

DEVELOPMENT OF FUNCTIONAL FIBROUS MATRICES VIA EMULSION ELECTROSPINNING

JIT PAL



**DEPARTMENT OF TEXTILE TECHNOLOGY
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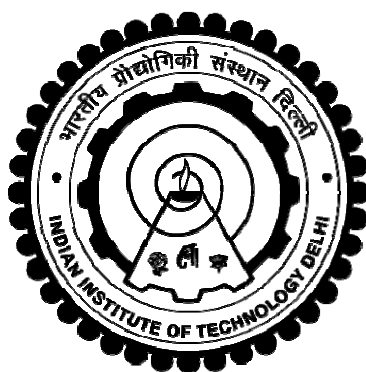
JIT PAL

Department of Textile Technology

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

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CERTIFICATE

This is to certify that the thesis titled “*Development of Functional Fibrous Matrices via Emulsion Electrospinning*” being submitted by **Mr. Jit Pal** to Indian Institute of Technology Delhi for the award of *Doctor of Philosophy* degree, is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and fulfilled the requirements for the submission of thesis which has attained the standard required for a Ph.D. degree of this institute. The work carried out to complete the thesis has not been submitted for degree or diploma in any institute in part or full.

(Dr. Rajiv K. Srivastava)

Associate Professor

Department of Textile Technology

Indian Institute of Technology Delhi

Date:

Place:

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ABSTRACT

The necessity of a polymeric biodegradable scaffold for successful tissue engineering has led to a surge in techniques that can be used to fabricate them. Electrospinning has emerged as one of the most popular methods due to its ease of operation, low processing cost and formation of fibrous scaffolds that mimic the extracellular matrix (ECM) of native tissues. The technique offers unlimited flexibility in selecting natural and synthetic polymers. The processing of polymers in solution form however is one major issue with electrospinning where solvent residues in the product results in cell toxicity and solvent removal adds time and cost to the overall process. Melt electrospinning may not be a feasible solution for polymers which are sensitive to high temperature.

Emulsion electrospinning can become a viable substitute for solution electrospinning where use of organic solvents can be significantly reduced in addition to easy control on the flow behavior of emulsions and carrying out electrospinning at reduced polymer concentration. Functionalized electrospun fibrous matrices of poly(ϵ -caprolactone) (PCL) are produced in the present research by emulsion electrospinning of PCL based oil-in-water emulsions. Poly(vinyl alcohol) (PVA) is used as a template polymer in continuous aqueous phase of the emulsion to enhance emulsion's stability and proper orientation and coalescence of dispersed oil phase droplets (comprised of PCL solution in toluene) to attain jet uniformity during electrospinning. The viscoelastic interaction between oil and water interface is tailored by varying polymer concentration in either phase. PCL electrospun matrices are functionalized with hydroxyapatite (HA) and unique strategies have been devised to accomplish high yield of HA in final matrices after post-electrospinning treatment. Tenside free emulsions are produced using acrylic acid grafted PCL (PCL-*g*-AA) and electrospinning of resultant

emulsions is carried out to obtain HA functionalized PCL-g-AA matrices. In-situ synthesized nano-HA is used in another approach to formulate PCL based oil-in-water emulsions and fabrication of nano-HA functionalized PCL fibrous matrices. Various generations of emulsion electrospun PCL fibrous matrices are assessed for their human breast cancer (MCF7) and osteoblast (MG63) cell attachment and growth efficacy. Cell proliferation is found to be better in emulsion electrospun matrices than those produced by solution electrospinning. Fabrication of uneven surfaced matrices by post-electrospinning wash of template polymer by DI water further enhanced the cell growth. Use of nano-HA and emulsion formulated without any emulsifier showed utmost cell proliferation and provided distinctive strategies to fabricate functionalized electrospun matrices from an emulsion.

सारांश

सफल ऊतक अभियांत्रिकी के लिए एक बहुलक जैव-निम्नकारिक आव्युह की आवश्यकता ने आव्युह को उत्पादित करने वाली तकनीकों में अनेक विकास किए हैं। आसान उत्पादन, कम प्रसंस्करण लागत और अंततः रेशेदार आव्युह का निर्माण करने के लिए विद्युतीय-कताई सबसे लोकप्रिय तकनीक के रूप में उभरी है, जिसके द्वारा निर्मित रेशेदार आव्युह ऊतकों के बाह्य आव्युह (ECM) में पाए जाने वाले तंतुओं से मेल खाते हैं। विद्युतीय-कताई के द्वारा प्राकृतिक और कृत्रिम, दोनों प्रकार के बहुलकों का चयन असीमित अवसर प्रदान करता है। तथापि, विद्युतीय-कताई में बहुलकों के विलयन का उपयोग एक प्रमुख समस्या है जिससे कोशिका-विषाक्तता और विलायक को हटाने में समग्र प्रक्रिया में अतिरिक्त समय और लागत लगते हैं। तरल विद्युतीय-कताई, उन बहुलकों के लिए जो उच्च तापमान के प्रति संवेदनशील हैं, एक व्यवहार्य समाधान नहीं हैं। पायसन विद्युतीय-कताई एक व्यवहार्य विकल्प बन सकता है जहाँ कार्बनिक विलयकों का कम उपयोग पायसन के प्रवाह व्यवहार पर आसान नियंत्रण तथा बहुलक के तनु विलयनों का उपयोग कारगर सिद्ध हो सकता है।

वर्तमान शोध में पॉली(कैपरोलेक्टोन) (PCL) के बहु-कार्यात्मक रेशेदार आव्युहों का पायसन विद्युतीय-कताई के दौरान धार की एकरूपता हासिल करने के लिए, पायसन की स्थिरता, उचित उन्मुखीकरण और तेल चरण बूँदों (PCL का Toluene में विलयन) का संघीकरण बढ़ाने के लिए पायसन के निरंतर जलीय चरण में पॉली(विनाइल अल्कोहल) (PVA) का उपयोग एक रूपता प्रदान करने वाले बहुलक के रूप में किया गया है। पायसन के तेल (अनिरंतर) और पानी (निरंतर) चरण के अन्तर्फलक के बीच के विस्को-इलास्टिक संपर्क, बहुलक के किसी भी चरण की सार के द्वारा नियंत्रित किया गया है। PCL के आव्युह को हाइड्रॉक्सी-एपेटाईट (HA) के साथ कार्यात्मक बनाया गया है और HA की अंतिम आव्युह में उच्च प्राप्ति के लिए एक अनन्य कार्यनीति विकसित गई है। पृष्ठसक्रियकारक मुक्त पायसनों का उत्पाद किया गया है और उन पायसनों की PCL-g-AA का

प्रयोग करके, विद्युतीय-कताई के द्वारा PCL-*g*-AA के HA के साथ कार्यात्मक आव्युह उत्पन्न किए गए हैं।

एक अन्य कार्य नीति में नैनो-HA का उपयोग करके PCL पर आधारित पानी-में-तेल पायसन विकसित किए गए जिनसे नैनो-HA के साथ PCL के कार्यात्मक आव्युहो का सर्जन किया गया है। पायसन विद्युतीय-कताई के द्वारा निर्मित PCL के विभिन्न आव्युहो का MCF7 और MG63 कोशिकाओं के संलग्निकरण और विकास की प्रभावकारिता के लिए मूल्यांकन किया गया है। पायसन विद्युतीय-कताई के द्वारा विकसित आव्युहो में कोशिका का प्रसार विलयन विद्युतीय-कताई के आव्युहो की तुलना में बेहतर पाया गया है। आव्युहो की असमान सतह जो उनको जल के साथ धोने के साथ प्राप्त की गई, में भी कोशिका का प्रसार बेहतर पाया गया है। नैनो-HA और बिना पृष्ठसक्रियकारक के द्वारा विकसित पायसनो से निर्मित आव्युहो में कोशिका का प्रसार अधिकतम पाया गया है जो कि पायसन विद्युतीय-कताई के द्वारा उत्पादित कार्यात्मक रेशेदार आव्युहो का विकास करने में एक विशिष्ट कार्यनीति प्रदान करती है।

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LIST OF SYMBOLS AND ABBREVIATIONS

$[\eta]$	Intrinsic Viscosity
2θ	Diffraction Angle
C^*	Critical Entanglement Concentration
d_m	Particle Diameter
G'	Storage Modulus
G''	Loss Modulus
h_m	Inter-Particle Distance
K	Consistency Coefficient
M_n	Number Average Molecular Weight
M_p	Peak Molecular Weight
M_w	Weight Average Molecular Weight
n	Flow Behavior Index
S_t	Total Surface Area
T_d	Decomposition Temperature
T_g	Glass Transition Temperature
T_m	Melting Temperature
γ_0	Applied Strain
η	Viscosity
η_0	Zero Shear Viscosity
η_s	Solution Viscosity
η_{sp}	Specific Viscosity

τ	Shear Stress
Φ_o	Volume Fraction of Oil
Φ_w	Volume Fraction of Water
$\Phi_{\max}$	Maximum Volume Fraction
ω	Angular Frequency
$\dot{\gamma}$	Shear Rate
$^1\text{H-NMR}$	Proton Nuclear Magnetic Resonance Spectroscopy
AA	Acrylic acid
ATR-IR	Attenuated Total Reflection Infrared Spectroscopy
BPO	Benzoyl Peroxide
Ca(OH)_2	Calcium Hydroxide
Ca/P	Calcium and Phosphorous ratio in Hydroxyapatite
CAD	Computer Aided Design
CDCl_3	Deuterated Chloroform
CL	ϵ -Caprolactone
DI water	Deionized Water
DLS	Dynamic Light Scattering
DMF	Dimethyl Formamide
DMSO	Dimethyl Sulphoxide
ECM	Extracellular Matrix
EDS	Energy Dispersive X-ray Spectroscopy
HA	Hydroxyapatite
H_3PO_4	Orthophosphoric Acid

KOH	Potassium Hydroxide
LVR	Linear Viscoelastic Region
MTT	3-(4,5-Dimethylthiazol-2yl)-2,5-Diphenyltetrazolium Bromide
nano-HA	Nano Hydroxyapatite
NH ₄ OH	Ammonium Hydroxide
NA	Information is not available
PBS	Poly(butylene succinate)
PCL	Poly(ϵ -caprolactone)
PET	Poly(ethylene terephthalate)
PGA	Poly(glycolic acid)
PHAs	Poly(hydroxy alkanoate)s
PHH	Poly(hydroxy hexanoate)
PHV	Poly(hydroxy valerate)
PLA	Poly(lactic acid)
PVA	Poly(vinyl alcohol)
ROP	Ring Opening Polymerization
SEM	Scanning Electron Microscopy
SFF	Solid Freeform Fabrication
SLS	Selective Laser Sintering
TEM	Transmission Electron Microscopy
TGA	Thermo-Gravimetric Analysis
THF	Tetrahydrofuran
WXR	Wide Angel X-Ray Diffraction