

**DEVELOPMENT OF ANTIMICROBIAL
POLYMERIC COATINGS FOR
TRACHEOESOPHAGEAL VOICE PROSTHESES**

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by

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Centre for Biomedical Engineering

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy
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Inspired by the teachings of

Guru Nanak

‘ਸਰਬੱਤ ਦਾ ਭਲਾ’

‘सरबत दा भला’

‘Sarbat Da Bhala’

(meaning - welfare of all)

Certificate

This is to certify the thesis entitled “**Development of Antimicrobial Polymeric Coatings for Tracheoesophageal Voice Prostheses**” submitted by **Mr. Manjeet Singh** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Biomedical Engineering is a record of bona fide research carried out by him. **Mr. Manjeet Singh** has worked under our guidance and supervision and has fulfilled the requirement for the submission of this thesis.

The results contained in this thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree or diploma.

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Manjeet Singh

Abstract

Tracheoesophageal voice prostheses (TEP) are known to fail in few weeks to several months of implanting due to clogging, caused mainly by microbial biofilm formation and is a cause of concern, resulting in frequent hospital visits, costs and inconvenience to patients. TEPs are soft polymeric devices and are used to restore voice of patients having undergone total laryngectomy (TL). TEPs are implanted on tracheoesophageal wall and come in direct contact with food, fluid and air. The environment of TEP implant is a budding ground for growth of microbes leading to biofilm formation on the TEP surfaces. To increase useful lifespan of TEPs, it is imperative to prevent biofilm formation.

Several attempts have been made to increase useful TEP lifespan. Various antimicrobial agents have been incorporated in TEPs to prevent biofilm formation, but these strategies have not produced prolonged favorable results, due to either short term release of antimicrobial agent or development of resistant bacteria cross-strain. Silver oxide as an antimicrobial agent was incorporated with various polymers but these strategies have not produced prolonged favorable results. Silver is also known to form silver resistant bacterial cross-strains. In other antimicrobial strategies investigated earlier, iodine as antimicrobial agent was complexed with base polymer but its prolonged stability is a concern. Antimicrobial coatings with drug eluting properties have been widely studied but development of drug-resistant strains and their release behavior remains a cause of concern.

The present research work is focused, firstly on developing a generic design tracheoesophageal voice prosthesis (TEP) using medical implant grade thermoplastic polyurethane (TPU) through injection molding technique. Secondly, development of two types of antimicrobial coatings for TPU, based on release and non-release antimicrobial system. The clinical evaluation of developed TEP was done after ethical approval.

In the first strategy to prevent microbial colonization was to use iodine as an antimicrobial agent. Iodine is a known broad spectrum antimicrobial agent and kills bacteria, virus, fungi etc. but its stability is an issue because of its sublime nature. The problem of iodine instability was addressed by complexing iodine with N-vinyl pyrrolidone (NVP) present as a comonomer in an acrylate based tercopolymer. NVP - acrylate tercopolymers were synthesized containing methyl methacrylate (MMA), butyl acrylate (nBA) and N -vinyl pyrrolidone (NVP) in various ratios by free radical polymerization. To optimize results, NVP ratio in tercopolymer was varied from 20%, 35% and 50% w/w while ratio of MMA and BA was kept equal. The three variants of tercopolymers were dissolved in tetrahydrofuran (THF) in 20%, 40% and 60% w/w concentrations before coating, in order to achieve optimum coating thickness. For iodine loading of tercopolymer coated TPUs, iodine solution was prepared in DI water by dissolving 4% NaI and 4% I₂ w/w. The tercopolymer coated TPUs were kept immersed in iodine solution for 2 hours under constant shaking at 120 RPM and 25 °C. The 24hour iodine release in PBS was calculated by observing iodine (I⁻) peak at 224 nm in UV-vis spectrophotometer. The minimum iodine concentration required to effectively kill >99% microbes was evaluated by agar plate method. The coatings were characterized using proton nuclear magnetic resonance (¹H NMR), Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), gel permeation chromatography (GPC) and differential scanning calorimeter (DSC) techniques. The water contact angle (WCA) and mechanical studies were performed to evaluate hydrophilicity, ultimate tensile strength and elongation. The results showed the TPUs coated with tercopolymer and loaded with iodine containing 35% NVP and having 20% solid content have good mechanical properties, good cytocompatibility and hemocompatibility and showed good long term antimicrobial properties till 2 months.

In the second strategy, to fulfil the research objective of prolonging useful TEP lifespan, an acrylate based crosslinked cationic coating was developed having contact mode of microbial killing mechanism. Acrylic tetracopolymers were synthesized using methyl methacrylate (MMA), butyl acrylate (nBA), dimethylaminoethyl methacrylate (DMEAMA) and hydroxyethylmethacrylate (HEMA), in various ratios by free radical polymerization. The TPUs were coated with tetracopolymer and were converted into cationic quaternary ammonium moieties by covalently bonding iodoctane with the tertiary nitrogen groups present in DMEAMA of tetracopolymer. The crosslinking of acrylate tetracopolymer was performed by using hexamethylene diisocyanate trimer (THDI) through urethane linkage with hydroxy group present in HEMA using dibutyltin dilaurate (DBTDL) as catalyst. The dried tetracopolymer coated TPUs were treated with iodoctane for 4 hours in an orbital shaker at 25°C in order to quaternize the DMAEMA followed by washing with hexane. The coatings were characterized using ¹HNMR, FTIR, GPC, DSC, FESEM and EDS techniques. The WCA and mechanical studies was performed to evaluate hydrophilicity, ultimate tensile strength and elongation. Antimicrobial evaluation was done through agar diffusion and contact killing method. The coatings were investigated for endurance under harsh conditions by washing repeatedly in 50% ethanol. Antimicrobial studies were performed by using mix culture of *E. Coli* (gram negative), *S. aureus* (gram positive) and *C. albicans* (fungi). The results showed tetracopolymer quaternized coatings on TPU containing 35% DMAEMA has excellent mechanical properties, biocompatibility and has antimicrobial properties for at least 180 days. The coating endured harsh condition of 100 washes with 50% ethanol and retained its antimicrobial properties. The quaternized tetracopolymer coating was found to be superior with long term antimicrobial properties as compared to NVP based coating developed earlier in the first strategy and was found to be an excellent choice for development of antimicrobial polyurethane based TEPs.

सार

ट्रेकियोसोफेगल वॉयस प्रोस्थेसिस (टीईपी) को मुख्य रूप से माइक्रोबियल बायोफिल्म निर्माण के कारण रुकावट के कारण प्रत्यारोपण के कुछ हफ्तों से लेकर कई महीनों में विफल होने के लिए जाना जाता है और यह चिंता का कारण है, जिसके परिणामस्वरूप बार-बार अस्पताल जाना पड़ता है, लागत और रोगियों को असुविधा होती है। टीईपी नरम बहुलक उपकरण हैं और इनका उपयोग कुल स्वरयंत्र उच्छेदन (टी एल) से गुजरने वाले रोगियों की आवाज को बहाल करने के लिए किया जाता है। टीईपी को ट्रेकियोसोफेगल दीवार पर प्रत्यारोपित किया जाता है और यह भोजन, तरल पदार्थ और हवा के सीधे संपर्क में आता है। टीईपी प्रत्यारोपण का वातावरण सूक्ष्मजीवों के विकास के लिए एक नवोदित भूमि है, जिससे टीईपी सतहों पर बायोफिल्म का निर्माण होता है। टीईपी के उपयोगी जीवनकाल को बढ़ाने के लिए, बायोफिल्म निर्माण को रोकना अनिवार्य है।

उपयोगी TEP जीवनकाल को बढ़ाने के लिए कई प्रयास किए गए हैं। बायोफिल्म निर्माण को रोकने के लिए टीईपी में विभिन्न रोगाणुरोधी एजेंट शामिल किए गए हैं, लेकिन इन रणनीतियों ने रोगाणुरोधी एजेंट की अल्पकालिक रिहाई या प्रतिरोधी बैक्टीरिया क्रॉस-स्ट्रेन के विकास के कारण लंबे समय तक अनुकूल परिणाम नहीं दिए हैं। रोगाणुरोधी एजेंट के रूप में सिल्वर ऑक्साइड को विभिन्न पॉलिमर के साथ शामिल किया गया था, लेकिन इन रणनीतियों ने लंबे समय तक अनुकूल परिणाम नहीं दिए हैं। सिल्वर को सिल्वर प्रतिरोधी बैक्टीरियल क्रॉस-स्ट्रेन बनाने के लिए भी जाना जाता है। पहले जांच की गई अन्य रोगाणुरोधी रणनीतियों में, रोगाणुरोधी एजेंट के रूप में आयोडीन को बेस पॉलिमर के साथ जटिल किया गया था, लेकिन इसकी लंबे समय तक स्थिरता एक चिंता का विषय है। ड्रग एल्यूटिंग गुणों के साथ रोगाणुरोधी कोटिंग्स का व्यापक रूप से अध्ययन किया गया है, लेकिन दवा प्रतिरोधी उपभेदों का विकास और उनका रिलीज व्यवहार चिंता का कारण बना हुआ है।

वर्तमान शोध कार्य सबसे पहले इंजेक्शन मोल्डिंग तकनीक के माध्यम से मेडिकल इम्प्लांट ग्रेड थर्मोप्लास्टिक पॉलीयूरेथेन (टीपीयू) का उपयोग करके एक सामान्य डिजाइन ट्रेकियोसोफेगल वॉयस प्रोस्थेसिस (टीईपी) विकसित करने पर केंद्रित है। आयोडीन एक जाना-माना ब्रॉड स्पेक्ट्रम एंटीमाइक्रोबियल एजेंट है और बैक्टीरिया, वायरस, फंगस आदि को मारता है, लेकिन इसकी स्थिरता इसकी उत्कृष्ट प्रकृति के कारण एक मुद्दा है। आयोडीन अस्थिरता की समस्या को एक ऐक्रिलेट आधारित टेरकोपॉलिमर में कॉमोनोमर के रूप में मौजूद एन-विनाइल पाइरोलिडोन (एनवीपी) के साथ आयोडीन को मिलाकर संबोधित

किया गया था। एनवीपी - ऐक्रिलेट टेरकोपॉलीमर को फ्री रेडिकल पॉलीमराइजेशन द्वारा विभिन्न अनुपातों में मिथाइल मेथैक्रिलेट (एमएमए), ब्यूटाइल ऐक्रिलेट (एनबीए) और एन-विनाइल पाइरोलिडोन (एनवीपी) युक्त संश्लेषित किया गया था। परिणामों को अनुकूलित करने के लिए, टेरकोपॉलीमर में एनवीपी अनुपात 20%, 35% और 50% वजन/वजन से भिन्न था जबकि एमएमए और बीए का अनुपात बराबर रखा गया था। कोटिंग से पहले टेर्कोपॉलिमर के तीन प्रकारों को टेट्राहाइड्रोफ्यूरान (टीएचएफ) में 20%, 40% और 60% वजन/वजन सांद्रता में घोला गया, ताकि इष्टतम कोटिंग मोटाई प्राप्त की जा सके। टेर्कोपॉलिमर लेपित टीपीयू की आयोडीन लोडिंग के लिए, 4% NaI और 4% I₂ वजन/वजन को घोलकर विआयनीकृत पानी में आयोडीन घोल तैयार किया गया था। टेर्कोपॉलिमर लेपित टीपीयू को 120 क्रांतियों प्रति मिनट और 25 डिग्री सेल्सियस पर लगातार हिलाते हुए 2 घंटे के लिए आयोडीन के घोल में डुबो कर रखा गया था। यूवी-विज़ स्पेक्ट्रोफोटोमीटर में 224 नैनो मीटर पर आयोडीन (I⁻) पीक को देखकर फॉस्फेट बफर खारा में 24 घंटे की आयोडीन रिलीज़ की गणना की गई। >99% सूक्ष्मजीवों को प्रभावी ढंग से मारने के लिए आवश्यक न्यूनतम आयोडीन सांद्रता का मूल्यांकन अगर प्लेट विधि द्वारा किया गया था। कोटिंग्स को प्रोटॉन न्यूक्लियर मैग्नेटिक रेजोनेंस (1एचएनएमआर), फूरियर ट्रांसफॉर्म इंफ्रारेड स्पेक्ट्रोस्कोपी विद एटेन्यूएटेड टोटल रिफ्लेक्शन (एटीआर एफटीआईआर), जेल परमीशन क्रोमैटोग्राफी (जीपीसी) और डिफरेंशियल स्कैनिंग कैलोरीमीटर (डीएससी) तकनीकों का उपयोग करके चिह्नित किया गया था। हाइड्रोफिलिसिटी, अंतिम तन्य शक्ति और बढ़ाव का मूल्यांकन करने के लिए जल संपर्क कोण (डब्ल्यूसीए) और यांत्रिक अध्ययन किए गए थे। परिणामों से पता चला कि टेरकोपॉलीमर के साथ लेपित और 35% एनवीपी युक्त आयोडीन से भरे हुए और 20% ठोस सामग्री वाले टीपीयू में अच्छे यांत्रिक गुण, अच्छी साइटोकम्पैटिबिलिटी और हेमोकम्पैटिबिलिटी है और 2 महीने तक अच्छे दीर्घकालिक रोगाणुरोधी गुण दिखाए।

दूसरी रणनीति में, उपयोगी टीईपी जीवनकाल को बढ़ाने के अनुसंधान उद्देश्य को पूरा करने के लिए, एक ऐक्रिलेट आधारित क्रॉसलिंकड कैटायनिक कोटिंग विकसित की गई जिसमें माइक्रोबियल किलिंग मैकेनिज्म का संपर्क मोड था। ऐक्रिलिक टेट्राकोपॉलिमर को मिथाइल मेथैक्रिलेट (एमएमए), ब्यूटाइल ऐक्रिलेट (एनबीए), डाइमिथाइलैमिनोइथाइल मेथैक्रिलेट (डीएमईएमए) और हाइड्रॉक्सीएथिमेथैक्रिलेट (एच-ई-एम-ए) का उपयोग करके विभिन्न अनुपातों में फ्री रेडिकल पॉलीमराइजेशन द्वारा संश्लेषित किया गया। टीपीयू को टेट्राकोपॉलिमर के साथ लेपित किया गया और टेट्राकोपॉलिमर के डीएमईएमए में मौजूद तृतीयक नाइट्रोजन समूहों के साथ आयोडोक्टेन को सहसंयोजक रूप से जोड़कर कैटायनिक क्वाटरनेरी अमोनियम मोइटीज में परिवर्तित किया गया। ऐक्रिलेट टेट्राकोपॉलीमर का क्रॉसलिंकिंग हेक्सामेथिलीन डायसोसाइनेट ट्रिमर (टीएचडीआई) का उपयोग करके एच-ई-एम-ए में मौजूद हाइड्रॉक्सी समूह के साथ यूरेथेन लिंकेज के

माध्यम से किया गया था, जिसमें उत्प्रेरक के रूप में डाइब्यूटिलटिन डाइलॉरेट (डीबीटीडीएल) का उपयोग किया गया था। सूखे टेट्राकोपोलीमर लेपित टीपीयू को 25 डिग्री सेल्सियस पर ऑर्बिटल शेकर में 4 घंटे के लिए आयोडोक्टेन के साथ उपचारित किया गया ताकि डीएमईएएम को क्वाटरनाइज किया जा सके और उसके बाद हेक्सेन से धोया जा सके। कोटिंग्स को 1एचएनएमआर, एफटीआईआर, जीपीसी, डीएससी, एफईएसईएम और ईडीएस तकनीकों का उपयोग करके अभिलक्षणित किया गया। हाइड्रोफिलिसिटी, अंतिम तन्य शक्ति और बढ़ाव का मूल्यांकन करने के लिए डब्ल्यूसीए और यांत्रिक अध्ययन किए गए। अगर प्रसार और संपर्क हत्या विधि के माध्यम से रोगाणुरोधी मूल्यांकन किया गया था। 50% इथेनॉल में बार-बार धोने से कठोर परिस्थितियों में धीरज के लिए कोटिंग्स की जांच की गई। ई. कोली (ग्राम नेगेटिव), एस. ऑरियस (ग्राम पॉजिटिव) और सी. एल्बिकेंस (फंगस) के मिक्स कल्चर का उपयोग करके रोगाणुरोधी अध्ययन किए गए। परिणामों से पता चला कि 35% डीएमईएएम युक्त टीपीयू पर टेट्राकोपोलीमर क्वाटरनाइज्ड कोटिंग्स में बेहतरीन यांत्रिक गुण, जैव-संगतता है और कम से कम 180 दिनों तक रोगाणुरोधी गुण होते हैं। कोटिंग ने 50% इथेनॉल के साथ 100 धुलाई की कठोर परिस्थितियों को सहन किया और इसके रोगाणुरोधी गुणों को बरकरार रखा। क्वाटरनाइज्ड टेट्राकोपोलीमर कोटिंग को पहले रणनीति में विकसित एनवीपी आधारित कोटिंग की तुलना में दीर्घकालिक रोगाणुरोधी गुणों के साथ बेहतर पाया गया और इसे रोगाणुरोधी पॉलीयूरेथेन आधारित टीईपी के विकास के लिए एक उत्कृष्ट विकल्प पाया गया।

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List of Abbreviations

μL	Micro Liter
μ-IM	Micro injection molding
AIBN	Azobisisobutyronitrile
AMP	Antimicrobial peptide
ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy
BA	Butyl acrylate
CFU	Colony-forming unit
CTRT	Chemo therapy radio therapy
DBDTL	Dibutyltin dilaurate
DMAEMA	Dimethylaminoethyl methacrylate
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
DOE	Design of Experiments
DSC	Differential scanning calorimeter
EPS	Extracellular polymeric substances
FBS	Fetal bovine serum
FDM	Fused deposition modeling
EDTA	Ethylenediaminetetraacetic acid
EDS	Energy dispersive spectroscopy
eV	Electron volt
FESEM	Field emission scanning electron microscope
GERD	Gastroesophageal reflux disease

GPC	Gel permeation chromatography
HEK	Human embryonic kidney 293 cells
HEMA	2-hydroxyethylmethacrylate
HNMR	Proton nuclear magnetic resonance
IM	Injection molding
IO	Iodoctane
kV	Kilo volt
LPM	liter per minute
Mg	Milligram
Min	Minute
Mm	Millimeter
MMA	Methyl methacrylate
MPa	Megapascal
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)
M_w	Weight average molecular weight
NaI	Sodium iodide
nm	Nanometer
NVP	N-vinyl-2-pyrrolidone
PBS	Phosphate buffer saline
PCL	Polycaprolactone
PEG	Polyethylene glycol
PEI	Polyethylenimine
PEO	Polyethylene oxide
pH	Potential of Hydrogen

PLA	Polylactic Acid
PMMA	Polymethyl methacrylate
PP	Polypropylene
Ppm	Parts per million
PVDF	Polyvinylidene fluoride
QoL	Quality of life
QAC	Quaternary ammonium compounds
RBC	Red blood cells
ROS	Reactive oxygen species
RP	Rapid prototyping
RPM	Revolutions per minute
RT	Radiation therapy
SHS	Super hydrophobic surface
SLA	Stereolithograph apparatus
SLS	Selective laser sintering
TEP	Tracheoesophageal voice prosthesis
THDI	Trimeric hexamethylene diisocyanate
THF	Tetrahydrofuran
T_g	Glass transition temperature
TL	Total laryngectomy
TPU	Thermoplastic polyurethane
UTM	Universal testing machine
UV	Ultraviolet
UV-Vis	Ultraviolet–visible spectroscopy
w/w	Weight per weight
XPS	X-Ray photoelectron spectroscopy
ZnO	Zinc oxide