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The haemocompatibility of polyurethane–hyaluronic acid copolymers

Fangmin Xu[a], John C. Nacker[b], Wendy C. Crone[a,b], Kristyn S. Masters[a,*]

[a]Department of Biomedical Engineering, University of Wisconsin-Madison, 1550 Engineering Drive, #2152, Madison, WI 53706, USA
[b]Department of Engineering Physics, University of Wisconsin-Madison, USA

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Abstract

Despite decades of research into haemocompatible biomaterials, there remain surprisingly few materials that can be used in blood-contacting applications. We have synthesized copolymers of polyurethane (PU) with hyaluronic acid (HA) with the goal of creating materials that incorporate an inherently non-thrombogenic, biological component into the bulk polymer structure. HA was incorporated into the polymer backbone as a chain extender during PU synthesis, and the physical and biological properties of the resulting copolymer were directly controlled by the HA content. Increases in HA content led to a linear increase in hydrophilicity ($R^2 = 0.993$) and corresponding increase in surface energy compared to PU controls. Elastic modulus also increased with HA content ($p < 0.001$), while surface roughness did not significantly differ from PU controls for most PU–HA formulations. Incorporation of HA resulted in negligible platelet adhesion to the PU–HA ($p < 0.001$), representing a 20-fold decrease in platelet adhesion compared to PU. Red blood cell adhesion also decreased with increasing HA content ($p < 0.001$). The PU–HA materials were cytocompatible and supported endothelial cell adhesion and viability. Thus, we have demonstrated the synthesis of a copolymer whose physical and biological properties are easily tailored, and whose potent anti-thrombogenic properties demonstrate its great promise for use in vascular applications.

Keywords: Polyurethane; Hyaluronic acid; Haemocompatibility; Thrombogenicity

1. Introduction

Materials that are resistant to platelet adhesion are needed for a wide range of applications, including vascular grafts, stents, heart valve replacements, pacemaker leads, hemodialysis tubing, and catheters. Moreover, as cardiovascular disease remains the leading cause of death in the US (41.4% of all deaths), there is a high demand for cardiovascular materials and blood-contacting devices, in the range of millions of devices per year in the US [1–3]. Yet, after several decades of research into haemocompatible biomaterials, there remain surprisingly few materials that can be used in blood-contacting applications without administering anticoagulant therapy to the patient [4–6]. Even materials viewed as haemocompatible have significant shortcomings—while Dacron and Teflon are the most widely used synthetic vascular graft materials, their failure due to thrombosis is almost immediate when used in small-diameter (<6 mm) applications, and 5-year patency rates are less than 50% even in large-diameter applications [7,8].

Regarding the performance of small diameter vascular grafts, it was recently noted that "The poor blood-compatibility of an artificial vascular graft is not simply because of its coagulation-stimulating or platelet-activating properties, but more due to its inability to *actively participate* in the prevention of blood coagulation and platelet deposition" [9] (emphasis added). To this end, efforts to improve the haemocompatibility of various materials have often concentrated on designing systems to elute anticoagulants, such as heparin [10,11]. Heparin is a naturally occurring glycosaminoglycan (GAG) with anti-thrombotic properties. Unfortunately, release of heparin from a biomaterial represents a relatively short-term solution to inhibiting thrombosis, as the delivery duration will be finite. Furthermore, the recent and significant troubles with some drug-eluting stents have illustrated risks

*Corresponding author. Tel.: +1 608 265 4052; fax: +1 608 265 9239.
E-mail address: kmasters@wisc.edu (K.S. Masters).

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of the strategy of non-covalently adding a non-thrombogenic coating to an existing surface [12,13]. Thus, a safer, alternative strategy to imparting anti-thrombotic activity upon a material would be to make the core material itself inherently non-thrombogenic. In this manner, the availability of the anti-thrombotic agent would not be transient, as the agent would be physically part of the material.

Polyurethane (PU) block copolymers have been widely used for numerous biomedical applications due to their excellent mechanical properties and biocompatibility [14]. In contrast to other materials used in vascular applications (Dacron, Teflon), PU-based materials support the growth of endothelial cells and possess mechanical properties that match that of the native vasculature [15]. Both of these characteristics are particularly important for applications such as vascular grafts, where the relatively rigid mechanics of Dacron and Teflon and their inability to support endothelialization are major contributors to the failure of these materials in small-diameter applications [8]. Their significant mechanical mismatch with adjacent arterial tissue (<0.4 MPa tensile modulus of elasticity for native artery vs. 500 MPa for Teflon) leads to significant problems at the graft anastomoses such as thrombosis and hyperplasia induced by migration and growth of fibroblasts and smooth muscle cells. Another advantage of PUs is the relative ease of modifying their structures; surface and/or bulk modification of PU via attachment of biologically active species is possible due to reactive groups which are part of the PU structure, and such modifications may be designed to control or mediate host responses [16–33]. Finally, PUs may be fabricated via a myriad of processing technologies, including casting, electrostatic and wet spinning of fibers and monofilaments, extrusion, dip coating, or spraying [14].

Despite the numerous favorable properties of PUs regarding their use in vascular applications, their marginal haemocompatibility has been a significant problem. As noted earlier, native GAGs such as heparin possess anticoagulant characteristics, and there have been numerous successful efforts to covalently modify PU surfaces with heparin in order to improve haemocompatibility [16–18,21,23,26,27,30,32,33]. While not as widely incorporated into vascular materials or devices as heparin, other native GAGs, such as hyaluronic acid (HA), similarly possess anti-thrombotic properties. HA is a particularly intriguing biomolecule for use in vascular applications, as it is not only non-immunogenic, but it also stimulates the proliferation of endothelial cells [34,35].

In this report, we describe the synthesis and characterization of new haemocompatible materials consisting of PU–HA copolymers. Our rationale in combining PU with HA was to: (1) take advantage of the beneficial properties of PU, such as its good mechanics and processibility, and (2) take advantage of the natural anti-thrombotic properties of HA, in order to (3) create biomaterials that are inherently non-thrombogenic and actively participate in the inhibition of platelet adhesion.

2. Materials and methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

2.1. Synthesis of polyurethane–hyaluronic acid (PU–HA) copolymers

2.1.1. HA degradation

Low molecular weight HA is both easier to handle (with respect to dissolution) and possesses more desirable biological properties, such as stimulation of endothelial cell growth, than native HA (~4 MDa). Thus, to create lower molecular weight HA, a 5 mg/ml solution of HA sodium salt in 20 ml diH_2O was prepared, and hyaluronidase (HyAse; from bovine testes, 1040 U/ml) was then added to this HA aqueous solution to achieve a final HyAse concentration of 500 U/ml. The solution was incubated in a 37 °C water bath overnight then heated in a 70 °C water bath for 30 min in order to inactivate the HyAse. After cooling to room temperature, the degraded HA solution was ready for ion exchange.

2.2. Formation of cetylpyridinium salt of HA

HA and its degradation products are soluble only in H_2O, but the synthesis of PU–HA copolymers requires that HA be dissolved in organic solvents such as dimethyl formamide (DMF). Thus, a simple ion exchange reaction was performed that replaces the sodium salt of HA with a cetylpyridinium salt of HA (HA-CP). A 0.48% (w/v) solution of cetylpyridinium chloride (CPC) in 2 ml diH_2O was added dropwise to 2 ml of HA solution, leading to formation of a white precipitate (HA-CP). Excess CPC will remain in solution. The precipitate was separated from the solution by centrifugation and washed twice with diH_2O. The precipitate was frozen and dried overnight. The product was HA-CP powder, which is soluble in DMF.

2.3. Synthesis of PU–HA

A 10% (w/v) solution of methylene di(*p*-phenyl isocyanate) (MDI; 2 mmol; MW = 250) in 5 ml of DMF was prepared in a 100 ml round-bottom flask and stirred at room temperature. A 10% (w/v) solution of poly(tetramethylene oxide) (PTMO or Terathane®; 1 mmol; MW = 1000) in 10 ml of anhydrous DMF was added, and the mixture was heated to 90 °C and held there for 3 h under argon. The reactor was cooled to room temperature before 1,4-butanediol (BD; 0.9 mmol; MW = 90) in 2 ml of anhydrous DMF was added. The HA-CP powder was dissolved in 4 ml anhydrous DMF and warmed to 50 °C. The HA-CP solution in DMF was then added to the reactor and incubated at 50 °C for 3 h under argon. The polymer solution was cooled to room temperature, precipitated in methanol, and dried naturally in a fume hood. This product is referred to as PU–HA in the present communication. The weight percent of HA in the copolymer was varied by using different ratios of HA-CP in the synthesis. The synthesis procedure is summarized in Fig. 1.

2.4. FTIR

Films of PU and PU–HA were created by pipetting 50 μl of a 15 mg/ml polymer solution in DMF onto 1 cm × 1 cm double-side polished silicon wafers, and allowing the solvent to evaporate. FTIR spectra of polymer films were obtained using a Bruker Tensor 27 FTIR Spectrometer (Bruker Optics, Inc., Billerica, MA) with a resolution of 4 cm^{-1} in absorbance mode. Each film was clamped between KCl plates and mounted on the sample holder. The KCl plate and silicon wafer were subtracted as background for each scan.