SYNTHESIS, CHARACTERIZATION AND BIOMEDICAL APPLICATION OF MICROPATTERNED POLYMERIC SURFACES

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Synthesis, characterization and biomedical application of micropatterned polymeric surfaces

by

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CERTIFICATE

This is to certify that the thesis entitled, "Synthesis, characterization and biomedical application of micropatterned polymeric surfaces" being submitted by Mrs. Meenakshi Verma to Indian Institute of Technology Delhi for the award of degree of Doctor of Philosophy is a record of bonafide research work carried out by her. Mrs. Meenakshi Verma has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my 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

Materials used for biomedical applications cover a wide spectrum and must exhibit specific properties. The most important property of materials used for fabricating implants is biocompatibility, followed by corrosion resistance. The main metallic biomaterials are stainless steels, cobalt alloy, and titanium and titanium alloys. Among these metallic biomaterials Ti alloys exhibit the highest biocompatibility, corrosion resistance, and specific strength (ratio of the tensile strength to density). Apart from metallic materials, biodegradable polymers have recently become as an influential platform for biomedical field to be used in various areas such as biosensor, therapeutics, targeted drug delivery, tissue engineering, biomedical implants, etc. due to their biodegradability, biocompatibility, controlled crystallinity, and adequate stability in biological environment. However, tailoring the properties of the polymeric surface widens the functionality, durability and applicability of the biodegradable polymers to be used for various applications. For example, the performance and functionality of the biodegradable polymer-based devices utilized for biomedical applications are significantly marked by the method of modification and its process parameters, emphasizing the need for understanding the methods used for modification.

This thesis explores the fabrication and application of biocompatible inorganic and biodegradable polymeric materials for biomedical implant applications. Chapter 2 discusses the synthesis of micropatterned PolyPEGMA nanobrushes on Titanium for enhancing antifouling and antimicrobial properties. A facile and scalable approach to create microtextured biocompatible and biofunctional polymer brushes with the ability to generate different type of protein patterns (e.g., line and circular) is reported. Nanosecond fibre laser was employed to generate around 30 micron wide patterns on the polymer brush (poly(ethylene glycol) methacrylate (polyPEGMA), a protein repellent brush) modified Ti alloy based substrate. PolyPEGMA brush of varying thickness (11-87 nm, measured by ellipsometry) was grafted on Ti alloy via surface initiated atom transfer radical polymerisation (SIATRP) using chloro functionalized ATRP initiator immobilized on Ti surface with initiator density (σ*) of 1.5 initiators/nm^2. Polymer brushes were then selectively laser ablated. Spatial orientation of biomolecules was first achieved by non-specific protein adsorption on areas ablated by the laser, via physisorption. Further, patterned brushes of polyPEGMA were modified to activated ester that gave rise to protein conjugation specifically on non-laser ablated brush areas. Moreover, the laser ablated brush modified

template forming alternate patterns was also demonstrated for generating alternate patterns of bacteria such as *E. coli*.

Chapter 3 details the development of micropatterned dual polymer brushes for biomedical implant and diagnostic applications. Micropatterned dual polymer brushes (PolyPEGMA and PolyDMAEMA) were grafted on PHBV surface via surface-initiated atom transfer radical polymerization (SIATRP). Immobilization of ATRP initiating sites were arranged with the help of hydroxyl groups generated via oxygen plasma on the PHBV surface. Presence of PolyPEGMA brushes on the surface helps to achieve antifouling properties in addition to spatial orientation of biomolecules in alternate patterns on the surface. These micropatterned dual polymer brush modified surfaces were also found to be an excellent source of attachment and detection of HeLa cells through patterned surfaces modified with aptamers immobilized on PolyDMAEMA brushes. In Chapter 4, the micropatterned dual polymer brushes were fabricated as domain selective cationic (PMETA) and anionic (PSPMA) polymer brushes on PLA for biomedical implant applications. The micropatterned dual ionic polymer brushes modified surface showed adsorption of different proteins taking advantage of their unique isoelectric point along with DNA adsorption at varying pH conditions and antimicrobial properties. Chapter 5 introduces synthesis of aliphatic biodegradable polylactide in combination with tartaric acid based copolymers with orthogonal functionality. Compared to the challenges involved for the formation of micropatterns on biodegradable surfaces, a new methodology was applied to fabricate cationic and anionic polymer brushes on copolymer matrix with patterned structure. Spatio-slective attachment of gold and silver microparticles along with the demonstration of spatial attachment of DNA was obtained. Based on this unique construction of polyelectrolyte brushes in one system, UV light enabled the introduction of new surface functionality. A particular advantage of this approach was that triple micropatterns of proteins were also obtained in facile manner. This work clearly establishes the preparation of polyelectrolyte surfaces with orthogonal reaction strategies to achieve multifunctional surface. Finally, Chapter 6 includes a comprehensive summary of all the works and highlights the significant outcomes while proposing directions for future research based on the findings of the thesis. Patterned polymer brushes were developed on both the inorganic biocompatible (Ti) and organic biodegradable polymeric substrates. The techniques used for patterning was broadly classified into nanosecond laser patterning, oxygen plasma treatment and click chemistry. Development of micropatterned surfaces offers promising strategies in the biomedical area to develop protein array, diagnostics, biosensors, *etc.* Nonetheless, development of such surfaces is a tedious process and require multistep synthesis. A facile, reproducible, and scalable approach to yield micropatterned surfaces having multifunctionality and non-toxicity needs to be developed for future benefits.

सार

जैव-चिकित्सा अनुप्रयोगों में उपयोग की जाने वाली सामग्री का एक व्यापक स्पेक्ट्रम होता है, जिसमें विशिष्ट गुणों का प्रदर्शन आवश्यक होता है। इम्प्लांट निर्माण के लिए उपयोग की जाने वाली सामग्रियों का सबसे महत्वपूर्ण गुण जैव-संगतता है, इसके बाद क्षरण प्रतिरोध का स्थान आता है। मुख्य धात्विक बायोमटेरियल्स में स्टेनलेस स्टील, कोबाल्ट मिश्रधातु और टाइटेनियम और इसके मिश्रधातु (Titanium and its alloys) शामिल हैं। इनमें टाइटेनियम मिश्रधातु उच्चतम जैव-संगतता, क्षरण प्रतिरोध और विशिष्ट शक्ति (ताकत और घनत्व का अनुपात) प्रदर्शित करते हैं। धात्विक सामग्रियों के अलावा, हाल के वर्षों में बायोडिग्रेडेबल पॉलिमर जैव-चिकित्सा क्षेत्र में बायोसेंसर, चिकित्सीय अनुप्रयोग, लक्षित दवा वितरण, ऊतक अभियांत्रिकी और जैव-चिकित्सा इम्प्लांट्स जैसे विभिन्न क्षेत्रों में महत्वपूर्ण भूमिका निभा रहे हैं, क्योंकि वे जैव-अवक्रमणीयता, जैव-संगतता, नियंत्रित क्रिस्टलीनिटी और जैविक वातावरण में पर्याप्त स्थिरता प्रदर्शित करते हैं। हालांकि, पॉलिमर की सतह के गुणों को अनुकूलित करके उनकी कार्यक्षमता, स्थायित्व और विभिन्न अनुप्रयोगों में उपयोगिता को बढ़ाया जा सकता है। उदाहरण के लिए, जैव-चिकित्सा अनुप्रयोगों के लिए उपयोग किए जाने वाले बायोडिग्रेडेबल पॉलिमर-आधारित उपकरणों की कार्यक्षमता संशोधन की विधि और प्रक्रिया मापदंडों पर निर्भर करती है, जिससे इन संशोधन विधियों को समझना आवश्यक हो जाता है। यह शोध प्रबंध जैव-संगत अकार्बनिक और बायोडिग्रेडेबल पॉलिमरिक सामग्री के निर्माण और जैव-चिकित्सा इम्प्लांट अनुप्रयोगों में उनके उपयोग का अन्वेषण करता है। अध्याय 2 में टाइटेनियम पर माइक्रोपैटर्न किए गए PolyPEGMA नैनोब्रशेस के संश्लेषण पर चर्चा की गई है, जिससे रोगाणुरोधी और एंटीफाउलिंग गुणों को बढ़ाया गया है। नैनोसेकंड फाइबर लेजर का उपयोग करके पॉली(इथाइलीन ग्लाइकॉल) मेथाक्राइलेट (PolyPEGMA) ब्रश वाले टाइटेनियम मिश्रधातु पर लगभग 30 माइक्रोन चौड़े पैटर्न बनाए गए। PolyPEGMA ब्रश की मोटाई (11-87 nm, एलिप्सोमेट्री द्वारा मापी गई) को सतह-आरंभित एटीआरपी (SIATRP) के माध्यम से टाइटेनियम मिश्रधातु पर जोड़ा गया, जिसमें क्लोरो-फंक्शनलाइज्ड एटीआरपी इनिशिएटर का उपयोग किया गया। इसके बाद, पॉलीमर ब्रश को लेजर द्वारा चयनात्मक रूप से हटाया गया, जिससे बायोमोलेक्यूल्स का स्थानिक अभिविन्यास (spatial orientation of biomolecules) प्राप्त किया गया। अध्याय 3 में माइक्रोपैटर्नेड डुअल पॉलिमर ब्रश (PolyPEGMA और PolyDMAEMA) के विकास का वर्णन किया गया है, जिन्हें PHBV सतह पर SIATRP के माध्यम से जोड़ा गया। ये माइक्रोपैटर्नेड डुअल पॉलिमर ब्रश बायोमोलेक्यूल्स की स्थानिक स्थिति और HeLa कोशिकाओं का पता लगाने में उपयोगी पाए गए। अध्याय 4 में PLA पर डोमेन-चयनात्मक कैटायोनिक (PMETA) और एनायोनिक (PSPMA) पॉलिमर ब्रशेस के निर्माण

को प्रस्तुत किया गया है, जो विभिन्न प्रोटीन और डीएनए अवशोषण के लिए अनुकूल हैं। अध्याय 5 में टार्टरिक एसिड-आधारित सह-पॉलिमर के साथ अलिफैटिक बायोडिग्रेडेबल पॉलीलैक्टाइड के संश्लेषण को शामिल किया गया है, जिसमें धातु कणों और डीएनए के स्थानिक अनुलग्नन को दर्शाया गया है। UV प्रकाश द्वारा नए सतही कार्यात्मकताओं की शुरुआत भी प्रदर्शित की गई है। अंततः, अध्याय 6 में संपूर्ण कार्य का सारांश प्रस्तुत किया गया है और भविष्य के अनुसंधान की संभावित दिशाओं का प्रस्ताव दिया गया है। इस शोध में नैनोसेकंड लेजर पैटर्निंग, ऑक्सीजन प्लाज्मा उपचार और क्लिक केमिस्ट्री जैसी तकनीकों का उपयोग करके माइक्रोपैटर्नेड बहुआयामी सतहों का विकास किया गया है, जो प्रोटीन एरे, बायोसेंसर और डायग्नोस्टिक उपकरणों के विकास में उपयोगी हो सकते हैं। हालांकि, इस तरह की सतहों का विकास एक जटिल प्रक्रिया है और इसके लिए कई चरणों वाली संश्लेषण विधियां आवश्यक होती हैं। इसलिए, भविष्य में गैर-विषाक्त, पुनरुत्पादनीय और स्केलेबल माइक्रोपैटर्नेड सतहों के निर्माण के लिए एक सरल दृष्टिकोण विकसित करने की आवश्यकता है, जो जैव-चिकित्सा इम्प्लांट प्रौद्योगिकी में महत्वपूर्ण प्रगति की राह खोल सके।

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Nanocapillary array membranes (NCAMs)

Poly(N-isopropylacrylamide) (PNIPAAm)

LCST (Lower Critical Solution Temperature)

Electron beam lithography (EBL)

Scanning probe lithography (SPL)

Nanoimprint lithography (NIL)

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1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam)

Poly(ethylene glycol)methyl ether methacrylate (polyPEGMA/PPEGMA)

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