An Antibacterial, Antioxidant Bioactive Composite Sponge Based on Biomimetic Gelatin and Alginate Dialdehyde for Hemostasis and Wound Healing

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Introduction: During major accidents and clinical surgeries, the failure to effectively control massive bleeding after severe trauma is the main cause of death. In order to save lives, rapid, safe, and convenient hemostatic materials are crucial. These materials are present in a variety of forms, including sponges, hydrogels, nanofibers, particles, sprays, etc. Among them, sponges are particularly effective due to their high porosity and large surface area. Their ability to accumulate red blood cells and platelets, enhances the local concentration of coagulation factors, thereby promoting efficient blood clotting. Anionic polysaccharides, such as alginate, are suitable candidate for hemostatic applications because of its high biocompatibility, hydrophilicity, and low cost. Since alginate is typically cross-linked with Ca^{2+}, it can exchange ions with body fluids, aiding in hemostasis. In this study, tannic acid (TA) was incorporated into an alginate dialdehyde-gelatin sponge, with Ca^{2+} introduced *via* $CaCO_3$ nanoparticles (NPs). TA facilated the integration and controlled release of Ca^{2+} into the alginate matrix, forming a composite hemostatic sponge to enhance matrix assembly.
Methods: Alginate dialdehyde (ADA) was synthesized by reacting $NaIO_4$ with sodium alginate. The reaction was stopped by adding 2 mL of ethylene glycol. The mixture was dialyzed and the aldehyde content was determined by alkaline hydroxylamine hydrochloride titration. Then, ADA was dissolved in water in the same concentration of gelatin (2%) and NPs were added to the solution. Finally, the samples were immersed in a TA solution and freeze dried.
Results: Different parameters, including the degree of oxidation of ADA, and concentrations of gelatin, ADA, NPs, and TA, were optimized to develop an ideal bioactive sponge with enhanced antibacterial and anti-inflammatory properties. The incorporation of TA not only improved the antibacterial properties of the sponge but also imparted antioxidant activity, contributing to suppression of inflammation during the wound healing process. Furthermore, *in vitro* hemocompatibility and hemostatis assessments have confirmed minimal hemolysis for the proposed sponge. These data suggest that both TA and Ca^{2+} play important roles in triggering blood coagulation due to their interaction with the coagulation pathway.
Conclusion: The synthesis of a new bioactive sponge from ADA, gelatin and TA was successfully obtained. Owing to the self-assembling properties of TA in solution and favorable molecular interactions between TA and gelatin, the hydrogel formation and crosslinking through multiple mechanisms were achieved. These include the egg-box structure formation with Ca^{2+}, Schiff base reaction between the aldehyde in ADA and the amine in gelatin and hydrogen interaction between TA and gelatin to form a hybrid hydrogel. Moreover, since the inherent antibacterial and antioxidant activity of TA, its incorporation significantly enhance the bioactivity of hybrid hydrogel and provide strong antibacterial activity and free radical scavenging capacity.
Keywords: bioactive molecules, biomimetic materials, hydrogels, sponges, wound healing, coagulation