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*School of Medicine
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January 14, 1993

Commander Peter Kent, MD
Office of Naval Research
Combat Casualty Care Research Area
Naval Medical Research & Development Command
Naval Medical Command, National Capitol Region
Code 405
Bethesda, MD 20814-5044

Subject: Periodic Report for Award N00014-90-J1797
Liquid Collagen Wound Coverings

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Dear Commander Kent:

Attached is a brief summary of research progress since our last report of May 15, 1992.

Yours sincerely,

J. Peter Bentley, PhD
Professor of Biochemistry
and Molecular Biology

cc: Administrative Grants Officer
Director, Naval Research Laboratory
Defense Technical Information Center
Office of Chief of Naval Operations
Bureau of Medicine and Surgery

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Freeze Dried Collagen Preparations

Sealing of flasks containing sterile lyophilized collagen, in plastic-lined foil pouches purged of atmospheric air which is replaced by dry nitrogen, has been successful.

Recently, difficulties with lyophilization of sealed-up pouches has been a problem, specifically an unacceptable amount of 'meltback' leading to evaporation rather than sublimation. Collagen which has been subjected to 'meltback' fails to rehydrate in an acceptable manner. The Research Department at Oregon Freeze Dry will work to pinpoint and correct the cause of this problem.

The successful use of a 'Gortex' membrane bag for lyophilization of blood products by Oregon Freeze Dry Research has given rise to the concept of a multichamber bag constructed on one side from the 'Gortex' membrane and on the other by clear impervious plastic to allow visual examination of the lyophilized and the rehydrated collagen. The final product is to be packaged in similar plastic-lined foil pouches with dry nitrogen replacing atmospheric air as presently used for flasks.

Vehicle for Growth Factors

We have conducted both in vitro and in vivo studies to determine the feasibility of using DOPA and/or iodine crosslinked bovine Type I collagen as a delivery vehicle for growth factors. The growth factor used is Basic Fibroblast Growth Factor (B-FGF) which has been provided to us through the generosity of Scios Corporation, Mountain View, California. This material has been shown to enhance wound healing when applied topically in a liquid vehicle.

In Vitro Studies

Human fibroblasts were plated in 24 tissue culture plates, together with appropriate medium, and containing 5 μ /ml of insulin. After two days aliquots of growth factor in isotonic saline (as a control) or pre-formed iodine crosslinked collagen gels, with and without varying amounts of FGF, were added to the wells. After another 20 hours the disks were removed and cells labeled for 4 hours with ^3H thymidine to measure cell growth. The disks were fixed and stained for histological evaluation. The cells were found to respond in a dose-dependent manner to the addition of FGF to the medium. However, when FGF was added to the collagen disks the results were quite variable. In

some cases, stimulation of growth was seen and in others, no effect. It was postulated that perhaps the cells in the medium had migrated into the disks and thus an apparent decrease in growth rate was seen when the remaining cells in the medium were tested. This experiment will be repeated.

In Vivo Studies

This study is to evaluate the effectiveness of a DOPA crosslinked collagen gel as a slow release vehicle for FGF in the rabbit ear cartilage model which we have developed. New Zealand white rabbits were anesthetized and a slit made through the inside of the ear. The skin was separated from the cartilage using blunt dissection. A 3 mm punch was made through the cartilage which was then removed. The cartilage plug was replaced with either nothing (control), cartilage, or a DOPA crosslinked collagen disk containing varying amounts of FGF. The wound was closed with sutures and the rabbit allowed to recover. After 1, 4, 7, or 14 days the animal was sacrificed and the wounds removed and processed for histology. As of this date the processing is incomplete, but on a preliminary basis we can state that there appears to be a technical problem in that some of the implants slide out of the hole or wound area. It is clear, however, that wherever the cartilage is adjacent to a collagen disk containing FGF the perichondrium responds strongly at 7 days and new tissue growth can be demonstrated. This clearly indicates that the methodology will permit the use of DOPA crosslinked collagen as a delivery vehicle and currently another study is planned which will permit us to implant the disk more firmly, and we will extend the observation time to 8 weeks.

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