MULTIFUNCTIONAL ELECTROSPUN FIBROUS SCAFFOLDS OF PICKERING - HIGH INTERNAL PHASE EMULSIONS

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by

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CERTIFICATE

This is to certify that the thesis titled ***"Multifunctional Electrospun Fibrous Scaffolds of Pickering - High Internal Phase Emulsions"*** being submitted by **Ms. Archana Samanta** to Indian Institute of Technology Delhi, for the award of ***Doctor of Philosophy*** degree, is a record of bonafide research work carried out by her. She 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.

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ABSTRACT

Electrospinning is the most popular and easy to operate technique to create micro- and nano-dimensional fibrous scaffolds suitable for many applications including tissue engineering. Electrospun fibrous scaffolds have inherently high specific surface area and mimic the fibrils found within extracellular scaffold (ECM) of native tissues that encourages cells to grow in proper lineage with fiber orientation. The technique offers unlimited flexibility in selecting natural and synthetic polymers, however processing of polymers in solution form is one issue with electrospinning where residual solvent in the scaffold results in detrimental effects on the cell growth. Complete solvent removal adds time and cost to the overall process. Melt electrospinning is not be a feasible substitute for polymers which tend to degrade under such conditions. Emulsions, as substitute for polymeric solutions, can be used to carry out electrospinning at reduced organic solvent content and provide better control on flow behaviour and formation of scaffolds at reduced polymer concentration. Emulsion electrospinning, on the other hand, often requires a template water soluble polymer and emulsion electrospun fibrous scaffolds show poor mechanical properties due to leaching of emulsifiers and template polymers in aqueous environment. Pickering emulsions, which do not require an emulsifier to stabilize the emulsion, can be considered as a viable choice to prepare electrospun fibrous scaffolds in place of conventional emulsions. Better emulsion stability at lower solid particle concentration, in a formulation free from emulsifier and template polymer, and prevention in deterioration of mechanical properties of scaffold in an aqueous environment are additional benefits of using a Pickering emulsion for electrospinning.

The focus of research in this thesis was to produce stable Pickering emulsions of an Food and Drug Association (FDA) approved polymer, poly(ε-caprolactone) (PCL), using four different Pickering agents viz. amphiphilic montmorillonite clay, hydrophobically modified silica, *in-situ* synthesized nano-silver and bioactive hydroxyapatite (HA). Functionalized fibrous scaffolds were fabricated from electrospinning of stable Pickering emulsions under controlled conditions. Fiber fineness was tailored by varying the polymer and Pickering agent content, volume fraction of oil and aqueous phase and electrospinning process conditions. Functionalized fibrous scaffolds generated from Pickering emulsions were found to have better thermo-mechanical properties than solution electrospun scaffolds of PCL. In addition, fibrous scaffolds developed from Pickering emulsions showed superior cell (osteoblast and fibroblast) growth than solution electrospun scaffolds of PCL. Nano-silver based Pickering emulsion electrospun scaffolds reflected significantly better performance as anti-microbial wound dressing than those developed using PCL solution. Alongside, electrospinning of PCL and poly(vinyl alcohol) (PVA) based high internal phase emulsion (HIPE) resulted in formation of a co-continuous fiber morphology and reduction in usage of organic solvent by 75%. Emulsions were invariably used as substitute for polymeric solutions to prepare fibrous scaffolds using electrospinning. Use of Pickering emulsions further provided a better approach to fabricate functionalized fibrous scaffolds for potential application in tissue engineering.

सारांश

विद्युतीय -कताई एक अत्यंत लोकप्रिय और आसान तकनीक है, जिससे उत्तक अभियांत्रिकी में प्रयुक्त होने वाले माइक्रो एवं नैनो -आयामी रेशेदार आव्यूहो का उत्पादन किया जा सकता है। विद्युतीय -कताई के द्वारा उत्पादित रेशेदार आव्यूहो मे उच्च विशिष्ठ सतह पाई जाती है, जो मानव शरीर के उत्तको के बाह्य आव्यूह (ECM) में पाए जाने वाले तंत्तुओ से मेल खाती है और तंतु उन्मुखिकरण के कारण कोशिकाओं को उचित दिशा मे विकसित करने मे सहायता करती है। यह तकनीक प्राकृतिक और कृत्रिम, दोनो प्रकार के बहुलकों के चयन का असीमित अवसर प्रदान करती है तथापि विद्युतीय -कताई में बहुलको के विलयन का प्रयोग एक समस्या है जहाँ आव्यूह में उपस्थित शेष विलायक कोशिका के विकास में हानिकारक परिणाम डालता है। विलायक को आव्यूह से पूर्णत: हटाने में अतिरिक्त समय और लागत लगता है. तरल विद्युतीय -कताई, उन बहुलकों के लिए जो उच्च तापमान के प्रति संवेदनशील है, एक व्यवहार्य समाधान नही है। बहुलक के विलयन की जगह पायसन का प्रयोग एक व्यवहारिक विकल्प बन सकता है, जिसमे कार्बनिक विलायों का कम उपयोग, पायसन के प्रवाह व्यवहार पर आसान नियंत्रण तथा बहुलक के तनु विलयनो का उपयोग करके आव्युहो का निर्माण किया जा सकता है। पायसन विद्युतीय -कताई मे, दूसरी तरफ अक्सर एक रुपता प्रदान करने के लिए जल घुलनशील बहुलक की आवश्यकता होती है तथा पायसन विद्युतीय -कताई के द्वारा निर्मित रेशेदार आव्यूह, पायसीकारक और एक-रुपता प्रदान करने वाले बहुलक की जल मे घुलनशीलता के कारण, हीन यांत्रिक गुण दर्शाते हैं. पिकरिंग पयसनो, जिसमे पायसन को स्थिर करने के लिए एक पयसीकारक की आवश्यकता नही होती, को विद्युतीय -कताई के लिए द्वारा रेशेदार आव्युहो का निर्माण करने के लिए परंपरागत पायसनो का एक उपयुक्त विकल्प माना जा सकता है। कम ठोस कण एकाग्रता में पायसन की बेहतर स्थिरता, पायसीकारक और रुपता प्रदान करने वाले बहुलक से विमुक्ति

और जलीय वातावरण में पायसन के यांत्रिक गुणो की गिरावट में रोकथाम से विद्युतीय -कताई में पिकरिंग पायसनों का उपयोग अतिरिक्त लाभ प्रदान करता है।

वर्तमान शोध कार्य का केंद्र एक फूड एव्म ड्रग एड्मिनिस्ट्रेशन (FDA) के द्वारा अनुमोदित बहुलक, पॉली(कॅप्रोलॅक्टोन) (PCL) के चार विभिन्न घटको- MMT, SiO2, नैनो- सिल्वर, HA का प्रयोग करके, स्थिर पिकरिंग पायसनों का उत्पादन करना था. स्थिर पिकरिंग पायसनों से कार्यात्मिक रेशेदार आव्युहो का नियंत्रित पारिस्थितियों में उत्पादन किया गया. विभिन्न पिकरिंग घटकों की मात्रा, तेल और जलिय चरण का परिमाण और विद्युतीय -कताई प्रक्रिया की स्थितियों को बदलकर तंतु की विरलता को नियंत्रित किया गया। पिकरिंग पायसन विद्युतीय-कताई के द्वारा निर्मित कार्यात्मक रेशेदार आव्युहो में उष्ण-यांत्रिकी गुण विलयन विद्युतीय-कताई के आव्युहो की तुलना में बेहतर पाए गए. इसके अतिरिक्त, पिकरिंग पायसन विधुतीय-कताई से निर्मित रेशेदार आव्यूहॉ में कोशिका (Osteoblast और fibroblast) का प्रसार विलयन विधुतीय आव्यूहॉ से श्रेष्ठतर पाया गया। नैनो सिल्वर आधारित पिकरिंग पायसन के रेशेदार आव्युहो, PCL के विलयन से निर्मित आव्युहो की तुलना में, सूक्ष्मजीवी-विरोधी मरहम पट्टी के तौर पर काफ़ी बेहतर प्रदर्शन किया. इसके अलावा, PCL और पाली(वाइनाइल आल्कोहॉल) (PVA) आधारित उच्च आंतरिक चरण पायसन (HIPE) की विद्युतीय -कताई में कार्बनिक विलयकों का 75% कम प्रयोग किया गया और परिणामस्वरूप एक सह-निरंतर तंतु आकारीकी प्राप्त की गयी। विद्युतीय -कताई के द्वारा रेशेदार आव्यूहॉ का निर्माण करने में पायसनों का उपयोग बहुलक विलयनों के विकल्प के रूप में किया गया है। पिकरिंग पायसनों का कार्यात्मक रेशेदार आव्युहो का निर्माण करने मे उपयोग, उत्तक अभियांत्रिकी में संभावित अनुप्रयोग के लिए एक बेहतर संभावना प्रदान करती है।

CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

LIST OF SYMBOLS AND ABBREVIATIONS

$\boldsymbol{A}$	Interfacial area
$\boldsymbol{B}$	Breadth of non-spherical Pickering stabilizers
$\boldsymbol{Cs}$	Concentration of stabilizer in the emulsion
$\boldsymbol{Cs'}$	Concentration of stabilizer in the continuous phase
$\boldsymbol{d}$	Average diameter of silica particles
$\boldsymbol{D}$	Average diameter of dispersed droplets
$\boldsymbol{E}$	Energy
$\boldsymbol{G/g}$	Acceleration due to gravity
$\boldsymbol{H^0{}_f}$	Enthalpy of fusion of 100 % crystalline sample
$\boldsymbol{H_c}$	Enthalpy of crystallization
$\boldsymbol{H_f}$	Enthalpy of fusion
$\boldsymbol{Hi}$	Value for a specific functional group for calculating HLB
$\boldsymbol{L}$	Length of non-spherical Pickering stabilizers
$\boldsymbol{M}$	Number of hydrophilic groups
$\boldsymbol{M_n}$	Number average molecular weight
$\boldsymbol{m_o}$	Mass of silica particles present in emulsion
$\boldsymbol{M_p}$	Mass of Pickering stabilizers
$\boldsymbol{N}$	Number of dispersed droplets
$\boldsymbol{\eta}$	Viscosity
$\boldsymbol{n_e}$	Number of particles
$\boldsymbol{n_o}$	Number of silica particles present in emulsion
$\boldsymbol{\phi}$	Volume fraction
$\boldsymbol{R}$	Radius of dispersed droplets

$\boldsymbol{R^*}$	Ratio of surface area of SiO_2 particles per unit area of oil/water interface
$\boldsymbol{R_{min}}$	Average minimum dispersed droplet diameter
$\boldsymbol{r_p}$	Radius of particles
$\boldsymbol{T}$	Absolute temperature
$\boldsymbol{T_d}$	Decomposition temperature
$\boldsymbol{T_g}$	Glass transition temperature
$\boldsymbol{T_m}$	Melting temperature
$\boldsymbol{V}$	Volume
$\boldsymbol{v_c}$	Creaming velocity
$\boldsymbol{v_o}$	Volume of silica particles present in emulsion
$\boldsymbol{v_s}$	Sedimentation velocity
$\boldsymbol{W}$	Work
$\boldsymbol{w_c}$	Crystallinity percentage
$\boldsymbol{\Upsilon}$	Interfacial tension
$\boldsymbol{\Delta G}$	Change in Gibbs free energy
$\boldsymbol{\Delta H}$	Change in enthalpy
$\boldsymbol{\Delta S}$	Change in entropy
$\boldsymbol{\Delta \rho}$	Density difference between dispersed and continuous phases
$\boldsymbol{\Theta}$	Angle formed by the particle at the interface
$\boldsymbol{\Phi_{aq}}$	Volume Fraction of aqueous phase
$\boldsymbol{\Phi_{max}}$	Maximum Volume Fraction of aqueous phase
$\boldsymbol{\Phi_o}$	Volume Fraction of oil phase
$\boldsymbol{\Gamma_{sat}}$	Excess saturation surface concentration of the stabilizer

2θ	Bragg's angle in WAXD
Ag	Silver
$AgNO_3$	Silver nitrate
Brij-58	Polyethylene glycol hexadecyl ether, $HO(CH_2CH_2O)_{20}C_{16}H_{33}$
Ca/P	Calcium to phosphorous ratio
$CaCl_2$	Calcium chloride
CNC	Cellulose nano crystals
CNT	Carbon nano tubes
$CHCl_3$	Chloroform
CL	ε-Caprolactone
Cremophor	Polyethylene glycol mono hexadecyl/octadecyl ether ($C_{47}H_{51}NO_{14}$)
DCM	Dichloromethane
DI water	Deionized water
DMF	Dimethyl formamide
DOX	Doxorubicin hydrochloride
DSC	Differential scanning calorimetry
ECM	Extra cellular matrix
EDS	Energy dispersive X-ray spectroscopy
FDA	Food and drug administration
HA	Hydroxyapatite
HCL	Hydrochloric acid
HFIP	Hexafluoroisopropanol
HIPE	High internal phase emulsion
HLB	Hydrophilic lipophilic balance

$K_2HPO_4 \cdot 3H_2O$	Potassium hydrogen phosphate trihydrate
KCl	Potassium chloride
kV	Kilo volt
$MgCl_2 \cdot 6H_2O$	Magnesium chloride hexahydrate
MH	Metformin hydrochloride
mMMT	Modified montmorillonite
MMT	Montmorillonite
MPEG–PLGA	Methoxy polyethylene glycol–poly(lactide-co-glycolide)
m-Silica	Modified silica
MTT	3-(4,5-Dimethylthiazol-2yl)-2,5-Diphenyltetrazolium Bromide
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
nano-Ag	Nano silver
P(LLA-CL)	Poly(L-lactide-*co*-ε-caprolactone)
PCL	Poly(ε-caprolactone)
PDLGA	Poly(D,L-lactide-*co*-glycolide)
PE	Pickering emulsion
PEO	Poly(ethylene oxide)
PET	Poly(ethylene terephthalate)
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLCG	Poly(D,L-lactide-*co*-glycolide)
PTFE	Poly(tetrafluoro ethylene)

PVA	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
ROP	Ring opening polymerization
SBF	Simulated body fluid
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SiC	Silicon carbide
Span 80	Sorbitan monooleate, $C_{24}H_{44}O_6$
TEM	Transmission electron microscopy
TGA	Thermo gravimetric analysis
TiO_2	Titanium dioxide
Tris	Tris(hydroxymethyl) aminomethane
UV	Ultraviolet
WAXD	Wide angle X-ray diffraction