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Peptide-functionalized poly[oligo(ethylene glycol) methacrylate] brushes on dopamine-coated stainless steel for controlled cell adhesion

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ABSTRACT

The modification of the surface of surgical implants with cell adhesion ligands has emerged as a promising approach to improve biomaterial-host interactions. However, these approaches are limited by the non-specific adsorption of biomolecules and uncontrolled presentation of desired bioactive ligands on implant surfaces. This leads to sub-optimal integration with host tissue and delayed healing. Here we present a strategy to grow non-fouling polymer brushes of oligo(ethylene glycol) methacrylate by atom transfer radical polymerization from dopamine-functionalized clinical grade 316 stainless steel. These brushes prevent non-specific adsorption of proteins and attachment of cells. Subsequently, the brushes can be modified with covalently tethered adhesive peptides that provide controlled cell adhesion. This approach may therefore have broad application to promote bone growth and improvements in osseointegration.

Statement of Significance

Stainless steel (SS) implants are widely used clinically for orthopaedic, spinal, dental and cardiovascular applications. However, non-specific adsorption of biomolecules onto implant surfaces results in sub-optimal integration with host tissue. To allow controlled cell-SS interactions, we have developed a strategy to grow non-fouling polymer brushes that prevent protein adsorption and cell adhesion and can be subsequently functionalized with adhesive peptides to direct cell adhesion and signaling. This approach has broad application to improve osseointegration onto stainless steel implants in bone repair.

1. Introduction

Stainless steel (SS) is used widely in medical devices for bone, dental, and cardiovascular applications [1,2]. Type 316 SS is attractive for orthopaedic applications such as fracture fixation by virtue of its high shear strength relative to that of other metals that are commonly used in such applications (e.g., titanium), along with its resistance to corrosion, cost effectiveness, and ease of manufacturing [1,3]. However, SS implants are subject to relatively high rates of failure over long periods of time due to implant loosening, inflammation and bone resorption [4,5]. For example, in osteoporotic fracture repairs, unstable fixation occurs in 5–23% of cases due to screw loosening and cutout [6–8]. Loosening rates of 18–27% have been reported for pedicle screws [9–11], and implant loosening results in pain, loss of spinal alignment and pseudoarthrosis. Various strategies have been explored to improve osseointegration of metal implants [12–17]. However, there is still a significant need to further improve performance.

Passive adsorption of cell adhesive proteins onto metallic implants provides biological coatings that promote osseointegration [18–20]. These proteins provide instructive cues to mediate cell attachment and differentiation [20–24]. However, passive adsorption of proteins onto the surface of the implant often leads to denaturation and loss of activity [25,26]. Pre-adsorbed proteins can also be replaced by other proteins that have stronger affinity for the surface [27]. Therefore, significant efforts have centered on the development of stable coatings that prevent non-specific protein adsorption and which present selected ligands to promote adhesion and stimulate function of specific cells [28–31].

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Self-assembled monolayers (SAMs) have long been explored to modify implant surfaces and thereby allow for control over protein adsorption and cell adhesion [28–33]. However, such coatings are often limited by the type of substrate. Most studies of SAMs have focused on gold and silver substrates, which are not ideal as implantable biomaterials. Another drawback of this approach is the limited resistance of SAMs to biofouling [28,29]. In contrast, films consisting of polymer brushes on metallic substrates can provide stable, non-fouling coatings that do not elicit negative inflammatory responses [18,34]. In "grafting to" approaches, presynthesized polymers are chemically attached to the surface. Whereas this has served as a successful strategy to attain control over protein adsorption and the attachment of cells, steric crowding between polymer chains limits the graft density and functionality [30,35,36]. On the other hand, "grafting from" approaches involve polymerization of monomers from an initiator that is bound to the substrate. The diffusion of small molecule monomers to the interface presents a low barrier for the preparation of dense polymer brushes. This strategy allows for the formation of dense films with control of the thickness by variation of polymerization conditions. Such "grafting from" polymerization of oligo(ethylene glycol)-based monomers on titanium provides robust polymer brushes that resist protein adsorption and biofouling [35].

Controlled radical polymerizations such as atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer (RAFT) provide facile routes for the growth of polymer brushes on metallic substrates [30,37–39]. Surface-initiated ATRP of oligo(ethylene glycol) methacrylate (OEGMA) provides a polymer brush that consists of a poly(methacrylate) backbone with pendant poly(ethylene glycol) (PEG) side chains. This provides the substrate with a robust hydrophilic coating that resists protein adsorption and cellular attachment [34]. The non-fouling properties of these brushes may be attributed to the presence of a high density of hydrated and dynamic PEG chains [40,41].

Xiao et al. recently described a "grafting to" approach to attach functionalized PEG chains on to SS surfaces [42]. They demonstrated that this modification resulted in a decrease in platelet adhesion to the surface. However, the presence of residual levels of adherent platelets on the surfaces might be attributed to the inherently low-density of the brush formed by the "grafting to" approach. In addition, the lack of reactive functional groups on the grafted polymer chains limits the opportunity to decorate these surfaces with adhesive peptides that direct cell adhesion and signaling. Here, we present a modified approach to graft polymer brushes from medical-grade SS using surface-initiated ATRP to provide a non-biofouling surface. The strategy relies on the covalent attachment of an ATRP initiator to a layer of polydopamine on the substrate surface. We further show that these brushes can be modified with peptide ligands that control cell adhesion. These peptide-modified surfaces could improve the osseointegration of SS implants, such as screws and rods. The wide use of SS in biomedical devices suggests that this approach may be of broad applicability.

2. Materials and methods

2.1. Materials

Foils (100 mm × 100 mm) of 316 SS and 316L polished SS (Goodfellow, Pittsburgh, PA, USA) were cut into 10 mm × 10 mm coupons and a 0.8 mm hole was drilled in one corner. 316L SS 20-gauge wire was obtained from Beadalon (Valley Township, PA, USA). Oligo(ethylene glycol) methacrylate (OEGMA), Cu(I)Br, 2,2′-bipyridyl, ethyl 2-bromoisobutyrate, dopamine hydrochloride, anhydrous pyridine, hydrogen hexachloroplatinate (IV) hexahydrate, anhydrous dimethylformamide (DMF), 10-undecen-1-ol, dimethylchlorosilane, 1-ethyl-3-(3-*N*,*N*-dimethylaminopropyl)carbodiimide hydrochloride, 2-bromoisobutyryl bromide and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Succinic anhydride was purchased from Alfa Aesar (Wardhill, MA, USA). For the polymerization of OEGMA, DI H_2O and methanol (MeOH) (VWR, Atlanta, GA, USA) were degassed by bubbling a stream of argon through the solvents for 3 h. Peptide ligands (RGD (GRGDSPC) or RDG (GRDGSPC) or RGD-FITC (GRGDSPK conjugated to fluorescein isothiocyanate(FITC))) were custom synthesized by GenScript (Piscataway, NJ, USA)

2.2. Preparation of polymer brush thin films

2.2.1. Preparation of SS surface

SS coupons were cleaned by soaking them for 1 min in H_2O and then acetone, and drying under a stream of N_2. The coupons were then placed in a ceramic slide holder and submerged into stirred piranha solution (3:1 conc. H_2SO_4:30% H_2O_2) for 1 h at room temperature. The coupons were soaked for 1 min in a large volume of H_2O twice, acetone twice, and for 10 s in methanol, 1 min in THF, and 1 min in hexane. They were then dried under a stream of N_2.

2.2.2. Deposition of dopamine layer on SS surface

Dopamine hydrochloride (91 mg, 0.47 mmol) was dissolved in a stirred solution of 10 mM Tris buffer (pH 8.5) at 60 °C. Freshly cleaned 316 SS coupons were suspended on a 316L SS wire and submerged into the heated solution of dopamine for 2 h. The suspended coupons were rinsed by immersion in MeOH (200 mL) for 10 min and DI H_2O (200 mL) for 10 min. The coupons were rinsed with MeOH for 10 s, and immersed in THF (1 min) and hexane (1 min), and dried under a stream of N_2 immediately prior to attachment of the initiator.

2.2.3. Covalent attachment of initiator

An oven-dried glass reactor was subjected to three cycles of vacuum and back-filling with argon. Anhydrous pyridine (1.6 mL, 20 mmol) and anhydrous THF (80 mL) were placed in the reactor under a stream of argon. The reactor was cooled in an ice bath for 1 h, and the wire-suspended dopamine-modified SS coupons were immersed into the solvent. 2-Bromoisobutyryl bromide (2.4 mL, 19 mmol) was added slowly to the solvent with stirring. The reactor was removed from the ice bath and the mixture was vigorously stirred for 1 day to prevent precipitate from depositing onto the surface of the steel.

2.2.4. Deposition of poly(OEGMA) brush polymer on 316 SS

A flask containing a mixture of MeOH (40 mL) and DI water (10 mL) under argon was immersed in liquid nitrogen and subjected to three freeze-pumpthaw cycles (freezing the solution in liquid nitrogen for 15 min, applying vacuum for 20 min, and then thawing the solution in a warm water bath until the evolution of gas bubbles had ceased). The degassed solution was frozen in liquid nitrogen and CuBr (0.44 g, 3.7 mmol), OEGMA (24 mL, 44 mmol), and 2,2′-dipyridyl (0.96 g, 6.3 mmol) were added under a flow of argon. The reaction mixture was allowed to warm to room temperature. The polymerization solution was subjected to three additional freeze-pumpthaw cycles. Cooling was performed stepwise, with cooling in an ice-cold water followed by an acetone-dry ice bath for 15 min prior to cooling in liquid nitrogen. This prevents the violent release of dissolved gas upon application of vacuum. Initiator-modified 316 SS coupons were suspended from a wire which was then mounted in a Soxhlet extraction thimble. This was placed atop the frozen polymerization solution under a strong flow of argon. The reactor was closed and subjected to three cycles of vacuum and back-filling with argon. The cooling bath was removed and the polymerization mixture was allowed to thaw.

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