A Keratin Based Hydrogel is Hemostatic in the Porcine Lethal Extremity Injury Model with Platelet Aggregation as the Presumed Mechanism of Action.

Luke Burnett[1], Jillian Rouse[1], Clinton Orebaugh[2], Joel Berry[3], Roy Hantgan[2] and Mark Van Dyke[1]

1.Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine

2. Department of Biochemistry, Wake Forest University School of Medicine

3. Department of Physics, Wake Forest University

Statement of Purpose: Penetrating trauma with uncontrollable bleeding remains a leading cause of death in civilian trauma cases and in battlefield mortality. Recent analysis of autopsy data from US personnel killed in Iraq and Afghanistan showed that 82% of lethal injuries could potentially be survivable (Holcomb JB, J Trauma 2006;60:397-401). These data further show that there is a treatment gap with respect to stabilizing internal bleeding from penetrating trauma. Recently our lab has developed a keratin based hydrogel that is hemostatic in a liver injury model (in press). Here we show that this keratin hydrogel is also hemostatic in the porcine lethal extremity injury model. We propose that the mechanism of action is platelet aggregation. Our hypothesis is that keratin is a pro-adhesive protein with structural and functional similarities to fibrin and collagen that promotes platelet binding, activation and aggregation mediated by integrin receptors.

Methods: Keratin was tested and compared to a gauze only control and the HemCon® dressing. Animals were sedated and maintained on 2-3% Isoflurane. The jugular vein and carotid arteries were exposed and cannulated to measure MAP, and a speenectomy was preformed to limit fluid compensation. Lactated Ringers + Hextend solution was given at 3x the weight of the spleen. The femoral artery was exposed and a 2x6mm puncture was made using a vessel punch. 10cc of keratin was applied to gauze and placed over the injury followed by 3 min of manual pressure, 3 min of no compression and a final 3 min of pressure. The animals were then monitored for 3 hours. In vitro platelet aggregation to keratin, fibrin and collagen was measured using light and scanning electron microscopy, microtider assays and under flow conditions. Keratin solutions were allowed to cross-link and gel to slides and platelet rich plasma was added dropwise and washed at various intervals. Electron microsopy was used to identify pseudopods on keratin-adherent platelets, indicative of activated cells. Static and shear-dependant systems were used with β1 functional blocking antibodies to identify the specific integrins involved in the keratin mediated platelet activation cascade.

Results: The results from this study confirmed previous results in a rabbit liver injury model showing that keratin hydrogel is hemostatic (in press). Pigs that received the keratin treatment for a femoral artery puncture had dramatically increased survival times (figure 1), decreased MAPs and decreased shock indexs. Once applied, the keratin formed a cake that integrated with the tissue and vessel that stopped the arterial bleeding (figure 2). Platelet binding studies showed that keratin increased platelet adhesion as well as collagen and better than fibrin (figure 3). Further studies have shown that β1 integrin function-blocking antibodies dramatically reduced platelet adhesion to keratin in both static and shear dependent systems.

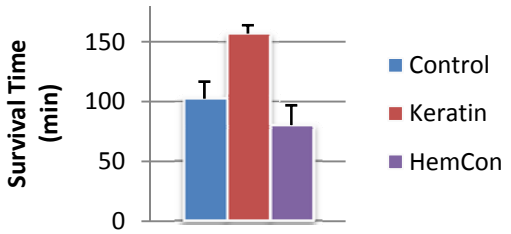

Figure 1. Preliminary data for 15 animals in three treatment groups; control gauze, keratin hydrogel and HemCon® shows that keratin increases survival times dramatically over the other groups tested.

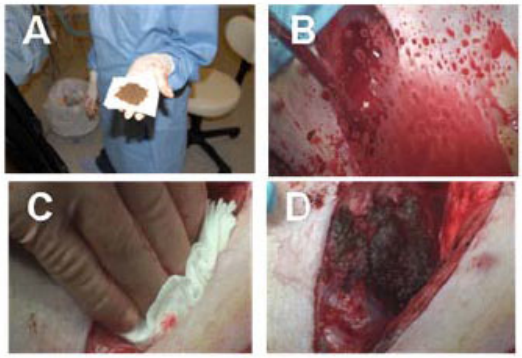

Figure 2. Keratin controls femoral artery puncture bleeding. A) 10cc of keratin hydrogel spread on 3x3 gauze. B) Artery is allowed to bleed for 45s, blood spray is blocked by a translucent face shield. C) Dressing is placed over the injury. D) Keratin gel shown after animal survived full 3 hours.

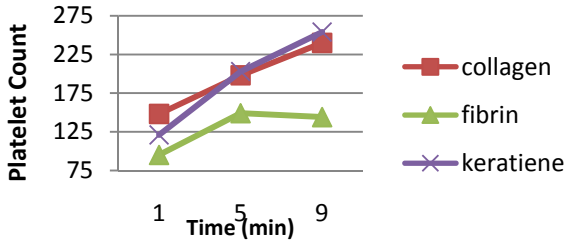

Figure 3: Platelet adhesion to slide coatings. Keratin increases platelet binding as well as collagen and better than fibrin coated slides.

Conclusions: These data show that keratin hydrogel is hemostatic in a porcine lethal extremity model. Keratin increased animal survival times and decreased both shock index and MAP. Analysis of the mechanism of action suggests that keratin acts by activating and aggregating platelets in processes mediated by the α2β1 or α5β1 integrins.