ~1 V) compared with lipid systems that commonly ruptured at 500 mV, indicating an enhanced mechanical stability in the polymers membranes. In addition, Langmuir isotherms were conducted to compress the copolymer films to measure film collapse pressures. We have previously shown that the polymer collapse pressures, in excess of 70 mN/m, were significantly higher than those observed in lipid systems (~50 mN/m).[12] With both the irreversible rupture and Langmuir collapse studies, increased polymeric mechanical strength over lipids was observed, and the polymers could withstand higher Langmuir surface compression pressures. As such, the biomimetic properties and robustness of the PMOXA–PDMS–PMOXA polymer make it an ideal engineering component for therapeutic nanofilms.

***In Vitro* Characterization of PolyDex Activity.** Advances in medical device technology have led to smaller and more complex implants that provide a greater standard of living to an increasingly aging population. Inflammatory response against implants, however, remains a problem to both tolerance and maintenance of function for a variety of these implants, ranging from cardiovascular devices (e.g., coronary stents) and electrical devices (e.g., pacemakers and glucose sensors) to prostheses (e.g., hip joint replacements).[11–16] Inflammation results from the infiltration of immune cells such as neutrophils and macrophages to the tissue–implant interface as these cells attempt to repair damage that occurs following implantation.[17]

A major mediator of the inflammatory responses in these immune cells is nuclear factor kappa B (NF-κB). During implantation, NF-κB is activated by a number of receptors that recognize implant particulates associated with tissue damage, resulting in the induction of inflammatory genes that promote the recruitment and activation of other immune cells and also lead to the degradation of foreign objects.[18–20] Because of the central role of NF-κB in inflammation, NF-κB inhibitors have been utilized as anti-inflammatory therapeutics.[21] The most notable of these NF-κB inhibitors are glucocorticoid steroids such as dexamethasone (Dex), which mediate their effects through activation of the nuclear hormone receptor glucocorticoid receptor (GR). While GR mediates its repression of NF-κB through multiple potential mechanisms, recent studies have demonstrated that direct interaction of activated GR with the NF-κB subunit p65 is capable of inhibiting NF-κB activity.[22,23] By inhibiting the interaction of NF-κB and these other transcription factors, GR inhibits the induction of a number of inflammatory genes that contribute to the recruitment and activation of immune cells.

In order to determine whether the Dex–copolymer composite (PolyDex) was capable of inhibiting inflammatory gene induction, RAW 264.7 macrophage cells were cultured in the presence or absence of PolyDex and treated for 6 h with 100 ng/mL lipopolysaccharide (LPS), a component of Gram-negative bacteria that induces increased inflammatory gene expression in the macrophages. As shown in Figure 2A, PolyDex potently inhibited LPS-mediated induction of the inflammatory genes interleukin 12 p40 subunit (*Il-12p40*), interleukin 6 (*Il-6*), tumor necrosis factor alpha (*TNF-α*), interferon gamma inducible protein 10 (*IP-10*), which contributes to the recruitment and activation of immune cells, and inducible nitric oxide synthase (*iNOS*), which contributes to nitric oxide (NO)-derived oxidant mediated degradation and damage of implant material. To confirm

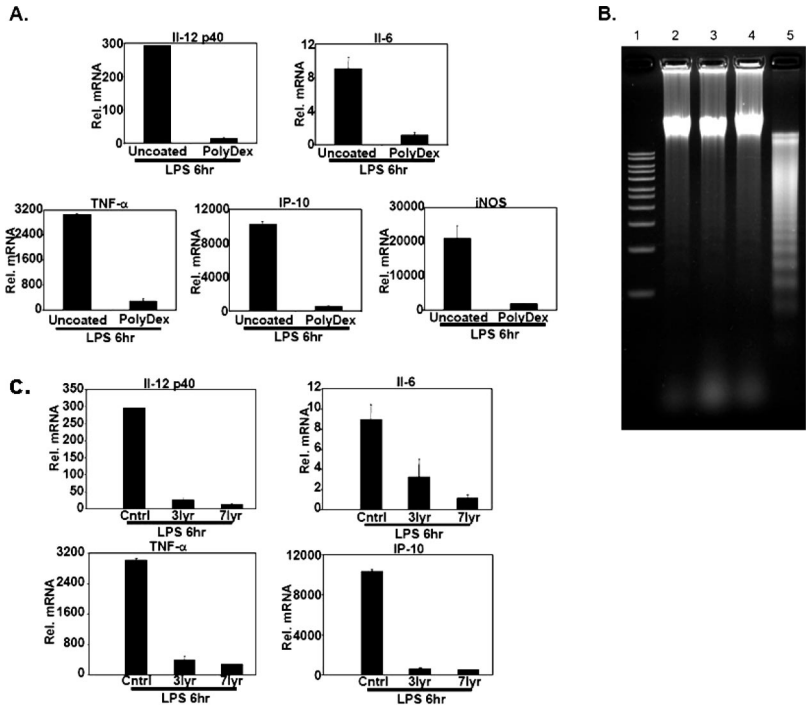

Figure 2. PolyDex coating prevents LPS induction of inflammatory genes in RAW 264.7 macrophage cells *in vitro*. (A) RAW 264.7 cells that were cultured on PolyDex-coated or uncoated glass slides were stimulated with LPS (100 ng/mL). After 6 h, RNA was isolated and analyzed by quantitative RT-PCR. (B) DNA fragmentation analysis showed that the PolyDex-mediated suppression of inflammation was due to the drug activity and not polymer-mediated cell death. Lane 1 was loaded with the DNA marker. Comparing the DNA fragmentation properties of cells cultured on glass (lane 2), polymer only (lane 3), and PolyDex (lane 4), we could see that no cell death was occurring due to the absence of DNA fragmentation. Doxorubicin, a potent cytotoxic agent that induced DNA fragmentation and cell death, was used as a control to provide a comparison and image of DNA fragmentation (lane 5). (C) RAW 264.7 cells that were cultured on films alone (Ctrl) or three layers or seven layers of PolyDex were stimulated with LPS (100 ng/mL). After 6 h, RNA was isolated and analyzed by quantitative RT-PCR.

that the inflammatory gene suppression was a result of the Dex and not material-induced cell death, studies of polymer-based activity upon inflammatory gene expression was also examined, and results demonstrated that the polymer did not induce adverse inflammatory gene effects (Supplementary Figure 1). Furthermore, cell culture/morphology studies showed that cell growth properties were not altered by the presence of the polymer in different concentrations (0.01 mg/mL and 0.1 mg/mL compared with 0 mg/mL, Supplementary Figure 2). Furthermore, DNA fragmentation assays were conducted to confirm that the neither the polymer alone nor the PolyDex induced cell death/apoptosis as shown by the absence of DNA fragmentation (Figure 2B). These conditions demonstrated that the suppression of inflammatory gene expression was due to Dex activity. Lane 1 was loaded with the DNA marker. Comparing the DNA fragmentation properties of cells cultured on glass (lane 2), polymer only (lane 3), and PolyDex (lane 4), we could see that no cell death was occurring due to the absence of DNA fragmentation. Doxorubicin, a potent cytotoxic agent that induces DNA fragmentation and cell death was used as a control to provide a comparison and example image of DNA fragmentation (lane 5). As such, the DNA fragmentation analysis showed that the PolyDex-mediated suppression of inflammation was due to the drug activity and not polymer-mediated cell death because no DNA fragmentation was caused by the polymer-only/PolyDex conditions. A key aspect of block copolymers is the ability to tailor composite film deposition to a specific number of Dex−copolymer layers, which allows for the controlled tuning of a range of drug storage and release levels. More specifically, the layer-by-layer deposition capabilities of the Langmuir–Blodgett thin film deposition modality enable the ability to determine the quantity of drug that is deposited on top of a substrate due to the number of film deposition cycles conducted. This serves as a useful feature of copolymer-mediated drug deposition as the sequential deposition or release of multiple drugs may be accomplished using this methodology. Gene expression analysis demonstrated that PolyDex was an effective anti-inflammatory agent where three layers and seven layers of Dex mixed with copolymer were deposited to demonstrate the varying degrees of inflammatory gene suppression depending on the number of deposition cycles conducted (Figure 2C). Additionally, seven layers resulted in a greater repression of Il-6 induction compared with three layers. This indicates that the inflammatory gene expression suppressing properties of PolyDex are adjustable. Thus, it appears that PolyDex allows for highly controlled release of Dex into macrophages that can be easily and rapidly tuned to result in a tailored inhibition of inflammatory gene induction.

Assessment of Innate Biocompatibility of Copolymer Matrix. Triblock copolymer solutions with concentrations of 0.01 and 0.1 mg/mL were incubated with the Raw 264.7 cell cultures. After cultures of adequate density were attained, cells were subsequently incubated with the copolymer solutions and monitored for growth at 4, 24, and 48 h. Quantitative RT-PCR analysis was performed to examine the gene expression of *TNFα*, *Il-6*, *Il-12*, and *iNOS*. Because basal levels of inflammatory gene expression were examined, no LPS was applied to the cell lines to induce inflammation. RT-PCR results examining the innate biocompatibility of the material *in vitro* showed that the cytokine mRNA levels of *Il-6*, *TNFα*, and *iNOS* were virtually unaffected, were within the normal limits of noninflamed cellular activity, and decreased slightly when compared with the controls where no polymer was added, further confirming quantitatively that cellular activity can be favorably interfaced with the biologically inert material *in vitro* (Figure 3A). To further determine the biocompatibility of

nanopolymers for potential use as a material for drug delivery in patients, the cell growth and morphology of the cells in response to incubation with nanopolymers in culture was investigated. Normal cellular morphology and growth characteristics further confirmed the biological amenability of the copolymer nanofilms.

Cellular inflammation at the genetic level was chosen as an indicator of innate material biocompatibility for several reasons including the fact that inflammatory cytokine secretion has been shown to play a role in defending the body against foreign materials that can cause the onset of adverse medical conditions but, when elevated chronically, can also generate potentially serious complications. The observation that the bare copolymer did not induce increased inflammatory cytokine expression was particularly significant in this context. For example, inflammation is instrumental physiologically for resistance to infection and for the protection of homeostatic body function from foreign bodies. However, the immune system-mediated blockage of implant functionality can become a significant hindrance when the introduction of foreign materials is beneficial to a patient, which may include transplantation or the implantation of a broad range of devices including stand-alone drug delivery systems, neural implants for Parkinson's disease, cardiac pacing leads, or stents, for example. Inflammation at the site of application leads to the recruitment of immune cells that surround the administered foreign material, thus eliminating interaction with the body itself and rendering the material nonfunctional. The generalized application of a foreign material that is not amenable toward prolonged interfacing with biological tissue can generate prolonged systemic inflammation, which when leading to imminent implant breakdown, results in complications with patient treatment regimens as well as decreased treatment efficacy. Prolonged inflammation can also cause apoptosis in various tissues, thus adding to the danger of implantation or administration of non-biocompatible materials. Some evidence also exists that prolonged inflammation may lead to tumorigenesis and the production of malignant tissues, which can vary depending on tissue type.[27] As such, the biological response to foreign materials can have serious consequences that can generate a predisposition to secondary medical disorders. An important consideration when determining the biocompatibility of the copolymeric nanofilms resides with postdrug elution conditions that may include bare material delamination or perpetual interfacing with surrounding tissue. Investigations into the cellular response following copolymeric integration provided important insight into innate nanofilm biocompatibility. As such, the observation that the copolymeric materials do not increase the expression of a range of cytokines is an indicator of their amenability at the genetic level as medically relevant coatings for implants or drug-delivery vehicles.

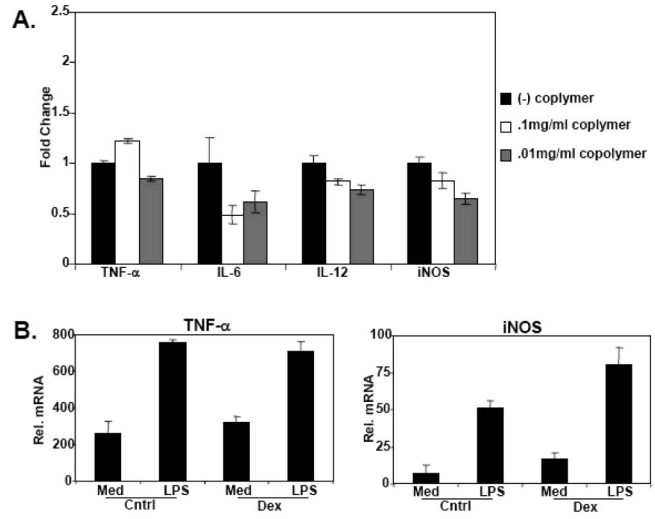

Figure 3. Both Dex and copolymer are required for the anti-inflammatory properties of PolyDex. (A) RAW 264.7 cells grown on glass slides coated with Dex alone were stimulated with LPS (100 ng/mL). After 6 h, RNA was isolated and analyzed by quantitative RT-PCR. (B) RAW 264.7 cells grown on glass slides coated with copolymer alone were stimulated with LPS (100 ng/mL). After 6 h, RNA was isolated and analyzed by quantitative RT-PCR.

To demonstrate the importance of the copolymer in maintaining drug−implant interfacing, gene expression analysis also revealed that the ability to sequester the Dex onto the implant surface as well as the resistance of the Dex to delamination following sustained washing steps required the application of the copolymer as a drug trapping/tethering element. Dex that was adsorbed to the implant surface without the copolymer was immediately delaminated as shown by the inability for implant surfaces with adsorbed Dex to suppress inflammatory cytokine expression (*TNF*α and *iNOS*) in LPS-stimulated macrophages (Figure 3B). This was expected because the copolymeric network serves as a robust matrix that is capable of supporting the embedding/integration of a broad collection of molecules and has previously been used as a robust material for the aforementioned reconstitution of membrane-based proteins. As such, copolymer-mediated Dex release as well as sustained integration with the implant surface served as the mechanism underlying the localized and sustained anti-inflammatory properties seen in PolyDex.

***In Vivo* Characterization of PolyDex Activity.** In order to determine the effect of PolyDex toward the inflammatory response against implants *in vivo*, uncoated and PolyDex-coated 5 mm diameter polyethylene disks were implanted dorsally into C57BL/6 wt mice. Following 7 days of incubation, disks and surrounding tissue were analyzed for inflammatory responses. Infiltration of immune cells to the site of implantation is a key aspect of the inflammatory response against implants that eventually leads to the degradation and disruption of functional implants due to implant encapsula-

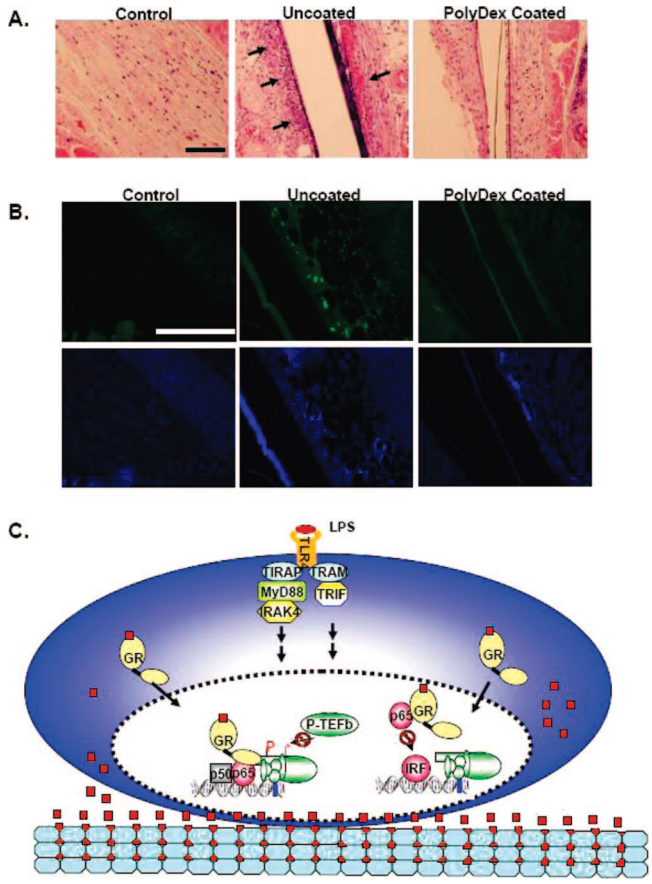

Figure 4. PolyDex coating prevents accumulation of infiltrating immune cells to disk implantation site. (A) C57BL/6 mice ($n = 6$) were subcutaneously implanted dorsally with two polyethylene disks (uncoated or PolyDex coated). After 7 days, disks and surrounding tissue were excised, formalin fixed, and hematoxylin and eosin stained. Samples were analyzed at 10× magnification. Representative images are presented. Black arrows indicate the site of cell infiltration at the interface of the dermis and disk. Black bar represents 200 μm. (B) Fixed samples were stained for CD11b (green) and 4′,6-diamidino-2-phenylindole (DAPI, blue) and analyzed at 20× magnification. Representative images are presented. White bar represents 200 μm. (C) Model of PolyDex inhibition of inflammatory response. During inflammation, such as that mediated by LPS, NF-κB is activated. With other coactivators, such as IRF3 and pTEFb, NF-κB induces inflammatory genes. PolyDex inhibits this inflammation by eluting Dex into nearby cells. Dex activates GR, which translocates to the nucleus and binds to the NF-κB subunit, p65. This association prevents NF-κB interaction with its transcriptional coactivators and inhibits NF-κB-dependent inflammatory gene induction.

tion and the preclusion of its ability to interact with surrounding tissue. As depicted in Figure 4A, hematoxylin and eosin (HE) staining demonstrated a marked increase in cell infiltration at the tissue−implant interface in uncoated samples. Furthermore, PolyDex-coated disks exhibited minimal cell infiltration and were characterized by dispersed cellular nuclei and a lack of concentrated recruitment, properties that nearly mimicked those found in tissue that did not receive an implant at the same time point, suggesting that PolyDex was capable of cloaking the implant within the mouse and was effective in alleviating an inflammatory response following implantation. Neutrophils and macrophages are the major immune cell types to infiltrate sites of implantation during an inflammatory response. In order to determine whether the infiltrating cells seen by HE staining are neutrophils and macrophages, immunofluorescent staining was done for CD11b expression in these cells. CD11b is an adhesion molecule and immune receptor that is expressed on multiple activated phagocytic immune cells, including neutrophils and macrophages.[24–26] Analysis of CD11b expression at the tissue−implant interface revealed that CD11b expression was noticeably higher with implantation of uncoated disks compared with untreated controls and PolyDex-coated disks (Figure 4B), confirming the large-scale recruitment of innate immune cells, which is indicative of an adverse physiological response toward the implanted disk. Thus, PolyDex is potentially capable of serving as a protective anti-inflammatory nanofilm coating in implants.

Functionalized nanomaterials provide an ideal platform for adjustable targeted drug delivery with minimal disruption to host biological systems. We have presented a novel application of block copolymers as a platform for the formation of a copolymer mixed with the anti-inflammatory Dex therapeutic, PolyDex, as a nanofilm protective coating for implants. This is accomplished through activation of GR and inhibition of NF-κB-dependent inflammatory genes as Dex is eluted from PolyDex into neighboring cells (Figure 4C). As highly sensitive functional implants become more common, anti-inflammatory nanofilms such as PolyDex will be needed to prevent disruption and degradation of such implants. Thus, this work provides evidence that nanomedical products can contribute to safer and more effective application of medical devices. Furthermore, this work suggests that functionalized nanomaterials are highly versatile platforms upon which a number of therapeutic approaches can be done to serve multiple medical needs.

The use of nanoscale drug−copolymer coated implants *in vivo* has great potential in medicine. The composite nanofilms that have been developed from this work would serve as ideal foundational materials for implant coatings because they are significantly thinner than most coatings employed today. This study has confirmed the innate biocompatibility of the block copolymer substrates that decrease the potential of the inflammation of cells coming into contact with the material. In addition, various antibiotic and anti-inflammatory drugs can be added to the copolymer for reduction of infection and inflammation at the site of implantation, further adding to the utility of this material. To test the efficacy of functionalizing triblock copolymer coatings of materials, the Dex anti-inflammatory drug was codeposited along with the copolymer onto implant surfaces for *in vitro* quantitative and *in vivo* trials. Continued work will harness the broad applicability of this functionalized material to expand

this investigation into suppressing the inflammation of astrocytes and other cell types, thus leading to the minimization of invasiveness of neural probes and other emerging implant technologies as well as increasing the lifespan of these devices via nanoscale medical technologies employed at the biotic−abiotic interface.

MATERIALS AND METHODS

Fabrication of PolyDex. The polymethyloxazoline−polydimethylsiloxane−polymethyloxazoline (PMOXA−PDMS−PMOXA) copolymer with acrylate end groups was custom synthesized and multiple forms of characterization including size exclusion chromatography and NMR were conducted to determine material purity and functionality (Supplementary Figures 3 and 8; Polymer Source, Quebec, Canada). The polymer materials were solubilized to a concentration of 10 mg/mL in chloroform. Solutions were applied to the surface of a Langmuir–Blodgett trough (KSV 2000, KSV Instruments, Finland) to the desired surface pressure (5 mN/m for Dex tethering), and chloroform was evaporated over a period of 10 min. Dex was prepared to a concentration of 1 mg/mL and then added in a controlled dropwise fashion (100 ng) to the air–water interface. Following 20 min of incubation and film equilibration, the hybrid membranes were compressed at a rate of 1 mm/min to a surface pressure of 25 mN/m and deposited at a rate of 1 mm/min with the desired number of layers. For Dex deposition monitoring studies, FITC-conjugated Dex was utilized for fluorescence imagery following Langmuir–Blodgett film deposition.

Quantitative RT-PCR. For quantitative real-time PCR (Q-PCR), total RNA was isolated with TRIZOL (Invitrogen) according to manufacturer's protocol. Following RNA isolation, cDNA was synthesized with iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol.[27] PCR was then performed using the iCycler thermocycler (Bio-Rad). Q-PCR was conducted in a final volume of 25 μL containing Taq polymerase, 1× Taq buffer (Stratagene), 125 μM dNTPs, SYBR Green I (Molecular Probes), and fluoroscein (Bio-Rad), using oligo-dT cDNA or random hexamer cDNA as the PCR template. Amplification conditions were 95 °C (3 min), then 40 cycles of 95 °C (20 s), 55 °C (30 s),and 72 °C (20 s). The primer sequences used are available upon request.

Animal Experiments. Age and sex matched 6–9 week old mice were used for all experiments. C57/B/6 mice were obtained from Jackson Laboratory. Uncoated or PolyDex-coated 5 mm diameter polyethylene disks were implanted dorsally into C57BL/6 Wt mice ($n = 6$). Following 7 days of incubation, mice were sacrificed. Sharp biopsy punch (8 mm diameter) was applied with a rotary motion and moderate hand pressure to the site of implantation. Plugs containing disk and surrounding tissue were gently lifted and excised with a sharp scalpel. The resulting sample was analyzed by histological analysis. All experiments were conducted within the parameters of approved protocol by the UCLA Animal Research Committee.

Histology. For HE staining, liver samples were fixed in formalin for 48 h. HE staining was performed by UCLA Tissue Procurement Core Laboratory (TPCL). For CD11b staining, tissue sections were deparaffinized with xylene and rehydrated. Trypsin epitope retrieval was done followed by washing, and sections were blocked in normal rabbit serum blocking solution. Following blocking, sections were washed and incubated for 3 h at RT with FITC-conjugated rat anti-CD11b antibody (1:50) (BD Pharmingen). Sections were washed and incubated with rabbit anti-FITC (1:200) (Invitrogen) for 1 h at RT. Sections were washed, counterstained with DAPI, and analyzed for CD11b expression.

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Supporting Information Available: Additional genetic analysis and polymer characterization data. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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