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Poly(glycidol) Coating on Ultra-High Molecular Weight Polyethylene for Reduced Biofilm Growth

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Abstract

Semi-branched poly(glycidol) (PG-OH) and poly(glycidol allylglycidyl ether) (PG-Allyl) coatings were formed on ultra-high molecular weight polyethylene (UMWPE) in a unique two-step process which included radiation of UHMWPE followed by grafting of PG-OH or PG-Allyl to the surface via free radical crosslinking. Resulting surfaces were extensively characterized by FTIR-ATR, XPS, fluorescent microscopy and contact goniometry. The performance was evaluated using the most prominent biofilm-forming bacteria *Staphylococcus aureus* for 24 and 48 h. The PG-Allyl coating demonstrated a 3 Log reduction in biofilm growth compared to non-coated control, demonstrating a promising potential to inhibit adherence and colonization of biofilm-forming bacteria that often develop into persistent infections.

Graphical abstract

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Supporting Information. Further characterization can be found in supporting information. This material is available free of charge via the Internet at <http://>

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Keywords

Biofilm; polyglycidol; ultra-high molecular weight polyethylene; implant coating

INTRODUCTION

The development of novel polymeric biomaterials for reducing bacterial adhesion and biofouling is a pressing concern.^{1–3} Out of 1 million total hip and knee replacement surgeries every year in the United States, over 59,000 cases fail due to bacterial infection from biofilm growth and require revision surgeries within the first 10 years.⁴ Ultra-high molecular weight polyethylene (UHMWPE) is strong, inert, stable and the most commonly used polymer for artificial hip and knee replacements.⁵ However, its strong hydrophobic surface chemistry leaves it susceptible for bacterial adhesion and biofilm formation, which is known to be the first conditioning step for infections.⁶ The vast majority of recent advances in biomaterial technology involve techniques that alter the surface chemistry from hydrophobic to hydrophilic with polymer coating techniques such as layer-by-layer (LBL) deposition or self-assembled monolayers (SAM), which use van der Waals secondary or electrostatic forces.⁷ It has been more recently shown that a covalent bond however is more ideal to impart both structural stability and effective hydrophilicity to a biomedical implant surface.⁸ Aside from the most prominent hydrophilic polymers like PEG or zwitterionic coatings, it has been recently realized that poly(glycidol) has potential to achieve bioinertness with more advanced functionality. Poly(glycidols) (PG) are comparable in structure to poly(ethylene glycol) (PEG) but offer tremendous advantages in terms of increased thermal stability, oxidative stability and can form a hydrophilic barrier to biological adsorption.⁹ Poly(glycidol) structural branching can be easily tuned by synthetic reaction temperature¹⁰ and copolymerized with pendant alkene groups¹¹ to remain hydrophilic even at 20% allyl incorporation. Due to the chemical inertness of polyolefin, harsh activation methods such as UV-irradiation or plasma treatments are often required to modify the surface.^{12–13} Haag and coworkers put forth an elegant “graft-to” approach using dendritic poly(glycidols) that were post-modified with amines in three synthetic steps, followed by grafting to plasma-brominated polypropylene.¹⁴ In light of demonstrating effective protein resistance, the highly branched structures may limit surface coverage due to steric hindrance. Bucio and coworkers demonstrated that gamma-ray induced grafting of poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) onto polyethylene (PE) films could be achieved by first exposing polyethylene to ionizing radiation, followed by exposure to high concentrations of monomer in solution.¹⁵ Inspired by both Haag and Bucio, we sought to achieve an effective anti-biofilm coating with fewer processing steps using a

“graft-from” approach with irradiated PE and our unique semi-branched poly(glycidols) to coat the most commonly utilized biomaterial for hip-and-knee implants, ultra-high-molecular weight polyethylene (UHMWPE).¹⁶ Characterization of the resulting coatings along with their capabilities to resist bacterial biofilm growth were investigated.

RESULTS AND DISCUSSION

Radiation grafting and characterization

An overview of the grafting process with scanning electron microscopy images are given by Figure 1. The “graft-from” method was initiated by trapped radicals throughout a radiated surface of the polyethylene by a mechanism described in Figure S3 (See Supplementary Information). Briefly, the ionizing radiation from Cesium-137 decays to generate gamma rays that cause homolytic cleavages of C-H bonds on polyethylene (PE). This generates carbon and hydrogen radicals that undergo chain transfer to poly(glycidol) during exposure. Finally, the radical PE and PG undergo radical association producing PE-PG, PG-PG and hydrogen gas. This process occurs multiple times on the surface leaving a crosslinked poly(glycidol) coating attached to the polyethylene surface. We hypothesized that the copolymer poly(glycidol allyl glycidyl) ether (20% allyl) would graft more effectively on the surface due to increased reactivity of pendant alkene groups compared to poly(glycidol) homopolymer. Our hypothesis was tested by conducting a time-dependent study of pre-irradiated chips exposed to equivalent concentrations of each polymer in solution and analyzing the surface results by scanning electron microscopy (SEM) at 5, 7 and 15 day time points after thorough washing to remove non-grafted material. Figure S4 (see Supplementary Information) shows images of the surface of PE chips before and after exposure to the polymer graft solution, and one can see the gradual decrease of observed crevasses, particularly after 15 d, due to the coating formation. One may also note the slightly darker and denser image associated with the PG-Allyl coating compared with the PG-OH after 15 d exemplified by Figure S5, which we attribute to the increased reactivity of the pendant allyl groups. This evidence was corroborated by grafting pre-irradiated PE with a fluorescent N-hydroxysuccinimide (NHS) ester dye conjugated to PG-Allyl (See Supplementary Information S1 for synthesis and characterization) in solution compared to a solution of simple dye mixed with PG-OH and control solution of dye alone (negative control) at equivalent dye concentrations for 15 days. The grafting was followed by careful washing to remove non-grafted materials. The fluorescent imaging results in Figure 2D indicate presence of dye, and more homogeneous coating was observed in the grafted PG-Allyl compared to PG-OH. We attribute this difference to the greater reactivity provided by the allyl groups.

Evidence of poly(glycidol) coating on UHMWPE was characterized initially by Fourier transform infrared-attenuated total reflectance spectroscopy (FTIR-ATR) as shown in Figure 2B, and the resulting spectra showed strong presence of PG-OH and PG-Allyl coatings characterized by the broad hydroxyl peak at 3400 cm^{-1} and ethylene peaks at 2915 and 2848 cm^{-1} . However, we could not distinguish a clear characteristic peak that would differentiate the PG-Allyl coating from the PG-OH, so we employed x-ray photoelectron spectroscopy (XPS) to obtain a more detailed view of the atoms present on the surface of our UHMWPE

chips. In Figure 2C, both PG-Allyl and PG-OH coating samples showed elevated presence of hydroxyl groups compared to a control UHMWPE surface which confirmed a coating was present. Additionally, the PG-Allyl coated sample showed 36% $-\text{CH}_2-$ character while the PG-OH coating showed only 25%. This difference can be attributed to the higher presence of ethylene groups on the PG-Allyl polymer structure compared with the PG-OH.

An important feature of the PG-OH and PG-Allyl polymer coatings include the hydrophilic characteristic they can impart on an implantable substrate. We employed contact-angle goniometry to quantify the wettability of the coated surfaces by measuring the angle created at the liquid-vapor interface when a water droplet is deposited onto the surface. The results for UHMWPE, PG-OH coating, and PG-Allyl coating groups were 18.3 and <10 , respectively, as shown by Figure 2A. The results from measuring contact angle (Θ , degrees) on PG-Allyl coating came to <10 degrees, which was below the detection limit, compared to PG-OH coatings that measured 18.3 degrees. While the non-coated surface data suggests significant ($p < 0.05$) increase in hydrophilicity from both treatment groups, the PG-Allyl coating created a surface that was more hydrophilic than the PG-OH treatment. Despite a lesser percentage of hydroxyl groups, the PG-allyl chips possessed a more complete coating which can be attributed to its increased reactivity during the graft step.

Biofilm in vitro assay

The ability for *S. aureus* cells to adhere and proliferate on medical implants has been a major challenge in the biomedical field. *S. aureus* cells are able to tolerate high doses of antibiotics and the ability to inhibit infection on implanted devices has yet to be solved.¹⁷ Infections can be potentially avoided by inhibiting adherence of bacteria on the surface in the first place, thus eliminating the possibility for an infection to persist on the implant and spread to the surrounding area (See Figure 3A). As proof of principle, samples of polymer-coated and non-coated UHMWPE chips were inoculated with 10^5 CFUs/cm² UAMS-1 strain *S. aureus* cells in solution and incubated at 37°C for 24 and 48 hours in a 24-well plate to assess the ability for poly(glycidol) coating to inhibit *S. aureus* cell adherence and growth. After the incubation period, any adherent cells were separated from the surface and counted. The results suggest that chips that were coated with PG-Allyl inhibit any and all cells from adhering to the surface during the 24- and 48-hour incubations (see Figure 3B). Similarly, the bacterial growth on PG-OH samples exhibited significant ($p < 0.05$) reduction of bacteria growth compared to the significantly-infected non-coated UHMWPE control (see Figure 3B). *S. aureus* colonization of non-coated control samples exceeded 10^6 CFU/cm² after 48 h which suggests these implant surfaces would be prone to chronic infection as supported by the literature^{18–19}. It is important to point out that we continued the bacterial incubation period to 48 h when previous groups who studied anti-biofilm coatings stopped at 90 min or 24 h before counting cells as can be found in reports by Puértolas and coworkers¹⁸ and Haag and coworkers¹⁴, respectively. We wanted to ensure enough time for a biofilm to develop on UHMWPE, which is why our experiments went 48 h. To the best of our knowledge, the PG-allyl demonstrated to be one of the best performing anti-adherence coatings for *S. aureus* cells in recent literature. Additionally, we analyzed the 24-well plate surfaces which were used to incubate *S. aureus* with the coated PG-Allyl and PG-OH samples to predict how the coatings would affect colonization of bacteria in surrounding areas. As shown in Figure 3C,

the quantified cell counts of *S. aureus* cells in well surfaces that were surrounding PG-Allyl coated samples indicate a significantly decreased adherence to the well-plate surface when compared to wells that incubated PG-OH samples. Figure 3C shows the PG-Allyl coating exhibited an interesting proximity effect by reduction of bacteria colonization in the well-plate that will be a focus of future experiments. It is important to note that PG-Allyl and PG-OH have previously demonstrated strong biocompatibility by Harth and coworkers¹¹ and Brooks and coworkers²⁰, respectively, among others. The proof-of-concept *in vitro* experiments suggest that the PG-Allyl coating on the surface of an implantable material demonstrated a promising potential to inhibit adherence and colonization of biofilm-forming bacteria that often develop into persistent infections.

CONCLUSION

In conclusion, we presented a simple pre-irradiation graft-from method for coating the most commonly used material for hip-and-knee implants, UHMWPE, with semi-branched poly(glycidols) in efforts to reduce infectious biofilm growth. Both PG-Allyl and PG-OH surface coatings were characterized by FTIR, XPS, SEM and fluorescent microscopy and tested against the most prominent biofilm-forming bacteria, *S. aureus*. Coated samples either inhibited or delayed *S. aureus* cell colonization of a UHMWPE chips after 24 and 48 hours of incubation. The PG-Allyl coating led to 3 log reduction in colonization of *S. aureus* cells compared to the highly infected non-coated UHMWPE control sample when incubated for 24 and 48 hours, indicating a strong potential for preventing infection in biomaterials that contain UHMWPE.

MATERIALS AND METHODS

Materials

Semi-branched poly(glycidol) (GPC $M_n = 2828$ Da, $M_w/M_n = 1.57$ using PEG standards) and poly(glycidol allylglycidyl ether) (PG-Allyl, 20% Allyl, GPC $M_n = 3218$ Da, $M_w/M_n = 1.42$ using PEG standards) were synthesized as previously reported.^{11, 21} Allyl glycidyl ether (AGE), tin(II)triflate ($\text{Sn}(\text{OTf})_2$) and glycidol (GLY) were purchased from Sigma Aldrich, USA. GLY was purified *via* Kugelrohr distillation before use. L- α -phosphatidylcholine (Egg-PC) was purchased from Avanti Polar Lipids (Alabaster, AL). 2,2-Azobis(2-methyl-*N*-(2-hydroxyethyl)propionamide (VA-086, 98%) was purchased from Wako Chemicals USA, Inc. and used without further purification. Dialysis tubing SnakeSkin® (molecular weight cutoff (MWCO): 1 kDa, 16 mm dry I.D) were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). A sheet of ultra high molecular weight polyethylene (UHMWPE, ~3000 kDa, opaque white, standard tolerance, ASTM D4020, 0.125" thickness, 12" width, 12" length) was purchased from Amazon, Inc (Amazon.com) and machined into 1 × 1 cm squares with 16-gauge holes strategically placed in the corner of squares by the Vanderbilt Machine Shop. Sulfo-Cyanine5 NHS Ester (Cy5) dye was purchased from Lumiprobe Corporation (Hallandale Beach, FL). Unless mentioned otherwise, all other solvents or reagents were purchased from Sigma Aldrich and used as received. Milli-Q water was used in these experiments and ultra-purified by a Modulab Water Systems USA with 0.2 μm filter with minimum resistivity of 18.2 M Ωcm at 25 °C.

Instrumentation

The UHMWPE sample chips were irradiated using a Mark I 68A Cs-137 Irradiator, manufactured by JL Shepherd and Associates (San Fernando, CA). X-ray photoelectron spectroscopy data was acquired with a Physical Electronics (PHI) XPS 5000 Versaprobe. Samples were sputter-coated with 1-2 nm of gold before scanning electron microscope (SEM) images were acquired on a Zeiss Merlin Scanning Electron Microscope operating at 10 keV and 8 mm working distance. Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) was performed using a Nicolet™ iS™ 5 FT-IR Spectrometer with an iD5 ATR Accessory equipped with OMNIC™ FTIR Software (Thermo Scientific, USA). Samples were pressed against the diamond tip, and resulting spectra represent the average over 32 scans. Epi-fluorescent images of Cy5 dye coatings were collected on a Nikon AZ100 Upright Wide Field Microscope equipped with NIS-Elements Software with a 5× objective and Cy5 filter at 3.0× zoom (0.72 μm/pixel).

Pre-irradiation grafting of polyglycidol to UHMWPE

The UHMWPE chips were cleaned by rinsing both sides first with methanol (5 s), then acetone (5 s) and dried under vacuum before submitting to the Cs-137 irradiator. Samples were then exposed to irradiation for 15.5 hrs (1 kGy of absorbed irradiation) and quickly transferred to polymer graft solutions while unused chips were stored under Argon until later use. Solutions of 40 wt % poly(glycidol) and 40 wt % poly(glycidol allylglycidyl ether) in methanol were prepared in 6 DRAM vials and bubbled with Argon for 20 min before submerging the pre-irradiated chips, outfitted with copper wire hooks to stabilize, into the vial solutions. A razor was used to score a line at the solution-air interface as an indicator in order to locate the resulting coating edge later *via* SEM (See Supplementary Information Figure S5). The 6DRAM vials were sealed with parafilm, and the samples incubated at room temperature for up to 15 days. Following incubation, samples were thoroughly rinsed in milli-Q water for 5 minutes to ensure any non-grafted material was removed before characterization. After washing, the coated chips were dried under gentle stream of nitrogen for at least 3 hours before drying overnight in ambient conditions.

Fluorescent dye conjugation

In a 0.5 DRAM vial, 15.0 mg of poly(glycidol allylglycidyl ethylamine) ether (PG-CC-N, 11 μmol, 3 amines per polymer, see Supplementary Information for complete synthesis) was combined with 5.0 mg of Sulfo-Cyanine5 NHS Ester (Cy5) dye (6.4 μmol) in 284 μL of total d₆-DMSO (with 1% v/v trimethylamine) solvent. The mixture was stirred by magnetic flea stir bar at 1500 rpm for 16 h at room temperature in the dark. At completion, the solution was diluted 100-fold in methanol and transferred to 1 kD MWCO dialysis tubing and dialyzed against 500 mL of methanol for 3 days, and dialysate was changed twice daily. The resultant product was dried down under vacuum overnight (dry yield = 15 mg, 75%) and analyzed by proton NMR. Characteristic proton signals in the aromatic region (6.2-8.4 ppm) confirmed successful attachment of the dye to polyglycidol (See Supplementary Information for labeled spectrum)²². Overall dye functionalization was determined to be 9% by NMR.

Fluorescent dye coating

UHMWPE was machined into rectangular cubes ($1 \times 0.3 \times 0.3$ cm) with holes on one end and thoroughly cleaned with methanol and acetone. Following cleaning, samples were dried and irradiated to 1 kGy (15.5 h) using a Cs-137 irradiator. Immediately after irradiation, samples were oriented using common sewing thread inside 0.5-DRAM vials with 200 μ L solutions of free Cy5 dye (1.5 mg, the control) in MeOH, Cy5 dye (1.5 mg) with roughly 400 mg of polyglycidol (un-functionalized, 40 wt%) in MeOH, or 15 mg of polyglycidol-Cy5 conjugated (10 % functionalized, effectively 1.5 mg of dye) with 385 mg of polyglycidol (unfunctionalized, 40 wt% total polymer) in MeOH. Graft solutions were bubbled with Argon for 20 min before submerging UHMWPE samples. Samples were allowed to graft for 15 d. Following graft, samples were thoroughly washed for 5 min in milli-Q water to remove unattached dye or polymer material. Samples were allowed to dry overnight in the dark and imaged on a Nikon AZ100 upright wide-field microscope as described in the instrumentation section.

Contact angle measurement

The contact angle measurements were conducted using a Rame-Hart manual contact angle goniometer with a microliter syringe. A 5 μ L droplet of DI water was added to 1 cm² chips of untreated UHMWPE, semibranched poly(glycidol) film, or semibranched poly(glycidol allylglycidyl ether) film on UHMWPE surfaces, and the angle (Θ) was measured between the solid surface and liquid-air interface of the static droplet. Measurements were performed in triplicate.

X-ray photoelectron spectroscopy (XPS)

XPS analyses were performed using an Ulvac-PHI Versaprobe 5000. The three samples were mounted onto one large sample holder using stainless steel masks. The mounted samples were pumped in the intro chamber over a weekend to minimize outgassing of any remaining water or other volatile species in the analysis chamber. Monochromatic Al K α x-rays (1486 eV), a 100 μ m diameter x-ray spot rastered over a 600 μ m by 400 μ m area, and a takeoff angle of 45 degrees off sample normal were used in each acquisition. Pass energies of 187.7 eV and 23.5 eV were used for the survey and high-resolution acquisitions, respectively. Charge neutralization was accomplished using 1.1 eV electrons and 10 eV Ar⁺ ions. Binding energies were calibrated to -CH₂- type bonding in the carbon 1s spectrum of 284.8 eV. Relative atomic concentrations were calculated using peak areas and PHI handbook sensitivity factors.²³

Bacterial strain and reagents

Staphylococcus aureus strain UAMS-1 was used for the biofilm assay due to its elevated ability to form biofilms.²⁴ Bacterial cell cultures were grown in 5 mL Tryptic Soy Broth (TSB) overnight shaking at 180 rpm. Bacterial cells were quantified by optical density at 600 nm (OD₆₀₀).

***S. aureus* in vitro adherence assay**

Standard assay procedures were performed as described previously.²⁵ To ensure sterility, samples were submerged in 70% EtOH for 2 seconds and placed in sterile PBS prior to inoculation. Samples were placed in 24-well plates with 1 mL of TSB containing 10⁵ CFUs/mL *S. aureus* and incubated at 37°C for 24 and 48 hours. Plates were rotated on an orbital shaker at 80 RPM to encourage mixing and allow for adherence. Following incubation, samples were extracted from bacterial solution and washed 3 times with sterile PBS to ensure that any cells that were not adhered to the surface were removed. Sonication of samples was performed for 5 minutes in a fresh 24 well plate containing 1 mL of sterile PBS for each sample to separate adhered cells from the surface of the sample. Similarly, surfaces of the 24-well plates that were used during incubation were also washed 3 times with sterile PBS and sonicated for 5 minutes using 1 mL of sterile PBS. Sonicated media was then serially diluted and plated on Tryptic Soy Agar (TSA) plates to quantify CFUs per surface area of sample substrate.

Statistics

Data analysis and statistics were performed with the Data Analysis Tool from Microsoft Excel 2016 for Mac. A student one-tailed t-test was performed using two-sample unequal variance parameters. In all cases, a p-value < 0.05 was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Pre-irradiation grafting of Polyglycidols to UHMWPE. **A)** Sample of UHMWPE were exposed to Cesium-137 source to initiate trapped radicals in the plastic. **B)** We then use the trapped radicals within UHMWPE to graft poly(glycidol) homopolymer (PG-OH) or poly(glycidol allylglycidyl ether) (PG-Ally) to the surface for 15 d. **C)** Upon developing the samples through exhaustive washings in water to remove the non-crosslinked polymer and thorough drying, we characterized the surface coatings by scanning electron microscopy. White scar bars indicate 1 μm .

Figure 2.

A) Contact angle Θ (degrees) versus test sample group for control non-coating (UHMWPE), poly(glycidol) (PG-OH) coating and poly(glycidol allylglycidyl ether) (PG-Allyl). A significant reduction in contact angle was observed in the PG-Allyl coating compared to the PG-OH which can be attributed to the more effective coating. Data represent mean $\pm$ SD ($n=3$) with significance ($p < 0.05$) indicated by asterisk. B) FTIR-ATR data confirms presence of polyglycidol coated on the polyethylene, particularly in the samples circled which show the strong broad hydroxyl peak at 3400 cm^{-1} from polyglycidol along with the sharp ethylene peaks from polyethylene at 2915 and 2848 cm^{-1} . C) Top row of plots indicate XPS full spectral results for control UHMWPE, poly(glycidol) and poly(glycidol allylglycidyl ether) chip samples. The bottom row represents zoomed in spectral data between 282 - 291 eV for each of the same sample types. D) Fluorescent imaging was performed at the fill line after washing the non-grafted material away. No detectable signal was observed in the control (top), while some dye molecules were entrapped within the PG-

OH coating (middle), and a substantial fluorescence was observed in the PG-Allyl-Dye coating (bottom). The white scale bars indicate 100 μm .

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Figure 3.

A.) *S. aureus* cells are the most prominent biofilm-forming bacteria that are found to adhere and proliferate on UHMWPE medical implant surfaces and create an infection. Our coating has been shown to inhibit adherence, thus eliminating the ability for bacterial cells to form an infection. B.) This plot indicates adherent *S. aureus* bacterial counts from the surface of coated and non-coated test samples after 24 and 48 h incubation periods. The dotted line indicates the limit of detection (LOD) at 2 log₁₀(CFU's/cm²). C.) Plot shows the adherent *S. aureus* cells that remained in surrounding well plate of test samples after 24 and 48 h incubation. Interestingly the PG-Allyl wells exhibited less bacterial growth than PG-OH wells and will be a focus of future experiments. All data represent $\pm$ mean SD (n=3).