STUDIES ON MANUFACTURING & PRE-CLINICAL TESTING OF CARDIOVASCULAR STENT MATERIALS

RAMYA AHUJA

DEPARTMENT OF MECHANICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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by

RAMYA AHUJA

Department of Mechanical Engineering

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This thesis is dedicated to my loving & supportive family and my alma mater: Columbia University, USA & Vasant Valley School, India

Certificate

*This is to certify that the thesis entitled "**Studies on Manufacturing and Pre-Clinical Testing for Cardiovascular Stent Materials"** submitted by **Ms. Ramya Ahuja** to Indian Institute of Technology, Delhi, for the fulfilment of award of the degree, **Doctor of Philosophy**, is a record of original research carried out by her under my supervision and guidance. This thesis has been prepared as per the rules and regulations of the Indian Institute of Technology Delhi, New Delhi.*

In my opinion, this thesis is worthy of consideration for the award of the degree of Doctor Philosophy in accordance with the institute regulations. To the best of my knowledge, the results embodied in the thesis have not been submitted to any other University or Institute for the award of any other Degree or Diploma.

(**Naresh Bhatnagar**)

Professor

Department of Mechanical Engineering

Indian Institute of Technology, Delhi

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Ramya Ahuja

Abstract

Cardiovascular stents have been developed and have evolved over the last few decades to clinically address the burgeoning problem of heart disease due to atherosclerosis around the world. In the latest iteration, fully resorbable scaffolds have been envisioned and deployed with the hypothesis that they will support the coronary arteries till local healing is complete. Within this domain, the present work aims to address a particular issue of the reformation of the endothelial layer over the stent and artery that occurs within days of implantation. Based on the literature surveyed and the clinical needs identified for rapid and efficient stent endothelializtion post implantation, modification of a polylactic acid (PLA) based scaffold with Magnesium and polycaprolactone (PCL) up to 5% by weight was attempted and tubes from the resultant materials were fabricated by a biaxial extrusion method for enhanced radial strength, previously patented in-house. *In vitro* and *in vivo* studies with L929 mouse fibroblasts and rat subcutanea respectively were conducted to check capsular formation around implant materials as a proxy for endothelialization. Higher cell proliferation was seen on PCL-modified samples *in vitro* at all time points, however, visually, limited cytoplasmic extensions and only bidirectional processes were seen. Mg- containing samples showed promise due to the defined spindle-like morphologies and cytoplasmic extensions, up to ~60μm, by 168 hours. Angiogenesis at the 7 day time point was not found significant to report *in vivo*, suggesting late initiation of this process in the peri-implant capsule. Both PLA/PCL5% and PLA-Mg4%, however, in the same *in vivo* study had significantly higher chronic inflammatory cells (macrophages) as well as the inflammatory markers (TNF- α & IL-1 β) compared to PLA by 8 weeks. While IL-6 is purely inflammatory, TNF-α and IL-1β have been linked to fibroblasts. Even so, no significant effect of the heightened cytokine levels was seen on calculated capsule thicknesses of the modified materials calculated from both the Hematoxylin & Eosin and Masson's Trichome stained sections. Both modifying materials may be considered for future PLA based bioresorbable scaffolds, but synthesis or copolymerization of tailor-made materials rather than processing may be preferred for reduced inflammatory response *in vivo*. Material development for cardiovascular stents may be furthered by continued exploration of bioresorbable polymer & metal composites and use of low cost biopolymers to increase accessibility & affordability of this life saving device. The material systems presented in this thesis can be tested in the atherosclerotic swine model, after cutting of tubes to the final stent design, and the effectiveness for use in humans can then be extrapolated.

सार

दुनिया भर में एथेरोस्क्लेरोसिस के कारण हृदय रोग की बढ़ती समस्या का चिकित्सकीय समाधान करने के लिए कार्डियोवस्कुलर स्टेंट पिछले कुछ दशकों में विकसित और विकसित हुए हैं। नवीनतम पुनरावृत्ति में, पूरी तरह से पुनर्शोषित करने योग्य मचान की कल्पना की गई है और इस परिकल्पना के साथ तैनात किया गया है कि वे स्थानीय उपचार पूरा होने तक कोरोनरी धमनियों का समर्थन करेंगे। इस डोमेन के भीतर, वर्तमान कार्य का उद्देश्य स्टेंट और धमनी पर एंडोथेलियल परत के सुधार के एक विशेष मुद्दे को संबोधित करना है जो आरोपण के दिनों के भीतर होता है। सर्वेक्षण किए गए साहित्य और प्रत्यारोपण के बाद तेजी से और कुशल स्टेंट एंडोथिलिज़लाइज़ेशन के लिए पहचानी गई नैदानिक आवश्यकताओं के आधार पर, वजन के हिसाब से 5% तक मैग्नीशियम और पॉलीकैप्रोलैक्टोन (पीसीएल) के साथ एक पॉलीलैक्टिक एसिड (पीएलए) आधारित मचान में संशोधन का प्रयास किया गया और परिणामी सामग्रियों से ट्यूब बनाए गए। बेहतर रेडियल ताकत के लिए द्विअक्षीय एक्सट्रूज़न विधि द्वारा निर्मित किया गया था, जिसका पहले से घरेलू पेटेंट किया गया था। एंडोथेलियलाइजेशन के लिए प्रॉक्सी के रूप में इम्प्लांट सामग्री के आसपास कैप्सुलर गठन की जांच करने के लिए क्रमशः एल929 माउस फाइब्रोब्लास्ट और चूहे सबक्यूटेनिया के साथ इन विट्रो और विवो अध्ययन आयोजित किए गए थे। सभी समय बिंदुओं पर इन विट्रो में पीसीएल-संशोधित नमूनों पर उच्च कोशिका प्रसार देखा गया, हालांकि, दृष्टिगत रूप से, सीमित साइटोप्लाज्मिक विस्तार और केवल द्विदिश प्रक्रियाएं देखी गईं। एमजी-युक्त नमूनों ने परिभाषित स्पिंडल-जैसी आकृति विज्ञान और साइटोप्लाज्मिक एक्सटेंशन के कारण ~ 60μm तक, 168 घंटों तक वादा दिखाया। 7 दिन के समय बिंदु पर एंजियोजेनेसिस को विवो में रिपोर्ट करने के लिए महत्वपूर्ण नहीं पाया गया, जो पेरी-इम्प्लांट कैप्सूल में इस प्रक्रिया की देर से शुरुआत का सुझाव देता है। हालांकि, पीएलए/पीसीएल5% और पीएलए-एमजी4% दोनों में, विवो अध्ययन में पीएलए की तुलना में क्रोनिक इंफ्लेमेटरी कोशिकाएं (मैक्रोफेज) और साथ ही इंफ्लेमेटरी मार्कर (टीएनएफ- α और आईएल-1 β) काफी अधिक थे। . जबकि IL-6 पूरी तरह से भड़काऊ है, TNF-α और IL-1β को फ़ाइब्रोब्लास्ट से जोड़ा गया है। फिर भी, हेमटॉक्सिलिन और इओसिन और मैसन के ट्राइकोम दाग वाले वर्गों से गणना की गई संशोधित सामग्रियों की गणना की गई कैप्सूल मोटाई पर बढ़े हुए साइटोकिन स्तर का कोई महत्वपूर्ण प्रभाव नहीं देखा गया। दोनों संशोधित सामग्रियों को भविष्य के पीएलए आधारित बायोरेसोरबेबल मचानों के लिए विचार किया जा सकता है, लेकिन विवो में सूजन प्रतिक्रिया को कम करने के लिए प्रसंस्करण के बजाय दर्जी सामग्रियों के संश्लेषण या कोपोलिमराइजेशन को प्राथमिकता दी जा सकती है। कार्डियोवास्कुलर स्टेंट के लिए सामग्री विकास को इस जीवन रक्षक उपकरण की पहुंच और सामर्थ्य बढ़ाने के लिए बायोरेसोरबेबल पॉलिमर और मेटल कंपोजिट की निरंतर खोज और कम लागत वाले बायोपॉलिमर के उपयोग से आगे बढ़ाया जा सकता है। इस थीसिस में प्रस्तुत सामग्री प्रणालियों का एथेरोस्क्लोरोटिक स्वाइन मॉडल में परीक्षण किया जा सकता है, ट्यूबों को अंतिम स्टेंट डिजाइन में काटने के बाद, और फिर मनुष्यों में उपयोग के लिए प्रभावशीलता का अनुमान लगाया जा सकता है।

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Abbreviations

ASTM	American Society for Testing & Material (Standards)
BMS	Bare Metal Stent
BVS	Bioresorbable Vascular Scaffold
CNC	Computer Numerical Control
CoCr	Cobalt Chromium
DES	Drug Eluting Stent
CE	"European Conformity"
DPX	Dibutylpthalate Polystyrene Xylene
ELISA	Enzyme Linked ImmunoAssay
IL-6	Interleukin 6
IL-1β	Interleukin 1Beta
ISO	International Organization for Standardization
IVUS	IntraVascular UltraSound
MACE	Major Adverse Cardiac Event
Mg	Magnesium
OCT	Optical Coherence Tomography
PCL	Polycaprolactone
PDLLA	Poly-D-L-lactic acid

PLA	Poly lactic acid
PLGA	Poly lactic-co-glycolic acid
PLLA	Poly-L-lactic acid
SS	Stainless Steel
SMC	Smooth Muscle Cells
TiNOx	Titanium Nitrous Oxide
Ti6Al4V	Titanium 6-Aluminium 4-Vanadium (alloy)
TLR	Target Lesion Revascularization
TNF-α	Tumor Necrosis Factor Alpha
TVR	Target Vessel Revascularization
SPSS	Statistical Package for the Social Sciences
SD	Standard Deviation