# Development of Bioabsorbable Chitosan Hemostatic Wound Dressing

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## Introduction

Exsanguinating hemorrhage is the leading cause of death of soldier in wartime and the second leading cause of death in civilian traumatic injuries. Quickly accessing and stabilizing the wound with effective hemostatic techniques is the key for life saving in these events. Historically, HemCon® chitosan hemostatic dressings (composed of chitosan with degree of deacetylation above 78% that is not readily bioabsorbable) were developed to address severe hemorrhage for external applications. In this study an absorbable, biocompatible chitosan (ABC) is prepared with a lower and narrow range of degree of deacetylation to provide a chitosan dressing material with good hemostatic properties that bioabsorbs without biodegradation induced fragmentation, and without adverse cytokine mediated inflammatory responses. The open structure, freeze dried porous matrix ABC dressing of the study is designed to facilitate biodegradation by providing enhanced cellular infiltration & proliferation. This study investigates bioabsorption kinetics and biocompatibility of the ABC chitosan dressing when placed intraperitoneal on an injured liver lobe and subcutaneously in a rat model.

## Materials and Methods

Test articles: The ABC chitosan was prepared at 24.8±6% DDA. Dressings were prepared by foaming 1% w/w ABC chitosan with 0.25% gelatin in an aqueous acetic acid solution, freeze dried at -45 ° C in a 2.5" diameter Teflon coated aluminum mold with lyophilization removal of ice. They were thermally treated at 100 ° C to bake off residual acetic acid (≤ 2.7±0.25%). The final test article was thermally pressed at 60 ° C to 1.2 mm thickness (from its original 5 mm) and close to 0.09 g/cm3 density. Test pieces were aseptically die cut to 6 mm diameter x 1.2 mm from 10 mm x 10 mm x 1.2 mm pieces that had been ethylene oxide sterilized.

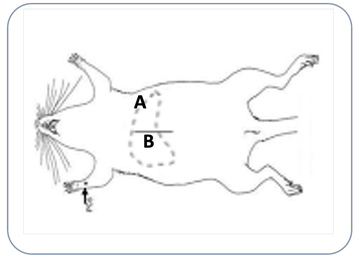


**Figure 1.** Illustration of position of implants of chitosan articles; A, Subcutaneous implantation, and B, Hepatic implantation.

| Group | 2d | 14d | 28d | 60d | 90d | **Total** |
|---|---|---|---|---|---|---|
| Number | 4 | 3 | 3 | 3 | 3 | 16 |

**Table 1.** Group and Number

## Animal Model

Sixteen female Sprague Dawley rats with body weight of 100-200 grams were used as models of intraperitoneal hepatic laceration injury and subcutaneous implantation(Figure1). Animal protocol was approved by OHSU IACUC under “Guide for the Care and Use of Laboratory Animals”.

**Subcutaneous implantation:** The upper abdominal area was shaved and an approximately 2 cm wide incision was made to create a ~2 cm long subcutaneous tunnel under the skin in the left lateral thoracic wall space. One 6.0 mm diameter dressing sample was placed into the left lateral thoracic wall subcutaneous space.

**Hepatic injury and repair:** A ~2 cm midline laparotomy was performed to expose the liver. A 2 mm long and 1mm deep laceration injury was created along the ventral surface of the right lobe of the liver. One 6.0 mm diameter sample of prototype dressing was placed on the hepatic injury. The test article was held in place for 2 minutes with light pressure to stop bleeding. The abdomen was closed with a 2-layer standard suture method and the rats were recovered from anesthesia.

Two 6.0-mm diameter test articles were implanted separately in subcutaneous space and on site of hepatic injury in each animal. The animals were monitored for 2, 14, 28, 60 and 90 days(Table1). At study endpoints, the animals were sacrificed and tissue samples were collected for histological evaluation.

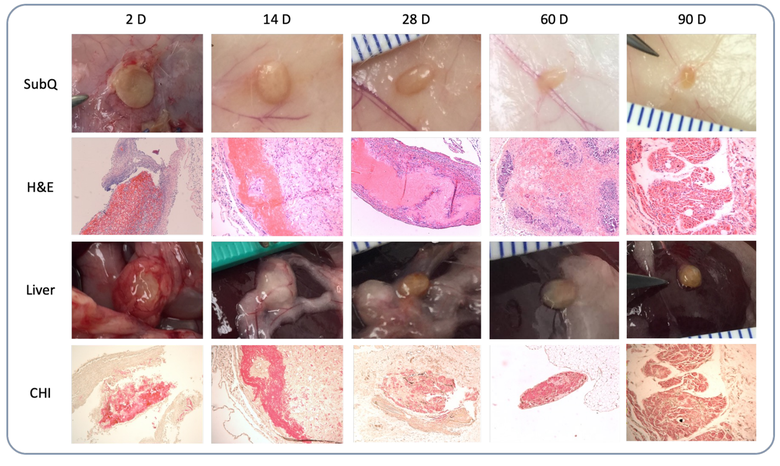


**Figure 3:** Pathology observation of the bioabsorbable Chitosan matrix in subcutaneous implantation (SubQ) and hepatic injury repair (Liver) in a rat model. The specimen were processed using Hematoxylin and eosin (H&E) stain and a chitosan-specific stain with Cibacron Brilliant Red-3BA and Iron Hematoxylin (CHI).

## Results

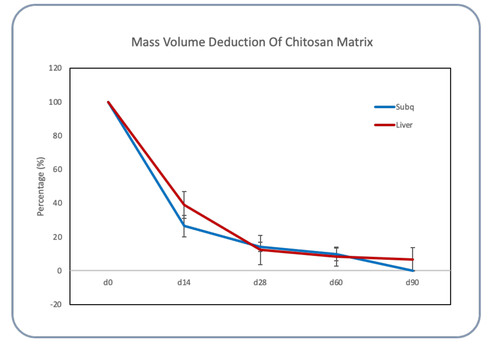


**Figure 4**. Percentage of mass volume deduction of Chitosan matrix over 90 days in subcutaneous implantation (Subq) and hepatic injury repair (Liver) in Rats.

All animals met their assigned study endpoints. There were no signs or symptoms of toxicity, allergic reaction, or infection. One animal in the 2-day group chewed through its sutures opening the incision after recovery from anesthesia. The incision was re-closed, and the animal survived without infection. One animal in the 14-day group demonstrated delayed wound healing, without any indication of infection. The delayed wound healing most likely was related to scratching or chewing of the wound by the recovering rat. The greater part (27/32) of the implanted samples were recovered and harvested from the animals. Individual implant samples were recovered substantially as tissue encapsulated single whole pieces. Gross examination at sample harvesting of the surrounding tissue area of the subcutaneous implants demonstrated absence of infection with normal wound healing response at all study points. At the hepatic injury sites, the injuries healed well in all study endpoints without any sign of local infection and tissue adhesion. All chitosan hemostatic dressings were dislodged from their original hepatic injury sites, relocating in the abdominal cavity where they were found wrapped in a thin layer of omentum. The size (estimated sample volume based on disc & ellipsoidal models) of the harvested samples decreased consistently with time of implantation (Figure 3&4). There was significant sample volume decrease at both subcutaneous and hepatic sites over the study timepoints.

## Conclusions

1. This study demonstrated bioabsorption kinetics and biocompatibility of the ABC chitosan matrices in both subcutaneous and intraperitoneal implantation model.
2. Learning objective 1: ABC chitosan dressing implant material was absorbed within 60-90 days in hepatic and subcutaneous implantation sites.
3. Learning objective 2: ABC chitosan dressing substantially meets biocompatibility requirements without systemic and local adverse responses.
4. Learning objective 3: ABC chitosan dressing does not result in surgical adhesion formation greater than Grade 2 (no surgical adhesions were observed) in the rat hepatic injury model.