

METHOD OF ACHIEVING HEMOSTASIS, INHIBITING FIBROPLASIA, AND PROMOTING TISSUE REGENERATION IN A TISSUE WOUND

BACKGROUND OF THE INVENTION

This is a continuation-in-part of our co-pending application, Ser. No. 251,321 filed April 6, 1981 now U.S. Pat. No. 4,394,373.

In applicants' earlier application, a method was described for achieving hemostasis. Since the filing of the co-pending application, the inventors have discovered that chitosan may be used to inhibit fibroplasia, and to promote tissue regeneration.

Science has long sought a method to inhibit the synthesis of collagen in wound healing. Much of the work directed to the inhibition of the synthesis of collagen has involved alteration of the biology of collagen by various chemical antagonists. Lower animals do not heal by scar tissue but generate normal structures from pre-existing cells. Usual wound healing begins with a blood clot containing a fibrin network, along which fibroblasts begin the process of fibroplasia. If blood loss is controlled in the presence of a fibrin clot, fibroblasts will be stimulated. Conversely, if blood loss can be controlled in the absence of a fibrin clot, fibroblasts may be not stimulated, and differentiated cells may have the opportunity to replace the lost tissue. It is has therefore been found that a material is needed to control blood loss absent the usual blood clotting factors, and to allow the ingrowth of normal tissue elements.

Prior art teaches that Chitin and some chitin derivatives accelerate tensile strength of wounds by speeding the fibroblastic synthesis of collagen in the first few days of wound healing. For example, see U.S. Pat. Nos. 3,902,268; 3,911,116 and 3,914,413. This topic is also discussed in the May 1970 issue of American Journal of Surgery, pages 560-564. The subject is further discussed in the June 1969 issue of S.G. & O., pages 1321-1326. It should be noted that the prior art only discusses Chitin and certain derivatives thereof which are entirely different than the de-acetylated chitosan employed in the method of this invention and which will be discussed in more detail hereinafter. The basic structure of natural Chitin is a polymer of N-acetylglucosamine. Although, Balassa describes several molecular modifications and shortenings of chain lengths, Balassa retains the N-acetyl structure on each monomer.

Therefore, it is a principal object of this invention to describe a method of treating a tissue wound so as to achieve hemostasis, to inhibit fibroplasia, and to promote tissue regeneration.

Still another object of the invention is to provide a method of treating a wound whereby chitosan solution, or solid chitosan sheets, powders, or fibers are placed in contact with a tissue wound thereby forming a coagulum to prevent bleeding and to negate the formation of a blood clot to prevent the formation of fibrin strands which in turn prevents the proliferation of fibroblasts and the synthesis of collagen, thus allowing the promotion of normal tissue regeneration.

Still another object of the invention is to provide a method of inhibiting fibroplasia and promoting tissue regeneration in vascular grafts by placing chitosan in contact with the graft or by incorporating the chitosan in the graft material.

Still another object of the invention is to provide a method of achieving hemostasis.

These and other objects will be apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart which illustrates the results of the chitosan hemostatic solution wound healing evaluation.

DESCRIPTION OF THE PREFERRED METHOD

As described in Balassa Pat. No. 3,804,949, the term "chitin" embraces naturally occurring chitin, synthetic chitin as well as poly(N-acetylglucosamine) and its epimerpoly(N-acetylgalactosamine). Suitable sources of chitin are from lobsters, shrimp, other crustacea and fungi. Chitosan is a derivative of chitin and the method of preparing chitosan is described in U.S. Pat. No. 3,533,940 and made a part hereof. Chitosan as used herein refers to a de-acetylated chitosan. Analysis of the chitosan material used herein reveals that most of the acetyls (78-92%) have been removed therefrom leaving a very reactive free amine group (NH₂) on the second carbon of most of the glucosamine monomers.

The chitosan used in this method is a partially deacetylated chitin and is a partially depolymerized chitin to form a polyglucosamine chain linked by Beta 1-4 glycosidic bonds with most acetyl groups removed from the number two positions to a 70-92% de-acetylation. Molecular weight determinations may be made by the method of Wu and Baugh (Journal of Chromatography 128, pages 87-99 (1976)). The degree of de-acetylation of the chitosan may be determined by the method of Hayes and Davies, Proceedings of The First International Conference on Chitin/Chitosan, 1978, pp. 193-199. Chitosan with defined physical and chemical properties may be prepared from any natural source of arthropod exoskeletons or fungal cell walls, by controlling the processes of de-acetylation and depolymerization.

The chitosan employed in this method may be purchased from Kypro, Inc., 208 Carlson Building, Bellevue, Wash. and identified as "CHITOSAN-High Viscosity". The chitosan employed in this method is a mixture of polymers with a molecular weight span from 10,000 through 2,055,000, and with individual molecules 78-92% de-acetylated. In those chitosans tested, the most abundant molecular species have a molecular weights of 1,487,000 to 1,682,000 and a number average of 129,000 to 322,000 with a dispersity of 5. The product is 78-92% de-acetylated with a mean of 85% deacetylation. Chitosan in solution, and solid chitosan as fibers, sheets or powders were used.

Although the chitosan employed in the various experiments set forth herein was purchased from Kypro, Inc., the term "chitosan" is used by several suppliers to denote a product derived by partially de-acetylating chitin. It is with the chitosan purchased from the various sources that the experiments herein began.

The methods of preparing the chitosan so it could be used in the various experiments are described in detail hereinafter with the preferred preparation methods and proportions being described. However, Table A set forth hereinbelow lists the characteristics (preferred and permissible) of the chitosan material of this invention.

Chitosans with various sources of origin, states of depolymerization and/or states of de-acetylation were dissolved at a concentration of 2 grams per liter, in