Angiogenic methacrylic acid copolymers adsorb different proteins and result in lower complement activation in comparison to poly (methyl methacrylate)

L.A. Wells[1], H. Guo[2], A. Emili[2], M.V. Sefton[2]
[1]Queen's University, [2]University of Toronto

Statement of Purpose: Methacrylic acid (MAA) copolymer beads and coatings promote an angiogenic healing response after their implantation in vivo through an unknown biological mechanism [1-3]. If identified and understood, factors that trigger this mechanism could be applied to the design of many biomaterial implants to improve their clinical outcomes. Proteins adsorb to biomaterials immediately after implantation, and these proteins subsequently influence the local wound healing response by interacting with nearby proteins and cells. As a result of protein adsorption, the complement cascade may also be activated; this protein cascade is part of the innate immune response. Most often when the complement cascade is unintentionally activated by biomaterials due to their charge or topography, there is a resulting cell-driven chronic inflammatory reaction. Activated complement proteins C5a and surface adsorbed C3b have been identified in promoting immune and inflammatory cell activation.

To assess the influence of protein adsorption and reactions in MAA healing responses, this work identifies the proteins that adsorb MAA copolymers and the resulting influence on complement activation.

Methods: Fresh human serum was incubated with either 45% MAA beads or PMMA beads – a control that does not promote the observed in vivo vascular healing response.

To identify which proteins adsorb to 45% MAA beads and PMMA beads, ion-trap shotgun mass spectrometry was performed on proteins that adsorbed from fresh human plasma and fresh human serum.

ELISAs for soluble complement proteins C5b9 and C3a were performed on fresh human serum incubated with both bead types (90 min, 37°C, 4 μmol EDTA added at 90 min).

Results: Over 30-40 different proteins adsorbed to MAA and PMMA beads (Figure 1). 45% MAA adsorbed primarily histidine rich glycoprotein, platelet basic protein, and numerous complement proteins, including C1q, C4a, complement factor H. PMAA adsorbed primarily apolipoproteins, albumin, and fibrinogen.

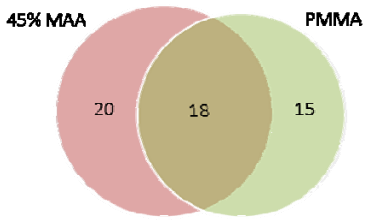

Figure 1. The Venn diagram illustrates the number of unique proteins adsorbed to each material.

As shown in Figure 2, SC5b9 concentrations were significantly lower in serum incubated for 90 min with MAA in comparison to PMMA (p=0.011), the negative control (p=0.045) and the positive control (p=0.003). There was no significant difference between MAA and the serum control (p=0.254) (n=3, different donors). In addition, C3a and C3a desArg concentrations were significantly lower in serum soaked with MAA in comparison to PMMA (p=0.003) and no significant differences between 45% MAA and the serum (p=0.212) and negative control (p=0.998) (n=3, different donors).

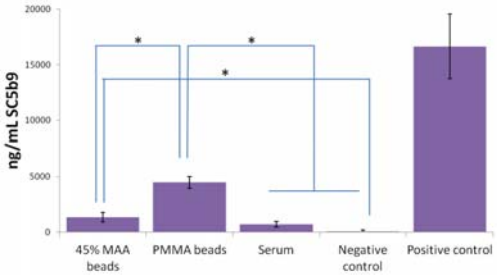

Figure 2. Complement activation was lower in serum incubated with 45% MAA beads.

Conclusions: Numerous complement proteins adsorbed onto 45% MAA beads and had minimal complement activation, as measured by C3a and SC5b9 activity. Conversely, PMMA beads primarily adsorbed common proteins such as apolipoprotein, albumin, and fibrinogen, and had significantly higher complement activation. The adsorbed C1q, C3 and C4 could have caused a decrease in complement activation or the adsorbed factor H could have increased complement inhibition. Studies show that lower complement activation reduces fibrotic responses to biomaterials. Furthermore, the binding of C1q to HUVEC has been shown to modulate cell behavior to potentially promote angiogenesis [4]. Therefore, complement inhibition is likely one mechanism contributing to the MAA-driven angiogenesis seen in previous studies.

While these in vitro studies used fresh serum to preserve complement protein activity, they do not include biological observations with cells or an in vivo wound healing environment. Therefore, future studies will focus on cell studies using complement inhibitors and on mouse models that include and exclude relevant complement factors to determine the impact of complement activation and inhibition on biological responses to 45% MAA.

References: 1) Martin DC, et al. J Biomed Mater Res A. 2010;93:484. 2) Eckhaus AA,et al. Plast Reconstr Surg. 2008;122:1361. 3) Wells, et al. I s J Chem. 2013;53:637. 4) Bossi et al. PNAS 2014;111:4209.