

**UNDERSTANDING CELL-CELL AND CELL-
MATERIAL INTERACTIONS IN 2D AND 3D FOR
TISSUE ENGINEERING APPLICATIONS**

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Understanding cell-cell and cell-material interactions in 2D and 3D for tissue engineering applications

by

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Dedicated to Mom and Dad

Certificate

This is to certify that the thesis entitled '**Understanding cell-cell and cell-material interactions in 2D and 3D for tissue engineering applications**' being submitted by **Mr. Akshay Joshi** to the Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him. **Mr. Akshay Joshi** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Abstract

Cells within the tissues or organs are organized in a highly structured microenvironment and this organization is considered to be a prime reason for functionality of the tissue. The microenvironment i.e. the cells and the ECM impose certain boundary conditions (in the form of geometrical or mechanical cues) that not only effect the cell organization but also its migration, proliferation and differentiation. The understanding of cell organization into a functional tissue has been a long-standing problem for tissue engineering (T. E.) and it is believed that cell-cell and cell-ECM interactions play a key role in directing the assembly of cells into a specific architecture.

Although, developmental biology has explained the formation of a tissue and an organ, the understanding of various cellular processes that results in complete assembly of cells into functional tissue *in-vitro* is lacking. This fundamental understanding of how cells assemble onto a substrate will enable development of functional tissues *in-vitro* and accelerate the field of tissue engineering. Thus the goal of this thesis was to study these cell-cell and cell-material interactions and utilize them to develop various models for tissue engineering applications.

Chapter 1 discusses the various challenges and recent developments in the field of tissue engineering. In **Chapter 2**, a simple model to co-culture cells without the use of tiresome lithographic techniques was developed by utilizing the cell-material interactions. The finding showed that by fabricating micropatterns of micrometers depth, the cell-material interactions can be enhanced along the corners of fabricated geometry. This force the cells to initially migrate and align along the corners. By letting the cells to occupy the corners, the study demonstrated how this type of phenomenon provides an easy way to generate voids that can be utilized for culture of another cell type.

Further in **Chapter 3**, the difference in the morphology of the cells present at the corners vs the center as seen in the previous chapter was utilized to guide a biological process such as differentiation of human mesenchymal stem cells into multiple lineage within the same geometrical pattern.

Previous chapter investigated the use of substrate biophysical cues towards the development of a 2D culture for controlling the differentiation of MSC's into multiple lineages within the same geometrical pattern. However, all these studies were carried out in 2 dimensional (2D) patterned surfaces. Studying differentiation in 3 dimensions (3D), which mimics *in-vivo* conditions is more relevant for clinical applications of tissue engineering. Typically, exogenous factors are used when cells are differentiated in 3D but that limits differentiation into multiple lineages in the same scaffold. If a strategy for controlling differentiation via scaffold or ECM-cell interactions can be developed, it will enable multi-lineage differentiation within the same scaffold. **Chapter 4** investigates substrate controlled differentiation of cells into multiple lineages without the use of exogenous factors and an attempt has been made to control the differentiation by controlling the scaffold chemistry.

Further, to seek application of modulating the biological process and more specifically cellular adhesions in tissue engineering, in **Chapter 5**, a micropatterned dressing was designed and the efficacy of these patterns towards enhancing the process of wound healing was demonstrated. **Chapter 6** discusses the conclusion and future outlook of the thesis.

सारांश

ऊतकों या अंगों के भीतर कोशिकाएं अत्यधिक संरचित माइक्रोएन्वायरमेंट में व्यवस्थित होती हैं और यह संगठन ऊतक की कार्यक्षमता का एक प्रमुख कारण माना जाता है। माइक्रोएन्वायरमेंट यानी कोशिकाएं और ईसीएम कुछ सीमा शर्तों (ज्यामितीय या यांत्रिक संकेतों के रूप में) को लागू करते हैं जो न केवल सेल संगठन बल्कि इसके प्रवासन, प्रसार और भेदभाव को भी प्रभावित करते हैं। एक कार्यात्मक ऊतक में कोशिका संगठन की समझ ऊतक इंजीनियरिंग (टीई) के लिए एक लंबे समय से चली आ रही समस्या रही है और यह माना जाता है कि सेल-सेल और सेल-ईसीएम इंटरैक्शन कोशिकाओं की असंबली को एक विशिष्ट आर्किटेक्चर में निर्देशित करने में महत्वपूर्ण भूमिका निभाते हैं।

हालांकि, विकासात्मक जीव विज्ञान ने एक ऊतक और एक अंग के गठन की व्याख्या की है, विभिन्न सेलुलर प्रक्रियाओं की समझ जिसके परिणामस्वरूप इन-विट्रो कार्यात्मक ऊतक में कोशिकाओं की पूरी असंबली होती है, की कमी है। कैसे कोशिकाओं को एक सबस्ट्रेट पर इकट्ठा करने की यह मौलिक समझ इन-विट्रो में कार्यात्मक ऊतकों के विकास को सक्षम करेगी और ऊतक इंजीनियरिंग के क्षेत्र में तेजी लाएगी। इस प्रकार इस थीसिस का लक्ष्य इन सेल-सेल और सेल-मटेरियल इंटरैक्शन का अध्ययन करना था और उनका उपयोग ऊतक इंजीनियरिंग अनुप्रयोगों के लिए विभिन्न मॉडल विकसित करना था।

अध्याय 1 ऊतक इंजीनियरिंग के क्षेत्र में विभिन्न चुनौतियों और हाल के विकास पर चर्चा करता है। अध्याय 2 में, सेल-मटेरियल इंटरैक्शन का उपयोग करके थकाऊ लिथोग्राफिक तकनीकों के उपयोग के बिना सह-संस्कृति कोशिकाओं के लिए एक सरल मॉडल विकसित किया गया था। खोज से पता चला है कि माइक्रोमीटर की गहराई के माइक्रोपैटर्न को गढ़ने से, गढ़े हुए ज्यामिति के कोनों के साथ सेल-मटेरियल इंटरैक्शन को बढ़ाया जा सकता है। यह कोशिकाओं को शुरू में माइग्रेट करने और कोनों के साथ संरेखित करने के लिए बाध्य करता है। कोशिकाओं को कोनों पर कब्जा करने की अनुमति देकर, अध्ययन ने प्रदर्शित किया कि कैसे इस

प्रकार की घटना रिक्त स्थान उत्पन्न करने का एक आसान तरीका प्रदान करती है जिसका उपयोग किसी अन्य सेल प्रकार की संस्कृति के लिए किया जा सकता है।

आगे अध्याय 3 में, पिछले अध्याय में देखे गए कोनों बनाम केंद्र में मौजूद कोशिकाओं के आकारिकी में अंतर का उपयोग एक जैविक प्रक्रिया को निर्देशित करने के लिए किया गया था जैसे कि एक ही ज्यामितीय पैटर्न के भीतर मानव मेसेनचाइमल स्टेम कोशिकाओं को कई वंशों में विभेदित करना।

पिछले अध्याय ने एक ही ज्यामितीय पैटर्न के भीतर एमएससी के कई वंशों में भेदभाव को नियंत्रित करने के लिए 2डी संस्कृति के विकास की दिशा में सबस्ट्रेट बायोफिजिकल संकेतों के उपयोग की जांच की। हालाँकि, ये सभी अध्ययन 2 आयामी (2D) पैटर्न वाली सतहों में किए गए थे। 3 आयामों (3डी) में भेदभाव का अध्ययन, जो इन-विवो स्थितियों की नकल करता है, ऊतक इंजीनियरिंग के नैदानिक अनुप्रयोगों के लिए अधिक प्रासंगिक है। आमतौर पर, बहिर्जात कारकों का उपयोग तब किया जाता है जब कोशिकाओं को 3डी में विभेदित किया जाता है लेकिन यह एक ही मंचान में कई वंशों में विभेदन को सीमित करता है। यदि स्कैफोल्ड या ईसीएम-सेल इंटरैक्शन के माध्यम से भेदभाव को नियंत्रित करने की रणनीति विकसित की जा सकती है, तो यह एक ही मंचान के भीतर बहु-वंशीय भेदभाव को सक्षम करेगा। अध्याय 4 बहिर्जात कारकों के उपयोग के बिना कई वंशों में कोशिकाओं के सबस्ट्रेट नियंत्रित भेदभाव की जांच करता है और मंचान रसायन को नियंत्रित करके भेदभाव को नियंत्रित करने का प्रयास किया गया है।

इसके अलावा, अध्याय 5 में जैविक प्रक्रिया और अधिक विशेष रूप से सेलुलर आसंजनों को संशोधित करने के लिए अध्याय 5 में, एक माइक्रोपैटर्न वाली ड्रेसिंग तैयार की गई थी और घाव भरने की प्रक्रिया को बढ़ाने के लिए इन पैटर्नों की प्रभावकारिता का प्रदर्शन किया गया था। अध्याय 6 थीसिस के निष्कर्ष और भविष्य के दृष्टिकोण पर चर्चा करता है।

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List of Abbreviations

°C	Degrees Celsius
µg	Microgram
µL	Microliter
2D	2 Dimensional
3D	3 Dimensional
ECM	Extracellular Matrix
PEGDMA	Polyethylene Glycol Dimethacrylate
PDMS	Polydimethylsiloxane
UV	Ultraviolet
SEM	Scanning Electron Microscopy
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
PI	Propidium Iodide
DAPI	4',6-diamidino-2-phenylindole
PFA	Paraformaldehyde
EDX	Energy Dispersive X-ray analysis
hMSC's	Human Mesenchymal Stem Cells
PEGDA	Poly(ethylene Glycol Diacrylate)
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration
TCP	Tissue Culture Plate
D ₂ O	Deuterium Oxide
Alg	Alginate
Alg-PO ₄	Phosphorylated alginate
BSA	Bovine serum albumin
Calcein AM	Calcein acetoxymethyl ester
COL2A1	Collagen type II alpha 1 chain
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ELISA	Enzyme-linked immunosorbent assay
IL-6	Interleukin-6
kDa	Kilodalton

MES	2-(N-morpholino)ethanesulfonic acid
mg	Milligram
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NHS	N-hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
OCN	Osteocalcin
PBS	Phosphate Buffered Saline
PEG	Poly ethylene glycol
TNF α	Tumor necrosis factor alpha
LPS	Lipopolysaccharide
SF	Silk fibroin
VEGF	Vascular endothelial growth factor
DLP	Digital Light Processing
GF	Growth factor
GelMA	Gelatin Methacryloyl
MN	Microneedle
CAD	Computer-aided design
W/o Hep	Without heparin
wHep	With heparin
TRITC	Tetramethylrhodamine
RPMI	Roswell Park Memorial Institute
H&E	Hematoxylin and Eosin
MT	Masson's trichrome
mL	Mililiter
mM	Millimolar
mm	Millimeter
nM	Nanomolar
T.E.	Tissue engineering
MSC	Mesenchymal stem cells
rpm	Rotations per minute
M.A.	Methacrylic anhydride