Ain Shams University
Faculty of Science
Biochemistry Department

Development of Tissue - Engineered Corneal Substitutes from Fish Scale - Derived Collagen

A Thesis

Submitted for the degree of Master of Science

As a partial fulfillment for requirements of the master of science

Dalia Ahmed Mohamed Hamza
(B.Sc. in Biochemistry 2010)

Under the supervision of

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Medical Biotechnology
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Declaration

I declare that this thesis has been composed by myself and that the work of which it is a record has been done by myself. This thesis has not been submitted for a degree at this or any other university.

Dalia Ahmed Mohamed Hamza

ABSTRACT

Dalia Ahmed Mohamed Hamza. Development of Tissue - Engineered Corneal Substitutes from Fish Scale - Derived Collagen. Unpublished Master of Science Thesis, Department of Biochemistry, Faculty of Science, Ain Shams University, 2015.

A tissue engineered corneal substitute is considered a promising solution to overcome the limitations of corneal replacement with allografts and drawbacks of keratoprostheses. The purpose of this study was to evaluate the suitability of collagen obtained from tilapia (*Oreochromis niloticas*) scales as an alternative source for common collagen sources including bovine skin and tendons, porcine skin and rat tail in preparation of tissue engineered corneal substitutes. Yield of pepsin solubilized collagen obtained from tilapia scales using a simple method consisting of four sequential steps on dry and wet weight basis was promising. The extracted fish scale collagen was identified using sodium dodecyl sulphate polyacrylamide gel electrophoresis which showed that collagen was composed of more than one subunit with high molecular weight. The pepsin solubilized collagen was dissolved in acetic acid to give an acidic transparent solution that used only and with other four crosslinking agents: glutraldehyde (GLU), tannic acid (TA), gallic acid (GA) and [1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/ N-hydroxysuccinamide (NHS)] to prepare five different formulations of collagen scaffolds. Optical and biological characterization was carried out for the five collagen scaffolds. Besides, thickness and water content of the five scaffolds were determined. The five collagen scaffolds revealed lower thickness in comparison with human cornea. The water content of three scaffolds (fish scale collagen scaffold, fish scale collagen scaffold crosslinked with EDC/NHS and fish scale collagen scaffold crosslinked with TA) was comparable with that of the human cornea. The light transmission of three scaffolds (fish scale collagen scaffold, fish scale collagen scaffold

crosslinked with GLU and fish scale collagen scaffold crosslinked with TA) was comparable with that of the human cornea. The biocompatibility of two scaffolds (fish scale collagen scaffold and fish scale collagen scaffold crosslinked with EDC/NHS) was superior to the positive control. These results were considered preliminary promising results in cornea tissue engineering.

Key Words: Cornea, Collagen, Tissue Engineering, Scaffold, Fish Scales, Tilapia (*Oreochromis niloticas*).

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LIST OF ABBREVIATION

ASC	Acid-solubilized collagen
PSC	Pepsin-solubilized collagen
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
WSCs	Water-soluble carbodiimides
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
NHS	*N*-hydroxysuccinimide
GLU	glutraldehyde
TA	Tannic acid
GA	Gallic acid
PBS	Phosphate Buffered Saline
MTT	3-(4,5-dimethylthiazoly-2-yl)-2,5-diphenyltetrazolium bromide
PBMCs	peripheral blood mononuclear cells
OD	Optical density
TE	Tissue Engineering
DMEM	Dulbecco’s modified Eagle's medium

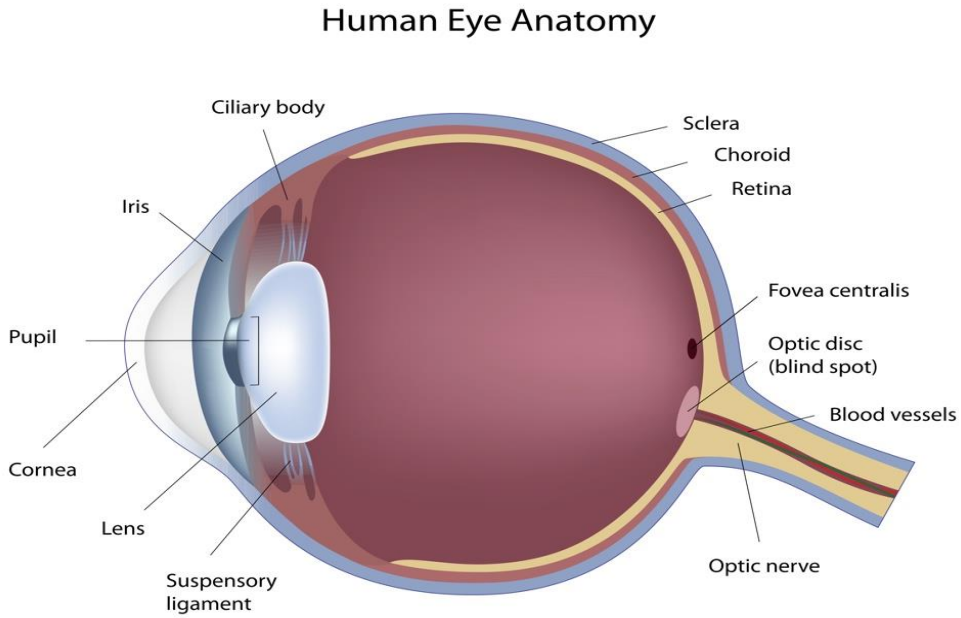
Introduction

1- Introduction

1.1 Anatomy and Functionality of the Cornea.

The human cornea constitutes the anterior, central portion of the eye that is responsible for most light transmission and refraction to the retina for vision (1). The cornea offers 75% of the refractive power of the human eye, allowing transmission of light through it to be focused onto the retina. As well as photo-protection, by significant absorbance of UV radiation (2), the cornea acts as a thick, elastic physical barrier protecting the internal ocular structures from outside insults, which may be physical, chemical or microbial. Furthermore, the cornea withstands changes in intraocular pressure (IOP) and curvature changes of the eye (1).

Figure 1.1 Anatomy of the eye and the cornea (3).



The cornea is a smooth, clear tissue with a thickness that is approximately 0.52 mm centrally and 0.65 mm peripherally and its average horizontal diameter is 11.7 mm (4). Unlike most tissues in the body, the cornea contains no blood vessels to nourish or protect itself against infection. Instead, the cornea receives its nourishment from the tears and aqueous humor that fills the chamber behind it. The cornea must remain transparent to refract light properly, and the presence of even the tiniest blood vessels can interfere with this process (5). To see well, all layers of the cornea must be free of any cloudy or opaque areas. Though the cornea is clear and seemingly lacks substance, it is densely innervated with sensory nerve fibers that make it one of the most sensitive tissues of the body (6). As shown in Fig.1.2, The human cornea consists of five distinct layers. From anterior to posterior they are 1) epithelium, 2) Bowman's layer, 3) stroma, 4) Descemet's membrane, and 5) endothelium.