SCULPTURING CORNEAL CONSTRUCTS USING MICROPATTERNED SURFACES AND DECELLULARIZED CORNEA

SHARDA

DEPARTMENT OF TEXTILE TECHNOLOGY

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by

SHARDA

Department of Textile Technology

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DEDICATED TO

MY

DAUGHTER

CERTIFICATE

This is to certify that the thesis entitled **"SCULPTURING CORNEAL CONSTRUCTS USING MICROPATTERNED SURFACES AND DECELLULARIZED CORNEA"** being submitted by **Ms. SHARDA** to the **Indian Institute of Technology Delhi** for the award of the degree of **"DOCTOR OF PHILOSOPHY",** is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which is to the best of my knowledge, has reached the required standard.

The material contained in this thesis has not been submitted in part or full to any other University or Institute for the award of any other degree.

Dr. Sourabh Ghosh
Associate Professor
Department of Textile Technology
Indian Institute of Technology Delhi

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ABSTRACT

Severe corneal damage due to any disease, infection or trauma; hampered the corneal transparency causing visual impairment in millions of individuals worldwide. Massive shortage of donors and immune rejection has led to the focus on generating tissue engineered replacement as a novel therapeutic substitute.

In the work presented here, different approaches of tissue engineering have attempted to accelerate the generation of tissue engineered corneal construct having close proximity to native ultrastructure. The first approach was to develop a decellularized biological matrix with an intact arrangement of collagen fibres having native topographic and ECM (extracellular matrix) cues for better cell-ECM interactions with the aim of generating a biological scaffold. A new method of direct perfusion with precisely controlled flow regime of detergent was developed to enhance the efficiency of decellularization with minimal damage of ECM. Various conventionally used decellularization methods including, TRITON (TRITON X-100) and sodium dodecylsulphate (SDS) detergent based orbital shaker method and physical procedures; liquid nitrogen and freeze-thaw, have also employed in order to do a comparative study with newly developed decellularization protocol of direct perfusion. The unidirectional fluid flow assists in the effective cellular removal as well as persuades the cells towards the apoptotic pathway. Further, spectroscopy of directly perfused cornea was found similar to typically observed in untreated corneas with abundant α-helical conformations while transitions towards random coils or β-sheet conformations have occurred in other decellularization protocols that emphasize the efficacy of direct perfusion over other methods. The optimized decellularized matrix was further reseeded

with corneal stromal cells in dynamic condition; matrix was found biocompatible, supporting adhesion and proliferation of reseeded cells. Thus, perfusion based decellularization protocol may lead towards the development of an appropriate biological scaffold to engineer a corneal equivalent.

The next strategy was based on simulation of stromal cell alignment along the topographical cues of patterned substrate. Thus, in the second objective, surface topography was optimized to spatially induce alignment of corneal stromal cells. Three different patterns of gelatin having average diameters 80 µm, 100 µm and 140 µm were deposited using direct-write assembly (DWA). However, corneal stromal cells cultured over patterned surfaces uniformly followed deposited polymer alignment only in the patterns having fibre diameter <100 µm, thus, translating the physical cues to the neighboring cells towards the physiologically relevant alignment.

Following these optimized pattern parameters, in the third objective, the customized thermoresponsive polymeric surfaces were generated in order to fabricate the carrier free cell sheets of functional keratocytes. The same technique i.e. DWA, was employed to deposit the tailored viscoelsatic polymers of silk fibroin (SF), gelatin, silk-PolyNIPA and gelatin-PolyNIPA in parallel patterns while the planar film casts from the same polymers were used as a control to assess the impact of surface topology, incorporation of PolyNIPA imparted thermoresponsive nature to the biopolymeric surfaces of silk and gelatin. Topographical cues from an aligned fibrous substrate of silk-PolyNIPA and gelatin-PolyNIPA were found to channel the cell alignment along the pattern while the rapid recovery of cell sheet was achieved in thermoresponsive films of these hybrids with the transition of the surface characteristic by altering temperature. Eventually, gelatin-PolyNIPA substrate was found more appropriate with enhanced metabolic activity and ECM gene expression of corneal cells.

Since, SF and keratin complimenting each other in transparency, mechanical, thermal stability and cell binding efficiency, thus, stable conjugates of SF and keratin, have a high potential as biomaterial for *in vivo* ocular surface regeneration. In that direction, in the fourth objective, a tyrosinase mediated crosslinked blend of SF and keratin was purposed to be a promising biomaterial with unique chemical structure and stability. Molecules of SF and keratin were found to interact strongly at the stoichiometric ratio of SF:keratin::2:1 with 25 and 28 interaction sites as predicted in 2SCPAB and 1RKEAB models respectively which resulted in the formation of energetically stable complex after tyrosinase cross-linking.

Taken together, bioengineering of corneal equivalent could be accelerated by using biological scaffold generated by perfusion based decellularization while simulation of corneal architecture could be achieved by guiding the parallel alignment of corneal cells with gelatin-PolyNIPA patterns. Generation of SF-K-T crosslinked blend could assist in development of biomaterial for future translational prospective.

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SDS	Sodium dodecylsulphate
TRITON X-100	t-octylphenoxypolyethoxyethanol
ECM	Extracellular matrix
SF	Silk fibroin
GAG	Glycosaminoglycans
sGAG	Sulphated glycosaminoglycans
PBS	Phosphate buffered saline
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
LiBr	Lithium bromide
FBS	Fetal bovine serum
EDTA	Ethylenediaminetetraacetic acid
DNA	Deoxyribonucleic acid
DAPI	4',6-diamidino-2-phenylindole
PolyNIPA	Poly(N-isopropylacrylamide)
Gel-PolyNIPA	Gelatin-Poly(N-isopropylacrylamide)
Silk- PolyNIPA	Silk fibroin- Poly(N-isopropylacrylamide)
Poly-HEMA	Poly(2-hydroxyethyl methacrylate)
LCST	lower critical solution temperature
UCST	Upper critical solution temperature
IPN	Interpenetrating polymer networks
Liq. Nit.	Liquid nitrogen

ELISA	Enzyme-linked immunosorbent assay
AFM	Atomic-force microscopy
SEM	Scanning electron microscope
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared spectroscopy
DWA	Direct-write assembly
CD	Circular dichroism
RT-PCR	Reverse transcription polymerase chain reaction
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulphoxide
DMMB	Dimethyl methylene blue
FACS	Fluorescence-activated cell sorting
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
NHS	N-hydroxysuccinimide
H&E	Hematoxylin and eosin
LDV	Leu-Asp-Val
RGD	Arg-Gly-Asp
ACAN	Aggrecan
COL	Collagen
PI	Propidium iodide
FITC	Fluorescein isothiocyanate
DMEM	Dulbecco's modified eagle medium

G’	Storage modulus
G’’	Viscous modulus
FGF	Fibroblast growth factors
TGF	Transforming growth factor
mg	Milligram
g	Gram
kda	Kilodalton
Min	Minutes
µm	Micrometer
µl	Microlitre
ml	Mililiter
mM	Milimolar
cm	Centimeter
mm	Millimeter
nm	Nanometer
h	Hours
α	Alpha
β	Beta