CHRONIC WOUND: IN VIVO GENE TRANSFER OF GROWTH FACTOR

Thesis

Submitted for Partial Fulfillment of M.D. Degree in Plastic Surgery

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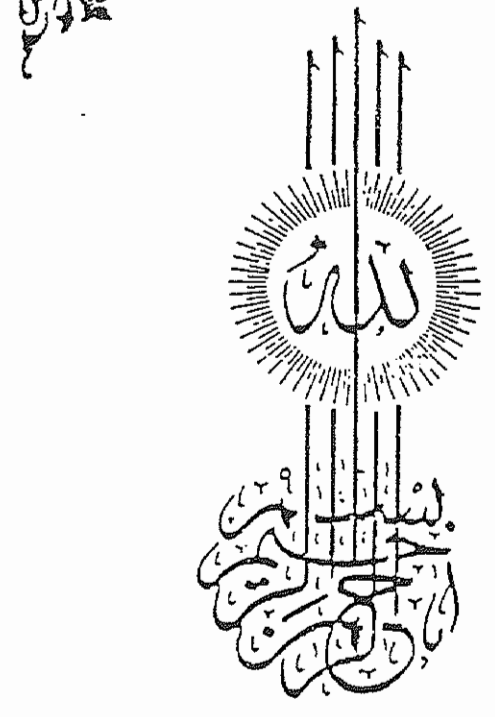

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ.

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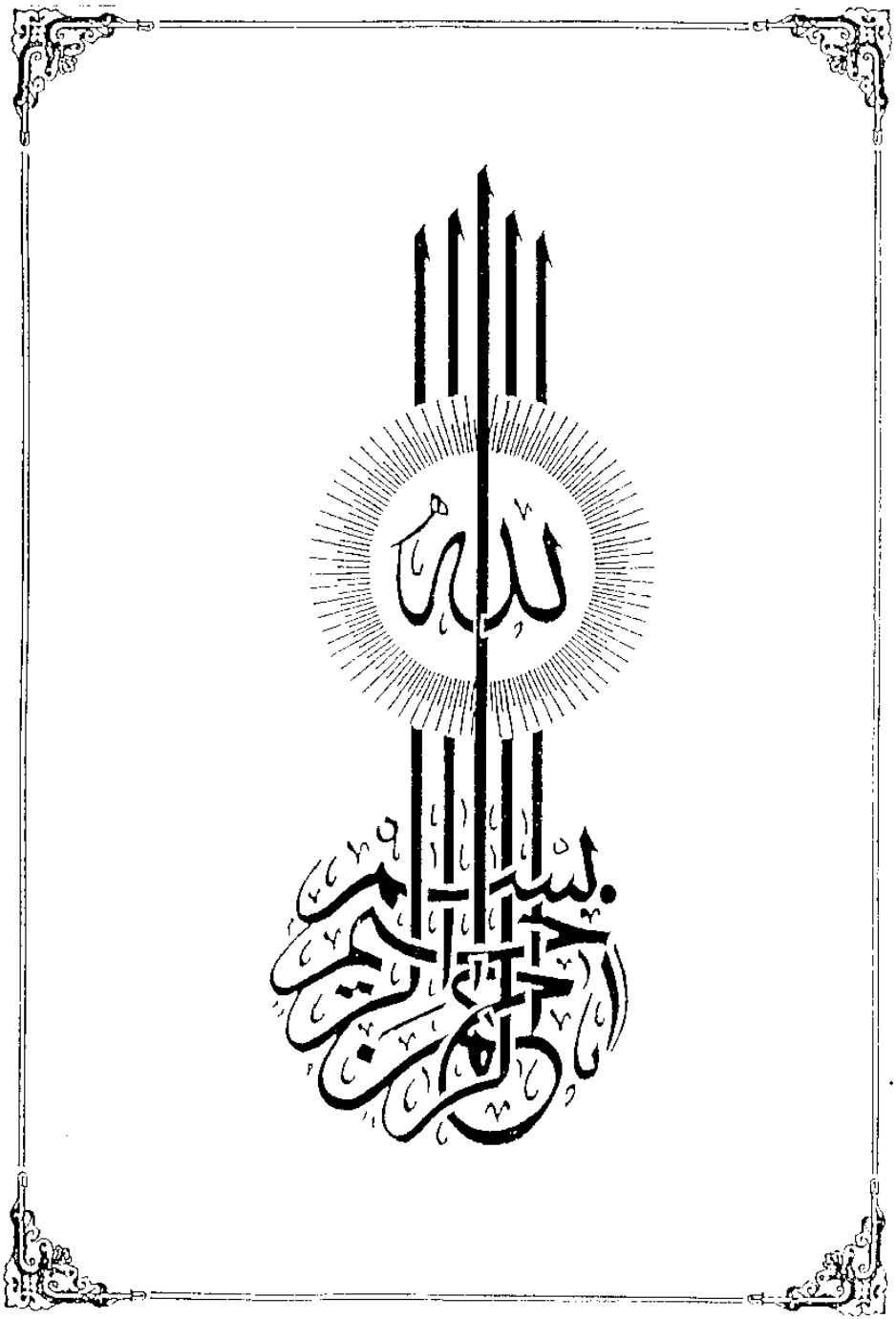


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TO my Family , Asem and Yara

TO my Family Asem and Yara


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I reserve my last words for my family, my husband and my daughter; whose belief, endurance, patience and great solicitude were necessary conditions for seeing this work through.

Samia M. A. Saied; 1999


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I. Introduction

In recent years, the ability to perform tissue and organ replacement has led to an improvement of quality of life and health care. Most of this success can be attributed to the interdisciplinary approaches to tissue engineering. Indeed, today scientists with very diverse backgrounds such as molecular, cellular and developmental biologists collaborate with bio-mechanical engineers to develop tissue analogues that allow physicians to improve, maintain and restore tissue function.

One of them involves the use of matrices containing specific cells and growth factors which can be used as tissue replacements.

In 1980, Yannas and Burke published the use of a bilayer "artificial skin" as a wound coverage after excision of the eschar in burned patients with major thermal injury (Yannas and Burke, 1980). This membrane was constructed as a bilayer consisting of a deep layer as dermal replacement made of a porous matrix of fibers of cross-linked bovine tendon collagen and glycosaminoglycan (chondroitin-6-sulfate). As "epidermal substitute" a silicone membrane was attached to the material on top (Fig. 1). This trial synthetic membrane was used to close excised burn wounds for periods up to 46 days before the silastic cover was removed and replaced with epidermal auto-grafts.

on the other hand, recent advances in molecular biology have resulted in the development of new technologies for the introduction and expression of the genes in human somatic cells. This emerging field, known as gene therapy, is broadly defined as the transfer of genetic material to cells/tissues/organs in order to achieve a therapeutic effect. Although this technology was originally developed as a potential means for the treatment of inherited diseases, gene therapy technologies are now under consideration for a variety of acquired diseases including cancer and infectious diseases.

There are numerous technologies has been developed for the delivery of genes. Genes can be delivered to cells either ex vivo or in vivo. The clinical success of these ex vivo and in vivo strategies will ultimately depend on their suitability for the treatment of specific diseases. Two primary considerations must be kept in mind for all ex vivo approaches. First, the target cell must be amenable to culturing ex vivo and adequate culture condition must be available to produce sufficient numbers of cells. Second, after the cells are genetically modified there must be an effective means for the delivery of the cells to the target organ (Morgan and Yarmush, 1998).

There are qualifications to permanent genetic modification, gene expression from these introduced genes can sometimes be variable, especially in vivo modified cells, expression of the genes can decline with time. This can be due to either down regulation of the genetic elements which control gene expression or loss (apoptosis, immune recognition) of the genetically modified cells. The net result is that the patient's genetic modification was only temporary. Allogenetic cells engrafted and made to secrete growth factors useful for tissue repair.

Gene transfer in tissue repair has been focused on growth factor gene expression. Studies have demonstrated that recombinant growth factor proteins have the potential to stimulate the wound healing response in various organ systems. However, an important feature to the efficiency of these proteins in vivo appears to be the sustained delivery to achieve optimal repair. Current limitations in the effectiveness of topically applied growth factors to non healing wounds might be rapid half-lives of the cytokines in vivo, toxicities at high doses and lack of bioavailability of growth factors from saline or cream vehicles (Roemer and Friedmann, 1992).

Genetic engineering of cells involved in tissue repair process has the potential to provide an effective means for the sustained delivery of these growth factor proteins and might therefore be superior to the topical application of these molecules. A cell-based method for delivery of

growth factor expression is directed to a specific type which provides control over the localization of protein expression and may lower the toxicity and improve the efficacy of the pharmaceutical reagent (Mulligan, 1993).

Growth factor gene therapy avoids cumbersome and expensive purification steps of the growth factor protein. In addition, cellular engineering combined with bio-materials holds a great potential to enhance the function of cell-biomaterial composites used in tissue reconstruction. The development of gene transfer strategies that may be used in conjunction with conventional medicine will provide new powerful therapeutic options for wound healing.

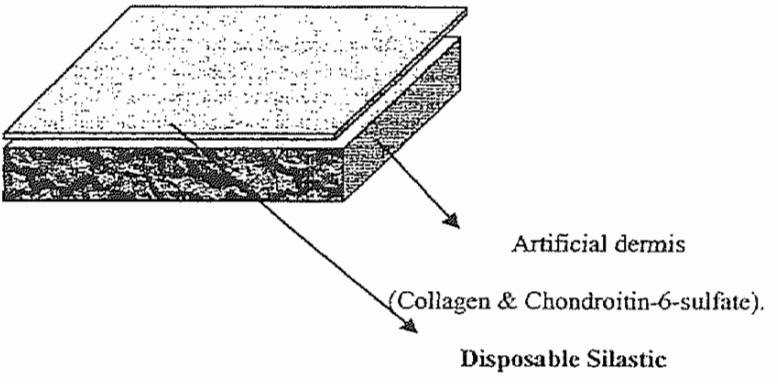

Fig. 1. Diagram of the bilaminar artificial dermis used in this study.