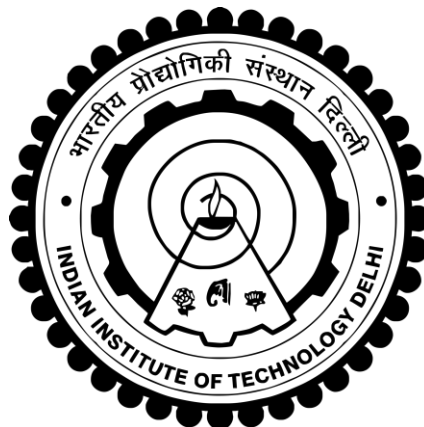


**EXPERIMENTAL INVESTIGATIONS INTO EMBEDDED DIRECT INK
WRITING OF TRIPLE-SHAPE MEMORY POLYMER BLEND FOR
BIOMEDICAL APPLICATION**

SHUBHAM SHANKAR MOHOL



DEPARTMENT OF MECHANICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MAY 2025

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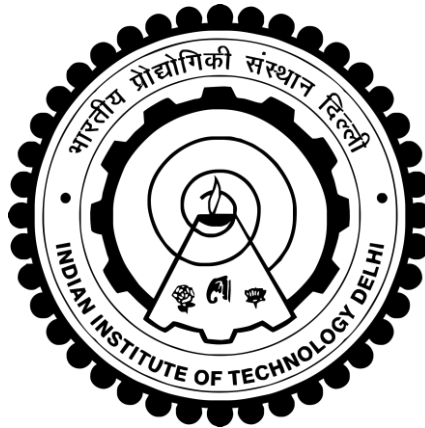
by

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DEPARTMENT OF MECHANICAL ENGINEERING

Submitted

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



INDIAN INSTITUTE OF TECHNOLOGY DELHI

MAY 2025

DEDICATED TO
LORD GANESH AND MY PARENTS

Certificate

This is to certify that the thesis entitled ‘**Experimental investigations into embedded direct ink writing of triple-shape memory polymer blend for biomedical application**’ submitted by **Mr. Shubham Shankar Mohol** to the Indian Institute of Technology Delhi, for the award of the degree of *Doctor of Philosophy*, is a record of the original bonafide research work carried out by him under my guidance and supervision. The results contained in it have not been submitted in part or full to any other institute or university for the award of any degree/diploma.



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(Shubham Shankar Mohol)

Abstract

Direct ink writing (DIW) technique is widely used for the fabrication of implants for biomedical applications owing to its ability to create personalized products. Also termed as 3D bioprinting, a variety of biomaterials that include natural and synthetic polymers are extensively utilized to 3D print tissue constructs. However, natural polymers fail to mimic the natural stiffness or ductility of the native organ but provide a suitable environment for the growth of the tissue. Some applications that include bone scaffolds, tracheal scaffolds, tracheal stents, ureter stents, etc. demand the need for both mechanical and biological properties appropriate for clinical restoration of tissue constructs. Also, conventional DIW is unsuitable for fabricating customized geometries with long, unsupported structures or overhangs. Hence, the fabrication of customized biomedical implants with desired material properties and intricate geometries is challenging.

In order to fill this research gap, a triple-shape memory polymer was developed by solution blending of polylactic acid (PLA) with polypropylene carbonate (PPC), which was 3D printed using the material extrusion principle. The blend of crystalline PLA and amorphous PPC enabled it to attain tunable mechanical properties. Morphological observations of blended PLA/PPC samples revealed a phase-segregated morphology, resulting in the appearance of two distinct glass transition temperatures, exhibiting a triple-shape memory effect (triple-SME). The PLA50/PPC50 composition achieved an optimum shape-fixity ratio and shape-recovery ratio, due to the existence of a co-continuous phase morphology. As proof of concept, 4D printing of the PLA50/PPC50 composition was demonstrated at body temperature as a potential biomedical application to facilitate minimally invasive surgery. An in vitro degradation study of the PLA50/PPC50 composition was performed over a period of 56 days. Finally, the in vitro cytotoxicity of all 3D printed PLA/PPC blends demonstrated excellent biocompatibility, proving its potential as an implant for tissue engineering applications. The elucidation of the

parameters influencing the selective actuation of triple-SME gained from this study is expected to open a wide range of possibilities for use in biomedical devices.

Further, a fabrication methodology was developed that provides flexibility to create freeform structures using synthetic polymers. An innovative approach of embedded 3D printing was developed, which allows the fabrication of freeform structures by directly 3D printing inside a supporting matrix. The polymer ink solution was prepared by blending PLA and PPC in 1,4-dioxane. Laponite RD was used to prepare the aqueous nano-silicate suspension, which exhibited yield stress and thixotropic properties due to its house-of-cards arrangement. Solidification of the 3D printed polymer ink occurred due to the hydrogen bonding between the oxygen atoms of dioxane molecules and the protons of water molecules. Particle image velocimetry study showed that the increasing nano-silicate concentration caused a reduction in yield region as a result of increasing yield stress. Dimensionless parameters (Oldroyd number and ratio of yield stress to hydrostatic pressure) for nano-silicate concentrations with values less than 1 were found to facilitate the nozzle movement without crevice formation, while the values greater than 1 were found to be unsuitable for printing. Different intricate structures such as tubular structures with varying geometry that mimic the native trachea, and a bifurcated tube were 3D printed as a proof-of-concept study. The proposed method introduced a novel approach by utilizing the solvent-water interaction capability to fabricate objects with overhanging geometry. This approach allows 3D printing of a wide variety of polymers by leveraging the miscibility between its corresponding solvent and supporting matrix.

The development of tissue-engineered tubular scaffolds was further investigated using statistical modeling and multi-objective optimization of embedded 3D process parameters. Design of experiments (DOE) was conducted using the central composite design to evaluate the effects of layer thickness, print speed, and polymer concentration on mechanical properties

and shrinkage. The mechanical properties were assessed by measuring the radial compressive load, while shrinkage was recorded to understand dimensional stability. The experimental results revealed that shrinkage was minimized at a combination of lower layer thickness, reduced print speed, and higher polymer concentration. Conversely, the radial compressive load exhibited a non-linear trend with print speed, increasing up to an optimal point before decreasing. Furthermore, the radial load was observed to increase with decreasing layer thickness and higher polymer concentration. A multi-objective optimization approach was implemented using a genetic algorithm (GA) to determine the optimum parameters for minimizing shrinkage and maximizing radial load. The optimized process parameters were validated through experimental trials, demonstrating improvement in the scaffold performance. To assess the practical utility of the optimized parameters, a case study was conducted by fabricating a tracheal scaffold using embedded 3D printing, and its mechanical properties were compared to those of a native goat trachea. The results confirmed that the scaffold achieved comparable mechanical properties, emphasizing the effectiveness of the proposed optimization strategy.

Finally, a study was conducted on the fabrication of PLA/PPC composite tracheal scaffold using embedded 3D printing to demonstrate its triple-shape memory functionality. The 4D printed scaffolds are investigated using mechanical, shape-memory, and biological assessment. Thermal characterization revealed that the Laponite nano-silicates on the PLA/PPC composite surface had a negligible effect on thermal transitions and shape-memory performance. Three scaffold geometries (cylinder, bellow, and spiral-shaped) were fabricated and tested for radial and longitudinal compression, with the bellow-shaped scaffold closely mimicking the mechanical properties of the native goat trachea. The PLA/PPC composite samples exhibited high shape-fixity and shape-recovery ratios. As a case study, the bellow-shaped scaffold was implanted inside the lumen of a native goat trachea, and the triple-shape

memory behavior was demonstrated. The degradation of PLA/PPC samples was investigated in PBS at physiological conditions (37 °C) for a period of 60 days. Additionally, Fourier-transform infrared spectroscopy was performed to assess the effect on various functional groups before and after the degradation test. To further assess the biocompatibility of the samples, MTT, live/dead, and cell adhesion assays were conducted using fibroblast cells. The in vitro cytotoxicity of the PLA/PPC composite demonstrated excellent biocompatibility, proving its potential as an implant for tissue engineering applications.

सार

डायरेक्ट इंक राइटिंग (DIW) तकनीक का उपयोग बायोमेडिकल अनुप्रयोगों के लिए प्रत्यारोपण के निर्माण के लिए व्यापक रूप से किया जाता है, क्योंकि यह व्यक्तिगत उत्पाद बनाने की क्षमता रखता है। इसे 3D बायोप्रिंटिंग भी कहा जाता है, जिसमें प्राकृतिक और सिंथेटिक पॉलिमर सहित कई तरह के बायोमटेरियल का उपयोग ऊतक निर्माण के लिए बड़े पैमाने पर किया जाता है। हालाँकि, प्राकृतिक पॉलिमर मूल अंग की प्राकृतिक कठोरता या लचीलापन की नकल करने में विफल रहते हैं, लेकिन ऊतक के विकास के लिए उपयुक्त वातावरण प्रदान करते हैं। कुछ अनुप्रयोग जिनमें अस्थि मचान, श्वासनली मचान, श्वासनली स्टेंट, मूत्रवाहिनी स्टेंट आदि शामिल हैं, ऊतक निर्माण की नैदानिक बहाली के लिए उपयुक्त यांत्रिक और जैविक दोनों गुणों की आवश्यकता होती है। इसके अलावा, पारंपरिक DIW लंबी, असमर्थित संरचनाओं या ओवरहैंग के साथ अनुकूलित ज्यामिति के निर्माण के लिए अनुपयुक्त है। इसलिए, वांछित सामग्री गुणों और जटिल ज्यामिति के साथ अनुकूलित बायोमेडिकल प्रत्यारोपण का निर्माण चुनौतीपूर्ण है।

इस शोध अंतराल को भरने के लिए, पॉलीएलैक्टिक एसिड (PLA) के साथ पॉलीप्रोपाइलीन कार्बोनेट (PPC) के घोल मिश्रण द्वारा एक ट्रिपल-शेप मेमोरी पॉलीमर विकसित किया गया था, जिसे मटेरियल एक्सट्रूजन सिद्धांत का उपयोग करके 3D प्रिंट किया गया था। क्रिस्टलीय PLA और अनाकार PPC के मिश्रण ने इसे ट्यूनेबल मैकेनिकल गुण प्राप्त करने में सक्षम बनाया। मिश्रित PLA/PPC नमूनों के रूपात्मक अवलोकनों ने एक चरण-पृथक आकारिकी का खुलासा किया, जिसके परिणामस्वरूप दो अलग-अलग ग्लास संक्रमण तापमान दिखाई दिए, जो एक ट्रिपल-शेप मेमोरी प्रभाव (ट्रिपल-SME) प्रदर्शित करते हैं। सह-निरंतर चरण आकृति विज्ञान के अस्तित्व के कारण PLA50/PPC50 संरचना ने एक इष्टतम आकार-स्थिरता अनुपात और आकार-पुनर्प्राप्ति अनुपात प्राप्त किया। अवधारणा के प्रमाण के रूप में, PLA50/PPC50 संरचना की 4D प्रिंटिंग को न्यूनतम इनवेसिव सर्जरी की सुविधा के लिए

संभावित बायोमेडिकल अनुप्रयोग के रूप में शरीर के तापमान पर प्रदर्शित किया गया था। PLA50/PPC50 संरचना का इन विट्रो डिग्रेडेशन अध्ययन 56 दिनों की अवधि में किया गया था। अंत में, सभी 3D प्रिंटेड PLA/PPC मिश्रणों की इन विट्रो साइटोटॉक्सिसिटी ने उत्कृष्ट जैव-संगतता का प्रदर्शन किया, जिससे ऊतक इंजीनियरिंग अनुप्रयोगों के लिए एक प्रत्यारोपण के रूप में इसकी क्षमता साबित हुई। इस अध्ययन से प्राप्त ट्रिपल-एसएमई के चयनात्मक क्रियान्वयन को प्रभावित करने वाले मापदंडों की व्याख्या से बायोमेडिकल उपकरणों में उपयोग की संभावनाओं की एक विस्तृत श्रृंखला खुलने की उम्मीद है।

इसके अलावा, एक निर्माण पद्धति विकसित की गई जो सिंथेटिक पॉलिमर का उपयोग करके फ्रीफॉर्म संरचनाएं बनाने के लिए लचीलापन प्रदान करती है। एम्बेडेड 3D प्रिंटिंग का एक अभिनव दृष्टिकोण विकसित किया गया था, जो एक सहायक मैट्रिक्स के अंदर सीधे 3D प्रिंटिंग द्वारा फ्रीफॉर्म संरचनाओं के निर्माण की अनुमति देता है। पॉलिमर स्याही समाधान 1,4-डायोक्सेन में PLA और PPC को मिलाकर तैयार किया गया था। लैपोनाइट RD का उपयोग जलीय नैनो-सिलिकेट निलंबन तैयार करने के लिए किया गया था, जिसने अपने हाउस-ऑफ-कार्ड व्यवस्था के कारण उपज तनाव और थिक्सोट्रोपिक गुणों का प्रदर्शन किया। 3D प्रिंटेड पॉलिमर स्याही का जमना डाइऑक्सेन अणुओं के ऑक्सीजन परमाणुओं और पानी के अणुओं के प्रोटॉन के बीच हाइड्रोजन बॉन्डिंग के कारण हुआ। पार्टिकल इमेज वेलोसिमीट्री अध्ययन से पता चला है कि बढ़ती नैनो-सिलिकेट सांद्रता ने उपज तनाव में वृद्धि के परिणामस्वरूप उपज क्षेत्र में कमी का कारण बना। 1 से कम मान वाले नैनो-सिलिकेट सांद्रता के लिए आयामहीन पैरामीटर (ओल्ट्रायंड संख्या और हाइड्रोस्टेटिक दबाव के लिए उपज तनाव का अनुपात) दरार गठन के बिना नोजल आंदोलन को सुविधाजनक बनाने के लिए पाए गए, जबकि 1 से अधिक मान मुद्रण के लिए अनुपयुक्त पाए गए। विभिन्न जटिल संरचनाएं जैसे कि अलग-अलग ज्यामिति वाली ट्यूबलर संरचनाएं जो मूल श्वासनली की नकल करती हैं, और एक द्विभाजित ट्यूब को प्रूफ-ऑफ-कॉन्सेप्ट अध्ययन के रूप में 3 डी प्रिंट किया गया था। प्रस्तावित विधि ने ओवरहैंगिंग ज्यामिति वाली वस्तुओं को बनाने के

लिए विलायक-पानी की परस्पर क्रिया क्षमता का उपयोग करके एक नया दृष्टिकोण पेश किया। यह दृष्टिकोण इसके संगत विलायक और सहायक मैट्रिक्स के बीच मिश्रणशीलता का लाभ उठाकर विभिन्न प्रकार के पॉलिमर की 3 डी प्रिंटिंग की अनुमति देता है।

ऊतक-इंजीनियर ट्यूबलर मचानों के विकास की सांख्यिकीय मॉडलिंग और एम्बेडेड 3D प्रक्रिया मापदंडों के बहु-उद्देश्यीय अनुकूलन का उपयोग करके आगे जांच की गई। यांत्रिक गुणों और सिकुड़न पर परत की मोटाई, प्रिंट गति और बहुलक सांद्रता के प्रभावों का मूल्यांकन करने के लिए केंद्रीय समग्र डिजाइन का उपयोग करके प्रयोगों का डिज़ाइन (DOE) आयोजित किया गया था। रेडियल कंप्रेसिव लोड को मापकर यांत्रिक गुणों का मूल्यांकन किया गया, जबकि आयामी स्थिरता को समझने के लिए सिकुड़न को रिकॉर्ड किया गया। प्रायोगिक परिणामों से पता चला कि कम परत की मोटाई, कम प्रिंट गति और उच्च बहुलक सांद्रता के संयोजन पर सिकुड़न कम हो गई थी। इसके विपरीत, रेडियल कंप्रेसिव लोड ने प्रिंट गति के साथ एक गैर-रैखिक प्रवृत्ति का प्रदर्शन किया, जो घटने से पहले एक इष्टतम बिंदु तक बढ़ गया। इसके अलावा, परत की मोटाई कम होने और उच्च बहुलक सांद्रता के साथ रेडियल लोड में वृद्धि देखी गई। सिकुड़न को कम करने और रेडियल लोड को अधिकतम करने के लिए इष्टतम मापदंडों को निर्धारित करने के लिए एक आनुवंशिक एल्गोरिथ्म (GA) का उपयोग करके एक बहु-उद्देश्यीय अनुकूलन दृष्टिकोण लागू किया गया था। अनुकूलित प्रक्रिया मापदंडों को प्रयोगात्मक परीक्षणों के माध्यम से मान्य किया गया, जो मचान प्रदर्शन में सुधार प्रदर्शित करता है। अनुकूलित मापदंडों की व्यावहारिक उपयोगिता का आकलन करने के लिए, एम्बेडेड 3डी प्रिंटिंग का उपयोग करके एक ट्रेकियल मचान का निर्माण करके एक केस स्टडी आयोजित की गई थी, और इसके यांत्रिक गुणों की तुलना देशी बकरी के श्वासनली से की गई थी। परिणामों ने पुष्टि की कि मचान ने तुलनीय यांत्रिक गुण प्राप्त किए, जो प्रस्तावित अनुकूलन रणनीति की प्रभावशीलता पर जोर देता है।

अंत में, एम्बेडेड 3D प्रिंटिंग का उपयोग करके PLA/PPC कम्पोजिट ट्रेकियल स्कैफोल्ड के निर्माण पर एक अध्ययन किया गया ताकि इसकी ट्रिपल-शेप मेमोरी कार्यक्षमता को प्रदर्शित किया जा सके। 4D प्रिंटेड स्कैफोल्ड की जांच मैकेनिकल, शेप-मेमोरी और जैविक मूल्यांकन का उपयोग करके की जाती है। थर्मल लक्षण वर्णन से पता चला कि PLA/PPC कम्पोजिट सतह पर लैपोनाइट नैनो-सिलिकेट का थर्मल संक्रमण और शेप-मेमोरी प्रदर्शन पर नगण्य प्रभाव था। तीन स्कैफोल्ड ज्यामिति (सिलेंडर, बेलो और सर्पिल-आकार) का निर्माण किया गया और रेडियल और अनुदैर्घ्य संपीड़न के लिए परीक्षण किया गया, जिसमें बेलो-आकार का स्कैफोल्ड देशी बकरी के श्वासनली के यांत्रिक गुणों की बारीकी से नकल करता है। PLA/PPC कम्पोजिट नमूनों ने उच्च आकार-स्थिरता और आकार-पुनर्प्राप्ति अनुपात प्रदर्शित किया। एक केस स्टडी के रूप में, बेलो-आकार के स्कैफोल्ड को देशी बकरी के श्वासनली के लुमेन के अंदर प्रत्यारोपित किया गया था, और ट्रिपल-आकार की मेमोरी व्यवहार का प्रदर्शन किया गया था। पी.एल.ए./पी.पी.सी. नमूनों के विघटन की जांच पी.बी.एस. में शारीरिक स्थितियों (37 डिग्री सेल्सियस) पर 60 दिनों की अवधि के लिए की गई। इसके अतिरिक्त, विघटन परीक्षण से पहले और बाद में विभिन्न कार्यात्मक समूहों पर प्रभाव का आकलन करने के लिए फूरियर-ट्रांसफॉर्म इन्फ्रारेड स्पेक्ट्रोस्कोपी का प्रदर्शन किया गया। नमूनों की जैव-संगतता का और अधिक आकलन करने के लिए, फाइब्रोब्लास्ट कोशिकाओं का उपयोग करके एम.टी.टी., जीवित/मृत और कोशिका आसंजन परख का आयोजन किया गया। पी.एल.ए./पी.पी.सी. कम्पोजिट की इन विट्रो साइटोटॉक्सिसिटी ने उत्कृष्ट जैव-संगतता का प्रदर्शन किया, जो उक्त इंजीनियरिंग अनुप्रयोगों के लिए एक प्रत्यारोपण के रूप में इसकी क्षमता को साबित करता है।

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Acronym

3D	Three dimensional
AM	Additive manufacturing
ANOVA	Analysis of variance
ASTM	American society for testing and materials
ATR	Attenuated total reflection
BPO	Benzoyl peroxide
CAD	Computer aided design
CCD	Central composite design
CNC	Cellulose nanocrystal
DBPH	2,5-dimethyl-2,5-di(tert-butylperoxy)-hexane
DCM	Dichloromethane
DF	Degree of freedom
DIW	Direct ink writing
DMA	Dynamic mechanical analysis
DMEM	Dulbecco's modified eagle medium
DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
DSA	Drop shape analyzer
DSC	Differential scanning calorimetry
DTS	Decellularized tracheal scaffold
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid

ETO	Ethylene dioxide
EVA	Ethylene vinyl acetate
FBS	Fetal bovine serum
FDA	Food and drug administration
FDM	Fused deposition modeling
FDSS	Freeze-dry sonication dodecyl sulfate
FESEM	Field emission scanning electron microscope
FRESH	Freeform reversible embedding of suspended hydrogels
FTIR	Fourier-transform infrared spectroscopy
GA	Genetic algorithm
GelMA	Gelatin methacryloyl
GO	Graphene oxide
HB	Herschel-Bulkley
hMSCs	Human mesenchymal stem cells
ISO	International organization for standardization
LAP	Laponite
LVR	Linear viscoelastic region
MS	Mean of squares
MSC	Mesenchymal stem cell
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
PBS	Phosphate buffer saline
PCL	Polycaprolactone
PCLDMA	Polycaprolactone dimethacrylate
PE	Polyethylene
PEG	Polyethylene glycol

PET	Polyethylene terephthalate
PGA	Polyglycolic acid
PIV	Particle image velocimetry
PLA	Polylactic acid
PLGA	Poly (lactic-co-glycolic acid)
PLMC	Poly (D, L-lactide-co-trimethylene carbonate)
PMAA	Polymethacrylic acid
PMMA	Polymethyl methacrylate
PP	Polypropylene
PPC	Polypropylene carbonate
PSO	Particle swarm optimization
PTMC	Poly (trimethylene carbonate)
PVAc	Polyvinyl acetate
RBC	Red blood cell
RSM	Response surface methodology
SD	Standard deviation
SEM	Scanning electron microscope
SS	Sum of squares
SSMP	Styrene-based shape memory polymer
TPU	Thermoplastic Polyurethane
TS	Temporary shape
UTM	Universal testing machine
XRD	X-ray diffraction

Nomenclature

α_0	Interception coefficient
α_i	Coefficients for linear terms
α_{ij}	Coefficients for interaction terms
$\dot{\gamma}$	Shear rate
ρ	Density
Δl	Change in length
τ	Shear stress
τ_0	yield stress
ΔH_m	Enthalpy of melting of sample
ΔH_0	Enthalpy of melting of 100% crystalline material
C=O	Carbonyl group
C-O-C	Ether bond
CH ₂	Methylene group
ε	Error
ε_X	Strain of shape X in its unloaded state
$\varepsilon_{X,rec}$	Strain after recovery to shape X
ε_Y	Strain of shape Y in its unloaded state
$\varepsilon_{Y,load}$	Strain from shape X to shape Y
E	Young's modulus
F_{value}	Fisher's value
f_1 , and f_2	Objective functions for outputs
g	Acceleration due to gravity
G'	Storage modulus

G''	Loss modulus
K	Consistency index
k	Number of process parameters
n	Flow index
OD	Optical density
Od	Oldroyd number
OH	Hydroxyl group
R^2	Coefficient of determination
R_f	Shape-fixity ratio
R_L	Radial Load
R_r	Shape-recovery ratio
T_g	Glass transition temperature
T_m	Melting temperature
T_c	Crystallization temperature
U	Print speed
V_{ACTUAL}	Actual volume
V_{CAD}	Volume of CAD model
V_e	Variance of the error in regression model
V_s	Volumetric Shrinkage
χ_c	Degree of crystallinity
$\hat{y}$	Response variable
$\hat{y}_p$	Predicted response values
$\hat{y}_{range}$	Variation range