Self-Assembling Peptide Nanofibers RADA16 and IEIK13 for Rapid Hemostasis

by
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Blood loss and trauma remain a significant cause of mortality in both the military and for civilians. This mortality can be reduced through the development of hemostatic bandages to reduce bleeding and prolong life in the prehospital setting. Many successful hemostat materials exist but there is still nothing that meets all the criteria of the ideal hemostat: easy to apply, no negative side effects, long term shelf stability, thermal stability, cost effective, and bioabsorbable. Self-assembling peptide nanofibers RADA16 and IEIK13 provide a unique possibility to create a hemostat that meets all of these criteria. These short peptides self-assemble into nanofibers that entangle and gel in solution. Previous work with RADA16 has shown that the fibers seem to promote hemostasis by acting as a sort of pre-formed artificial clot that is able to collect and concentrate blood components leading to more accelerated clot formation. Through the use of layer-by-layer (LbL) we can apply these hemostatic peptides to a bioabsorbable substrate in thin conformal layers to produce a bandage that is lightweight and stable for long periods at room temperature.

In this thesis, we developed and optimized a new LbL system using the self-assembling peptide IEIK13 and validated it alongside a RADA16 based system. Dipping LbL on flat substrates was optimized and used to validate stability and film growth. These findings were translated into a spray LbL process allowing for coating of a three dimensional gelatin sponge substrate. These spray LbL methods were optimized to produce robust hemostatic films.

We examined possible mechanisms for RADA16 and IEIK13 to accelerate hemostasis by examining the effects on the activity of several clotting factors as well as effects on clot formation. While some effects on the activity of individual clotting factors were observed, in vitro testing using whole blood showed no significant differences in clot formation. This data supports the hypothesis that the peptide nanofiber mechanism is based on physically collecting blood components rather than triggering specific factors in the clotting cascade. A swine liver injury model was used for in vivo pilot testing in which reduced blood loss from RADA16 based films was observed. This further indicates that the blood flow present in vivo plays a role in the mechanism through which the peptide nanofibers promote hemostasis.

Finally, we developed entirely new LbL systems to through the addition of adhesive molecules, catechol functional groups and chitosan, to improve interaction between the coated dressings and wound tissue. We were able to show the application of these LbL films produced significant increase in tissue adhesion. These findings lay the groundwork for the development of a successful hemostatic bandage based on LbL films contain self-assembling peptide nanofibers.

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