DEVELOPMENT OF PECTIN BASED GELS FOR WOUND CARE

MYTHILI TUMMALAPALLI

DEPARTMENT OF TEXTILE TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

MYTHILI TUMMALAPALLI

Department of Textile Technology

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CERTIFICATE

This is to certify that the thesis entitled "**Development of Pectin based Gels for Wound Care**" submitted by **Ms. Mythili Tummalapalli** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. Ms. Mythili Tummalapalli has worked under our guidance and has fulfilled the requirements for the submission of this thesis which has attained the standard required for a Ph.D. degree of this institute. 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.

(Dr. Bhuvanesh Gupta)

Professor

Department of Textile Technology

Indian Institute of Technology Delhi

Hauz Khas, New Delhi - 110016

(Dr. B. L. Deopura)

Professor

Department of Textile Technology

Indian Institute of Technology Delhi

Hauz Khas, New Delhi - 110016

(Dr. M. Sarwar Alam)

Professor

Department of Chemistry

Jamia Hamdard

Hamdard Nagar, New Delhi - 110062

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ABSTRACT

Wound management is an essential aspect of post-surgical patient care. For efficient wound care and management, it is necessary to develop highly effective antimicrobial materials that can exhibit prolonged infection control. In addition to infection control, it is desirable that the wound dressing leads to rapid wound healing with acceptable aesthetic features. With these objectives in mind, the current study was aimed at designing and developing pectin based wound care materials with all the features of an ideal wound dressing.

This investigation is associated with the development of wound dressings based on pectin, which is a natural polymer obtained from terrestrial plants. High methoxy citrus pectin was oxidized by periodic acid to prepare a dialdehyde functionalized material, oxidized pectin (OP). During the oxidation process, degradation of the polymer chains also takes place, leading to shorter chain lengths and reduction in the molecular weight. The effect of various reaction conditions, *viz.*, reaction time, reaction temperature, pH of the medium, periodic acid concentration and solvent composition on the oxidation process was investigated. With an increase in the reaction time, the aldehyde content increased. However, the intrinsic viscosity of the system decreased indicating that degradation takes place simultaneously with oxidation. The amount of generated aldehyde increased with an increase in the reaction time, reaction temperature and the concentration of periodic acid. Due to the polyanionic behaviour of pectin, higher aldehyde contents were obtained at lower pH. It was observed that the smaller chain lengths obtained upon oxidation led to crystalline perfection. Changes in surface topography supported this conclusion. Keeping all other reaction conditions constant, higher aldehyde contents were obtained in water-ethanol system as compared to pure aqueous medium. An increase in the ethanol content increased the amount of aldehyde generation. Fourier transform infrared (FTIR) spectra of OP systems show a carbonyl peak at 1734 cm^{-1}, indicating the formation of aldehyde groups. The reaction conditions were optimized to produce OP with an aldehyde content of 2.101

mmol/g. The final product was subsequently used to synthesize nanosilver and to form crosslinked network structures with gelatin.

An alternative route for facile synthesis of nanosilver using OP as the reducing agent as well as the stabilizing agent is reported. As a result of the reduction reaction, oxidized pectin-nanosilver (OP-NS) core sheath nanohydrocolloids are formed. The effect of reaction parameters on the nanoparticle formation was investigated. The structural and morphological features have been analyzed using X-ray diffraction (XRD) and high resolution transmission electron microscopy (HRTEM), respectively. The crystal size of nanosilver was calculated to be 28.76 nm. It was noticed that OP-NS nanohydrocolloids of different morphologies could be produced by controlling the reaction parameters. While the average size of the core sheath structure varied from 289 nm to 540 nm, the size of the silver nanoparticle entities at the core varied from 100 nm to 180 nm, with variation in reaction time. From the morphological examination, it could be seen that flower like nanostructures are formed with nanosilver in the core surrounded by a polymeric halo.

In the third step of the current research, crosslinked hydrocolloid networks were developed by *in situ* reaction of OP and gelatin, leading to OP-gelatin (OP-Gel) network. The reaction takes place through the formation of Schiff bases between aldehyde groups of OP and amine groups of gelatin. The effect of various process parameters, such as reaction time, reaction temperature, pH of the reaction and composition on the efficacy of the crosslinking was investigated. The aldehyde consumption reached a saturation level after 16 h, indicating the highest degree of crosslinking. With an increase in the reaction temperature, a reduction in crosslinking was observed. On the other hand, the aldehyde consumption gradually increased up to a pH of 6.4, beyond which it reduced. The polymer composition played a major role in the network formation, with the highest degree of crosslinking at 70/30 OP/Gel composition. Field emission scanning electron microscopy revealed that homogenous, single phase systems are obtained after the crosslinking of OP and gelatin. Glycerol, when used as a plasticizer, improved the flexibility and the handling characteristics

of the crosslinked hydrogels. Plasticized films retained good tensile strengths in the range of 19-48 MPa.

To fabricate OP-Gel biocomposite dressings, a nonwoven cotton fabric was chosen. The OP-Gel composition when coated onto the fabric resulted in water retention of 400%, and thus can exhibit excellent exudate absorption characteristics. Nanosilver and ciprofloxacin based OP-Gel interactive wound dressings have been fabricated. Nanosilver was synthesized *in situ* within the OP-Gel crosslinked matrix to develop OP-Gel-NS dressings. Ciprofloxacin hydrochloride has also been incorporated into OP-Gel matrix to produce OP-Gel-Cipro dressings. While OP-Gel-NS dressings exhibited 100% antimicrobial activity at extremely low loadings of 3.75 $\mu g/cm^2$, OP-Gel-Cipro dressings exhibited antimicrobial activity at 1% ciprofloxacin hydrochloride loading. The cytocompability and wound healing potential of OP-Gel-NS and OP-Gel-Cipro dressings were contrasted against a commercial dressing, Bactigras®. NIH3T3 mouse fibroblast cells were cultured on OP-Gel-drug and Bactigras® dressings and it was observed that OP-Gel-Cipro dressings were most conducive to cell growth and proliferation. On the other hand, OP-Gel-NS hindered cell growth, while complete lysis took place with Bactigras® treatment. Full thickness excisional wounds were created on C57BL6 mice and the wound healing potential of the OP-Gel-NS dressings led to complete healing within 12 d, while OP-Gel-Cipro dressings treated wounds at a rate similar to that of Bactigras®. Histological examination revealed that OP-Gel-NS and OP-Gel-Cipro treatment led to organized collagen deposition, neovascularization and nuclei migration. The OP-Gel-NS and OP-Gel-Cipro biocomposite dressings exhibited good hydrophilicity, sustained antimicrobial nature, promoted cell growth and proliferation, and led to rapid healing.

Apart from the synthetic drug based dressings, natural drug based OP-Gel biocomposite dressings were fabricated using aloe vera and curcumin as the bioactive agents. From the morphological examination of the dressings in contact with simulated body fluid, it was observed that leaching of the OP-Gel matrix takes place. The leverage of drug

concentration on the antimicrobial nature of the dressings was investigated. It was found that 40% loading of drug was necessary for effective antimicrobial activity. The optimized OP-Gel-Curcumin dressings exhibited ~80% free radical scavenging activity while OP-Gel-Aloe dressings exhibited ~10% activity. However, from the pre-clinical *in vivo* wound healing studies, it was observed that OP-Gel-Aloe dressings resulted in 80% wound contraction while OP-Gel-Curcumin treated wounds healed about 60% in 8 d. Histopathological studies were conducted on excised tissues. It was found that OP-Gel-Aloe treated wounds healed with reduced scarring, good collagen organization and deposition, and neovascularization. NIH3T3 mouse fibroblast cells were cultured *in vitro* in the presence of OP-Gel-Aloe and OP-Gel-Curcumin dressings. The OP-Gel-Aloe treated cells exhibited better cell viability compared to OP-Gel-Curcumin treated cells and Bactigras® treated cells. Hence, it was concluded that OP-Gel-Aloe biocomposite dressings could be used beneficially for effective and rapid wound management with improved aesthetics.

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ABBREVIATIONS

2,4-dinitrophenyl hydrazine	-	DNPH
Acid orange 7	-	AO7
Arginylglycylaspartic acid	-	RGD
Atomic absorption analysis spectrometer	-	AAS
Atomic force microscopy	-	AFM
Carboxymethyl cellulose	-	CMC
Carboxymethyl chitosan	-	CMCTS
Diphenyl picrylhydrazyl	-	DPPH
Dulbecco's modified eagle's medium	-	DMEM
Dynamic light scattering	-	DLS
Energy dispersive X-ray analysis	-	EDX
Epidermal growth factor	-	EGF
Escherichia coli	-	E.coli
Extracellular matrix	-	ECM
Fetal bovine serum	-	FBS
Field emission scanning electron microscopy	-	FESEM
Fourier transform infrared spectroscopy	-	FTIR
High resolution transmission electron microscopy	-	HRTEM
Lower critical solution temperature	-	LCST
Nitric oxide	-	NO
Oxidized pectin	-	OP
Oxidized pectin-gelatin	-	OP-Gel
Oxidized pectin-gelatin-aloe vera	-	OP-Gel-Aloe

Oxidized pectin-gelatin-ciprofloxacin	-	OP-Gel-Cipro
Oxidized pectin-gelatin-curcumin	-	OP-Gel-Curcumin
Oxidized pectin-gelatin-nanosilver	-	OP-Gel-NS
Oxidized pectin-nanosilver	-	OP-NS
Phosphate buffer saline	-	PBS
Poly(2-hydroxyethyl methacrylate)	-	PHEMA
Polyethylene oxide	-	PEO
Poly(ethylene glycol)	-	PEG
Poly(N-isopropyl acrylamide)	-	PNIPAM
Poly(N-vinylpyrrolidone)	-	PVP
Polypropylene	-	PP
Polyurethane	-	PU
Polyvinyl alcohol	-	PVA
Relative fluorescence units	-	RFU
Semi interpenetrating networks	-	semiIPNs
Simulated body fluid	-	SBF
Staphylococcus aureus	-	S.aureus
Ultraviolet-visible spectrophotometry	-	UV-Vis
Waterborne polyurethane	-	WBPU
X-ray diffraction	-	XRD