Platelet adhesion to chitosan mediates wound healing through proteoglycans and integrin receptors

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Introduction

Haemorrhage is a major cause of death in trauma patients accounting for almost 50% of combat fatalities and up to 80% of civilian trauma fatalities in the US. A natural polysaccharide-based material, chitosan, has a potent haemostatic activity. This project aims to understand how chitosan contributes to wound healing through the characterisation of blood component interactions with chitosan. Specifically, we aim to investigate mechanisms of platelet interactions with plasma protein (including proteoglycans) coated chitosan. Platelets are a potent source of heparanase which is released upon platelet activation. We hypothesize that the release of platelet heparanase allows controlled extracellular matrix remodeling and functional growth factor gradients at the injury site for tissue repair.

Methods

Platelets were freshly isolated from healthy adult donors by centrifugation. Platelet adhesion to chitosan coated with serum proteins was measured using an enzyme linked immuno-sorbent assay (ELISA), quartz crystal microbalance with dissipation monitoring (QCM-D) and fluorescence microscopy. Serum proteins investigated included fibrinogen, albumin, perlecan, type I collagen and vitronectin which were exposed to chitosan for 2 h at 37°C followed by incubation with platelets (2×10^7 platelets/ml) for 1 h. Platelet adhesion was detected by ELISA using a monoclonal anti-$GPII_bIII_a$ antibody (Eli-Lily) while platelets were stained with rhodamine-phalloidin to visualize the actin cytoskeleton by fluorescence microscopy. Integrin ($\alpha_2\beta_1$) and perlecan domain blocking antibodies were used to determine mechanisms of platelet adhesion to perlecan.

Results

Platelet adhesion to chitosan was enhanced in the presence of the plasma proteins fibrinogen and vitronectin compared to the perlecan-coated or uncoated material (Fig. 1).

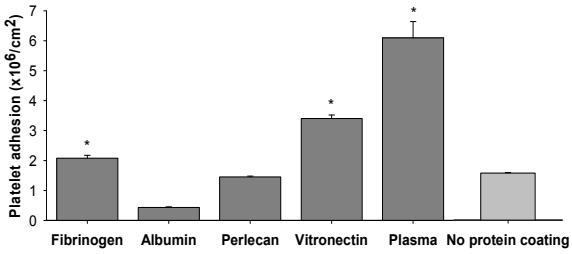

*Figure 1. Platelet adhesion to protein precoated chitosan. * = t test, $p<0.05$ compared to no protein coating.*

Platelet adhesion to perlecan was found to be enhanced after digestion of its heparan sulfate (HS) chains (Fig. 2). QCM-D analysis showed that platelet interactions with perlecan devoid of its HS (Fig. 2B) caused an increase in dissipation suggesting that the platelets were aggregating on the surface. This was confirmed by light microscopy (Fig. 2D).

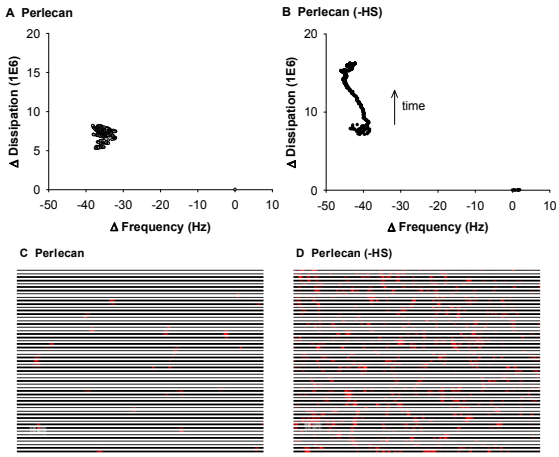

Figure 2. Analysis of platelet adhesion to perlecan using a quartz crystal microbalance with dissipation (QCM-D) monitoring (A and B) and actin staining visualized by fluorescence microscopy (C and D) in the presence (A and C) and absence (B and D) of heparan sulfate (HS).

Platelet adhesion to perlecan protein core was found to be mediated by the $\alpha_2\beta_1$ integrin (Fig. 3) which is more widely known as the cell receptor for collagen. Blocking the $GPII_bIII_a$ receptors resulted in a significant decrease in platelet adhesion for all three coated surfaces supporting the idea that platelet aggregation was downstream of platelet adhesion to collagen and perlecan.

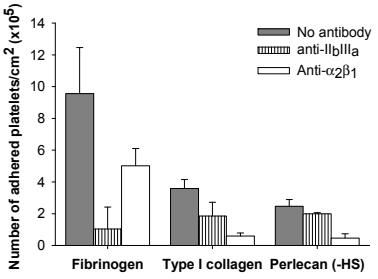

Figure 3 Platelet adhesion to proteins in the presence of antibodies that inhibit adhesion and activation.

Conclusions

Platelets adhere to plasma coated chitosan both in the presence of complex serum and isolated plasma proteins. Perlecan appears to have a modulating effect on platelet adhesion to chitosan which is dependent on the heparan sulfate chains and $\alpha_2\beta_1$ integrins. Future work will focus on identifying the specific adhesion/activation sites on perlecan protein core and the role of platelet heparanase on extracellular matrix remodeling for tissue repair.