Characterization of a Multi-Functional Tetronic Surgical Adhesive Containing Chitosan for Soft Tissue Applications

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Statement of Purpose: Previously, we demonstrated that bi-functionalization of Tetronic® T1107 with acryloyl chloride and N-hyrdoxysuccinimide (NHS), and chemical cross-linking with dithiothreitol (DTT) led to formation of a tissue adhesive that is highly compliant and stronger than those of other similar formulations[1]. To enhance its utility as a surgical sealant/adhesive, the present study aimed to add hemostatic function to the Tetronic hydrogel by conjugation of chitosan. Although chitosan has been known for superior biocompatibility and hemostatic activity[2,3], little has been achieved for development of a surgical adhesive that encompasses hemostasis and needed mechanical properties for soft tissue applications. Therefore, the objectives of the present study are to synthesize and characterize a multi-functional Tetronic adhesive comprising acrylate groups for bulk strength, NHS ends to activate the adhesive for strong covalent bonding with tissue and chitosan as a hemostatic agent.

Methods: *Preparation of multi-functional Tetronic:* T1107 ACR/NHS/CHIT was prepared by partial (2 of 4 arms) acrylation[1] and partial (2 of 4 arms) chitosan modification. Modification with chitosan was carried out by reacting T1107 ACR/NHS[1] with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and chitosan in 4-morpholino-ethane-sulfonic acid (MES) buffer (pH: 4.8) for 24 hours. The product was dialyzed against distilled water using a membrane (MWCO: 14,000) for 3 days and lyophilized.

Rheological analysis: The curing temperature of modified Tetronic hydrogels was characterized using rheological analysis. Specifically, TA Instruments AR1000 rheometer with parallel plate geometry (40mm diameter, 0.2mm gap) was used with a temperature sweep (4-40°C, 1°C min-1) and oscillatory shear strain (0.01) at constant frequency (0.1 Hz) to determine the gelation temperature of 400 µL of modified Tetronic, T1107 ACR, T1107 ACR/NHS and T1107 ACR/NHS/CHIT, hydrogels. The steep rise in hydrogel viscosity was used to determine gelation temperature.

Blood coagulation time: Lee-White's blood clot test method was adapted to examine the time it takes for various modified Tetronic polymer and hydrogels to coagulate fresh sheep blood. Briefly, fresh blood (1 mL) was mixed with either 50mg of modified Tetronic polymer or 100µL of hydrogel (no DTT) solution and incubated in 37°C water bath. Each vial was taken out of water bath every 15 sec and inclined (90°) to observe blood flow. The time when blood coagulates and does not flow was recorded.

Hemorrhaging testing: To test in vivo hemostatic properties of the modified Tetronic adhesive, a rat hemorrhaging liver model was used. After abdominal incision was made and securing the rat to a surgical board at 45° incline, the hepatic artery was clamped and a puncture on the liver was made using an 18G needle. Modified Tetronic adhesive (100µL) was immediately applied on the surface of the liver wound and the clamp was removed. After 3 min, the amount of blood absorbed on the filter paper was weighed (final wet weight) and reweighed after 3 days (final dry weight). No adhesive treatment was used as the control.

Results: FT-IR spectrum of multi-functional (T1107 ACR/NHS/CHIT) Tetronic indicates attachment of bi-functional (T1107 ACR/NHS) Tetronic onto the chitosan backbone. Rheology studies confirm all curing temperatures of modified Tetronic hydrogels are below physiological temperature (37°C) with T1107 ACR having a curing temperature at 14°C and multi-functional Tetronic hydrogel at 24°C. These results demonstrate the addition of chitosan within the modified Tetronic hydrogel provides a longer handling time before complete gelation. In coagulation studies, adding dried chitosan or modified Tetronic polymer to sheep blood significantly reduces the time to clot compared to no coagulant from 263 sec to 50-90 sec. Additionally, the multi-functional Tetronic hydrogel (128 sec) clotted blood significantly faster than bi-functional Tetronic adhesive (201 sec). The results of hemorrhaging testing revealed that the punctured liver with no adhesive treatment (positive control) bled an average of 600 mg (wet weight) and 147 mg (dry weight), whereas the adhesive treated punctures had minimal to no blood loss after accounting for the blood secretion from a non-punctured liver (negative control). Qualitatively, slight blood loss was observed from the liver treated with bi-functional adhesive, while the multi-functional adhesive maintained a strong bond with the tissue throughout the experimental period and there was no blood loss.

Conclusions: A multi-functional Tetronic adhesive was developed and characterized for its ability to stop blood loss from punctured liver. By incorporating chitosan within the bi-functional Tetronic adhesive[1], we not only added hemostatic functionality, but also improved the handling time for application of the hydrogel adhesive. However, further testing, especially in vivo, is needed to the determine cytotoxicity and biocompatibility of our multi-functional Tetronic tissue adhesive.

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