SUMMARY OF Ph.D. DISSERTATION

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Title

Study of the application of laser-induced stress wave-driven gene transfection to tissue engineering

Abstract

Gene therapy has a great potential for improving the outcome of tissue engineering. Several types of growth factors are known to enhance angiogenesis and wound healing. Thus, the secretion of such growth factors can be upregulated by gene transfection to enhance the adhesion of transplanted tissues. For successful gene therapy, however, it is necessary to develop a safe and efficient method to deliver foreign DNA into targeted cells and tissues. Although various kinds of technologies have been investigated for gene transfer, safe and efficient methods have not been developed yet.

Laser-mediated gene transfer is a promising nonviral method for targeted gene transfection because of the high spatial controllability of laser energy. In addition, since laser energy can be transmitted through an optical fiber, catheter-based gene transfer may come into practical use. For gene transfection, lasers have been used mainly for direct irradiation of cells or tissues to perforate cell membrane. However, the direct irradiation approach cannot be applied to deep tissue because optical penetration depth is limited. Gene can also be transferred by use of stress wave generated by irradiating a solid target with nanosecond laser pulses (laser-induced stress wave, LISW). By using this method, a large number of cells can be transfected simultaneously, and, in addition, deeper tissues can be treated because stress waves can propagate in optically high scattering tissue media much more efficiently than can laser light.

The goal of this dissertation is to apply LISW-mediated gene transfer to tissue engineering. *In vitro* experiments reveal the dependence of transfection efficiency on the laser irradiation conditions. Gene transfection of various types of cell lines is also demonstrated. Based on the results, therapeutic gene delivery is performed to demonstrate the usefulness of LISW-mediated gene transfer for tissue engineering. Delivery of a therapeutic vector construct carrying human hepatocyte growth factor (hHGF) gene to skin grafts of rats is investigated to demonstrate the enhancement of their adhesion.

Chapter 1 presents an overview of gene therapy and gene transfer, and describes the aim of this study. In Chapter 2, the theory of LISW generation and the experimental results of LISW used for gene transfection are described. Chapter 3 presents the gene transfer results into cultured cell lines by using LISW. The dependence of transfection efficiency on the LISW parameters is described. Chapter 4 presents the transfer of hHGF-gene into skin grafts of rats to study the enhancement effect of the adhesion of transplanted skin. The enhancement of angiogenesis in the grafted skins that were transfected using LISW is demonstrated. In Chapter 5, the LISW interaction with tissues and transgenes is described. The mechanism of LISW-driven gene transfer *in vivo* is discussed. In Chapter 6, the results of this study are summarized, and the conclusion and future perspective are given.