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HEMOSTATIC ACTIVITY OF CHITOSAN IN WOUND MANAGEMENT

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Research has continued on the preparation of a hemostatic agent in the form of a lyophilized sponge composed of chitosan glutamate and collagen. Characterization of the chitosan glutamate raw material has progressed; levels of metal and amino acid contamination were determined. Work was also initiated on the in vitro degradation of chitosan glutamate by lysozyme. Over 25 sample compositions of various chitosan glutamate/collagen sponges were evaluated for wet and dry strength, handling characteristics, and hemostatic activity in a rat bleeding sagittal sinus model. From these samples, two compositions were identified for further testing. Semex Medical was identified as the manufacturer of the sponge samples.

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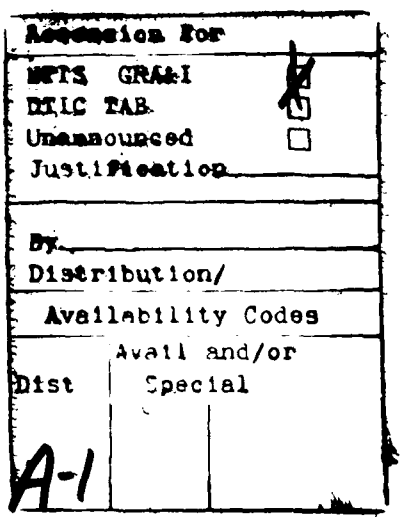

1.0 INTRODUCTION

This report covers progress during the second three months of the Office of Naval Research Contract N00014-89-C-0024. The various sections of the report are numbered and titled using the format of the original proposal.

2.0 PROGRESS REPORT

2.1 Task 1: Raw Material Characterization

During the past quarter, additional work has been completed on the characterization of chitosan glutamate supplied by Protan, Inc. 3M has also met directly with Protan in order to ensure that their manufacturing facilities for production of the ultrapure grade of chitosan glutamate are operating according to Good Manufacturing Practices. Compliance with GMP's will be essential since their product will be incorporated into an implantable device.

2.1.1 Subtask 1.1: Quality Assurance Testing

Characterization of ultrapure Protan chitosan glutamate (Protasan LV lot no. 808-572-01) has been completed by 3M Analytical Research. Heavy metal analysis was completed using Inductively Coupled Plasma Spectroscopy and Cold Vapor Atomic Absorption (for mercury). The following results (Table 1) indicate that there are not significant levels of heavy metal contamination in this ultrapure chitosan glutamate.

3M has continued to work on spectroscopic methods to determine the percent deacetylation of chitosan salts. It now appears that deacetylation measurements on the free base chitosan are more reliable than direct measurement on the chitosan glutamate salt. Furthermore, since these measurements are done by Protan prior to formation of the chitosan glutamate, 3M will rely on the vendor's determination. 3M will, however, try to secure retain samples of the chitosan free base of each lot so that an independent determination of the percent deacetylation will be possible.

TABLE 1. Heavy Metal Levels in Chitosan Glutamate Lot No. 808-572-01.

Element	Level Detected (ppm)	Element	Level Detected (ppm)
Ag	<7.5	Mo	<0.5
Al	<7	Na	112
As	<1.3	Ni	<1.0
Au	<1.1	P	<6.9
B	<2.4	Pb	<3.2
Ba	<0.19	Pt	<4.5
Ca	32	Sb	<2.3
Cd	<0.23	Se	<1.5
Co	<0.7	Si	<44
Cr	<4.1	Sn	<1.0
Cu	<5.2	Te	<2.1
Fe	76	Ti	<0.3
Hg	<0.6	V	<0.6
Mn	<0.8	Zn	<3
		Zr	<1.4

3M has also received analysis reports on the collagens (both Semed S and Semed F) used by Semex Medical in the preparation of sample hemostatic sponges. These reports include appearance, total solids, ash, pH of a 1% solution, nitrogen percent, fat, plate count, and hydroxy proline content. Semex Medical operates their facility according to the guidelines of GMP's so acceptable product traceability is already in place with this vendor.

2.1.2 Subtask 1.2: Protein Content Studies

Initial gel electrophoresis experiments on the chitosan glutamate indicate the absence of significant amounts of high molecular weight protein. The detection limits for this experiment were 200 micrograms protein/gram chitosan glutamate. Further experiments in which chitosan glutamate samples have been analyzed by HPLC for amino acid content have shown essentially no amino acid contamination except for the expected glutamic acid residues. Work continues in this area.

2.1.3 Subtask 1.3: Biodegradation Studies

3M has initiated experimentation to evaluate the in vitro lysozyme degradation of the chitosan glutamate used in the hemostatic agent. Using the methodology reported by Pangburn et al[1], 3M has results which indicate that the chitosan salt in solution is enzymatically degraded by lysozyme. Further experimentation will include examination of the degradation profile of a lyophilized sponge. In vivo studies await further developments in formulation and optimization of the product form (see Task 2).

2.1.4 Subtask 1.4: Molecular Weight Characterization

3M has been in contact with Professor Olav Smidsrod who is located at the Trondheim Institute of Technology, Norway, and a recognized expert in polysaccharide chemistry. His group has initiated some work on molecular weight determination of chitosans using chromatographic techniques. His experience thus far is that chitosan non-specifically binds to standard chromatographic separation media so that no reproducible and reliable information can be gained using standard techniques. This is similar to the results that 3M had obtained early in our work with chitosan. 3M is, therefore, not actively pursuing the accurate determination of chitosan molecular weights. Smidsrod anticipates initiation of a research program directed at this problem, and 3M will stay in contact with him to keep abreast of all the latest developments. As was shown earlier, the molecular weight of chitosan (as long as it's above an empirically determined minimum) has only a minimal effect on the time to stable coagulum formation in vitro. Therefore, 3M will continue to monitor molecular weight of samples with intrinsic viscosity measurements to ensure that samples are of sufficient molecular weight to coagulate blood. When more precise methods become available, 3M will incorporate them into its testing scheme.

2.2 Task 2: Formulation and Optimization

As described in the last quarterly report, the hemostatic agent is a lyophilized sponge composed of chitosan glutamate and collagen. Although 3M has continued to make a limited number of trial compositions in our laboratory, it has been decided to utilize Semex Medical as the source for all prototype lyophilized sponge compositions. This decision was based on

the high quality of the sponges delivered to 3M by Semex Medical and their ability to scale-up any composition for further testing.

During the past quarter, 3M evaluated 23 different sponges supplied to us by Semex (to our specifications) and 5 compositions prepared in our laboratory. The evaluations consisted of dry and wet strength testing, dry and wet handling properties, and hemostatic activity in the rat sagittal sinus bleeding model described in the grant proposal. Based on the results of these evaluations, two compositions from Semex Medical (S-1573 and S-1582) will receive further testing. Additional samples (80 square inches) of these compositions made by two different processes have been ordered from Semex and should be available during the second quarter. It is anticipated that the final product form for the sponge will be chosen from these samples, and, in doing so, 3M will have met the first project milestone identified in the project proposal. Once the final product form has been identified, in vivo work with the hemostatic agent can commence.

2.3 Task 7: Sponge Manufacturing Scale-Up and Process Validation

The decision to utilize Semex Medical as the supplier of the lyophilized final product sponge significantly affects the ease with which Task 7 will be completed during years two and three of the contract. The prototype sponges are being prepared by Semex with processes which are known to be applicable to larger scale manufacturing. The scale-up and process validation for this product, therefore, should be rather uncomplicated and not require as long as originally anticipated in the original proposal. This also results in 3M testing prototype products with the added assurance that any composition can be scaled-up, if appropriate.

3.0 CONCLUSIONS

Research has continued on the "Hemostatic Activity of Chitosan in Wound Management" project. Laboratory work toward the characterization of chitosan glutamate has progressed. Over 25 prototype compositions of lyophilized sponge hemostatic agents have been evaluated during the last quarter. From these sample compositions, two have been selected for further evaluation. It is anticipated that the final product form will be selected

from these compositions. Semex Medical has been identified by 3M as the short and long term supplier of lyophilized sponge materials.

4.0 REFERENCES

1. Pangburn, S.H., Trescony, P.V., and Heller, J., "Lysozyme Degradation of Partially Deacetylated Chitin, Its Films and Hydrogels," Biomaterials, 3, 105 (1982).