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Cold Plasma Surface Modified Electrospun Microtube Array Membrane for Chitosan Immobilization and Their Properties

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***Abstract*—**Electrospun microtube array membranes (MTAMs) made of PLLA (poly-L-lactic acid) have wide potential applications in tissue engineering. However, their surface hydrophobicity and poor biocompatability have limited their further usage. In this study, the surface of PLLA MTAMs were made hydrophilic by introducing extra functional groups, such as peroxide, via an acetic acid plasma (AAP). UV-graft polymerization of acrylic acid (G-AAc) was then used to produce carboxyl group on MTAMs surface, which bonded covalently with chitosan through EDC / NHS crosslinking agents. To evaluate the effects of the surface modification on PLLA MTAMs, water contact angle (WCA) measurement and cell compatibility tests were carried out. We found that AAP treated electrospun PLLA MTAMs grafted with AAc and, finally, with chitosan immobilized via crosslinking agent, exhibited improved hydrophilic and cell compatibility.

***Keywords*—**Plasma, EDC/NHS, UV grafting, chitosan, microtube array membrane.

I. Introduction

ELECTROSPINNING is a straightforward method of producing ultrafine fibers from micro- to nano-meter range diameters and with controlled surface morphology. Electrospun fibrous substrates were often used in drug delivery, artificial dressings, and cell enzyme immobilization. MTAMs made of PLLA hollow fibers have possessed wide potential applications in tissue engineering [1]-[3]. However, their hydrophobicity and poor biocompatibility have limited such usages [4].

Chitosan is a non-toxic and biodegradable material, which exhibits good antibacterial activity and cell adhesiveness, and has been widely utilized to prepare microspheres for anticancer drug delivery system [5].

Surface modification by plasma deposition and graft polymerization is often used to solve the adhesion problem between substrate surfaces and coatings. Plasma deposition can provide free radicals and peroxide groups on the substrate surfaces [6]. Plasma-deposited surface film has many advantages such as good adhesion to substrates, excellent uniformity, easy for thickness control, low porosity, and pin hole-free formation [7]. Graft polymerization on the plasma-treated surface further adds radicals to the surface for enhancing the chemical property of the surface [8]-[11].

PLLA MTAMs surface was modified by an AAP to introduce functional groups and subsequently treated by UV-graft polymerization of AAc to facilitate the immobilization of chitosan. This study employed a plasma deposition and a graft technique to modify the surface of PLLA MTAMs to facilitate the immobilization of chitosan. an oxygen plasma treatment on the PLLA MTAMs is expected to introduce peroxide groups on the substrate surface. Subsequent UV grafting with AAc on the plasma-treated PLLA MTAMs was applied to polymerize carboxylic groups on the surface. EDC/NHS crosslinking agents were used to combine the functional groups on the grafted PLLA MTAMs surface with chitosan. biocompatability of electrospun PLLA MTAMs was evaluated by MTT assay using NIH3T3 cells.

II. Experimental

A. Materials

Porous MTAMs were fabricated by co-axial electrospinning (Fig. 1) [1]-[3]. The chemicals used in this study includes AAc and ammonium peroxodisulfate (APS) from Wako Co., Japan.

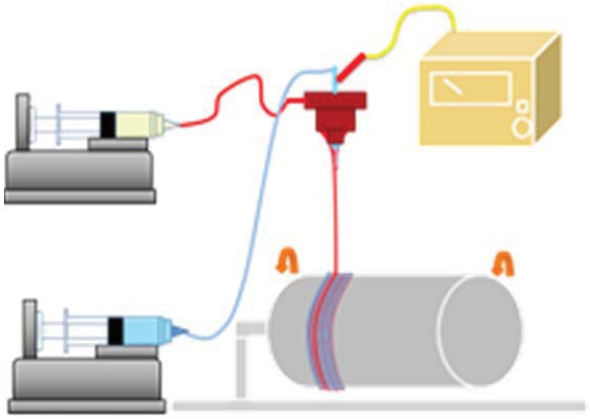

Fig. 1 Fabrication of porous PLLA MTAMs by co-axial electrospinning

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B. Processes for Immobilizing Chitosan on PLLA MTAMs

The reaction steps for the immobilization of chitosan on PLLA MTAMs by plasma and grafting treatments are shown schematically in Fig. 2.

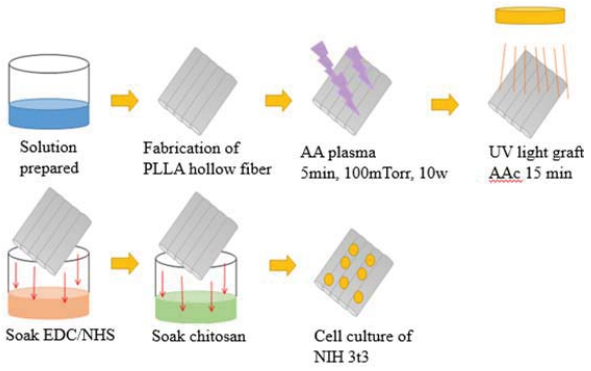


Fig. 2 Schematic reaction steps for the immobilization of chitosan on MTAMs by plasma and grafting treatments are shown

PLLA MTAMs were subjected to an AAP pretreatment (Fig. 3). The cleaned PLLA MTAMs were placed on the lower electrode of the reaction chamber before vacuumed. The reaction chamber was firstly evacuated to less than 30 mTorr. After the pressure was stable, acetic acid monomer was introduced into the reaction chamber and maintained at a constant pressure by adjusting the micro-throttle value. After the pressure has become stable, the plasma glow discharge treatment was employed. Substrate temperature was kept at room temperature during the treatment. The treatment was performed under the following conditions: AAP pretreatment at 100 mTorr, 10 W, and 5 min.

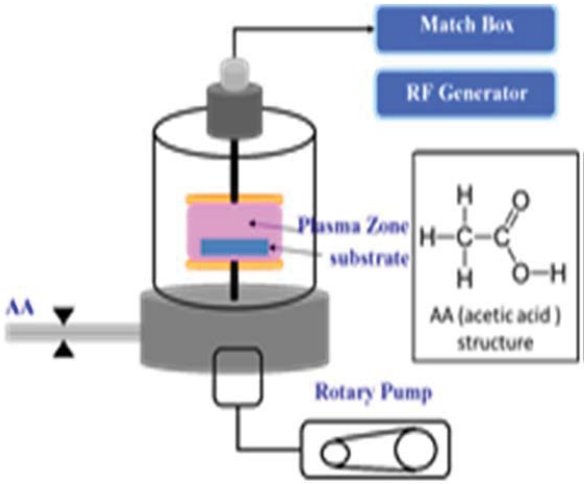


Fig. 3 Schematic diagram of AAP reactor system

Subsequently, the plasma-modified PLLA MTAMs were grafted by UV-radiation using a 10 mmol AAc solution containing 0.1 mmol APS under high-pressure mercury lamp at 1000 W for 20 min. The grafting solution of AAc was composed by: AAc 1.5ml, water 13.5ml, Vitamin B2 4ml, APS 0.0212g.

Finally, the AAc-grafted PLLA MTAMs were cross-linked with EDC/NHS to immobilize chitosan by mixing 1 wt% glutaraldehyde and 1 wt% chitosan with MTAMs at room temperature for 1 day. EDC: 5mg/ml, NHS: 1.25mg/ml, Chitosan: 2N.

Bio-compatibility: MTT assay with NIH3T3 cells. Cells were cultured on the surface of the different steps of preparation, quantitative to observe the situation NIH3T3 mouse fibroblasts grown on the surface of the material after 1,3,5, day by (MTT assay) method as mentioned previously [8], [9].

III. Results and Discussion

A. Wettability of PLLA MTAMs

Surface wettability of the modified substrate can be measured by the WCA.

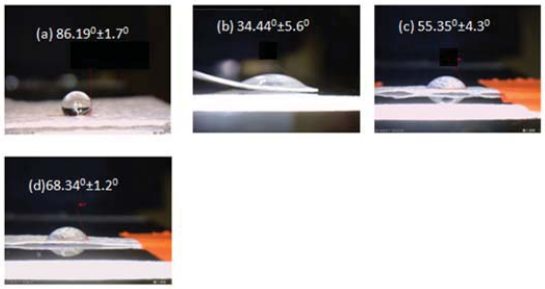


Fig. 4 WCAs of MTAMs (a) untreated, (b) P.D. acetic acid(c) S-Graft AAc (5%), and (d) chitosan Immobilized

Fig. 4 shows the WCA results of plasma treated, grafted acrylic polymer and immobilized chitosan. The WCA decreased from 59.2°±1.7° (untreated) to 34.4°±5.6° (AAP treated), then rose to 55.4°±4.3° (grafted with AAc polymer), and eventually became more hydrophobic (68.3°±1.2°) after chitosan immobilization.

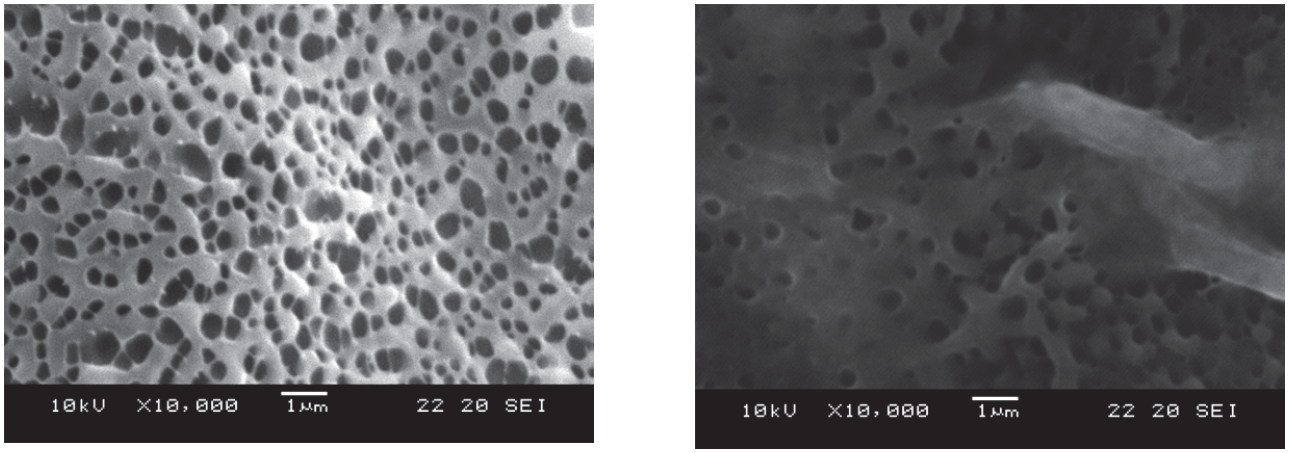
TABLE I
HYDROPHOBICITY (WCA, DEGREE) OF SURFACE TREATED ELECTROSPUN PLLA MTAMS SUBSTRATES.

	Plasma	EDC/NHS + Chitosan
Untreated	86.0	75.0
PAA	48.0	46.0
PAA+G-AAc	40.0	38.0

It can be observed that PLLA MTAMs have become more hydrophilic after plasma and grafting treatments however showed slightly hydrophobic than untreated PLLA MTAMs after immobilization of chitosan through EDC/NHS crosslinking agents.

B. Surface Morphology of Modified MTAMs

Surface morphology of PLLA MTAMs by scanning electron microscope (SEM) is showed in Figs. 5 and 6. A relatively thick layer of polypropylene acid and chitosan was observed.



(a) (b)

Fig. 5 SEM of PLLA MTAMs (a) untreated, and (b) PAA+ EDC/NHS+chitosan (X10000)

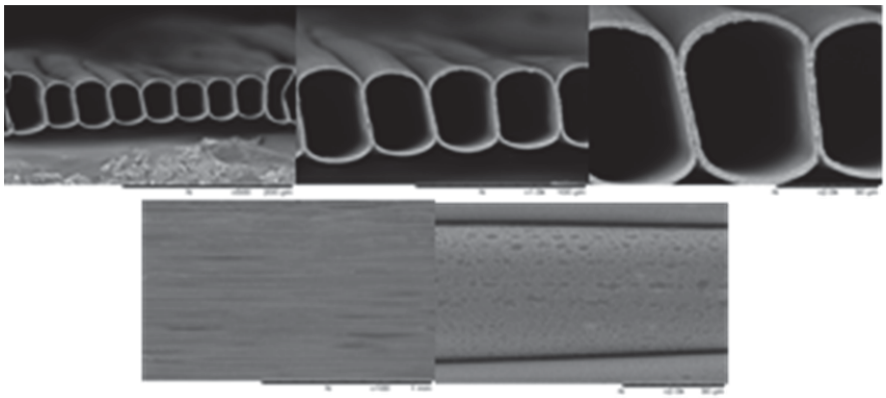


Fig. 6 Scanning electron microscopy images (surface and cross section) of electrospun PLLA MTAMs

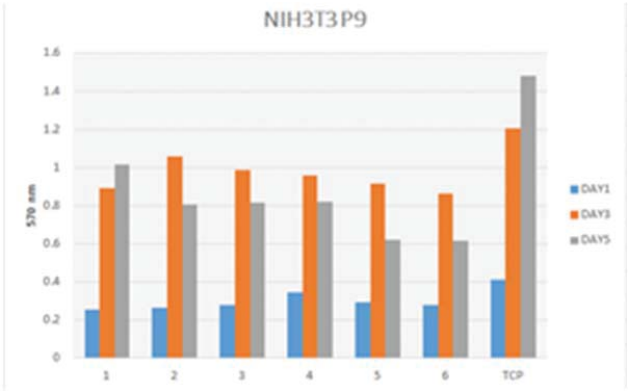


Fig. 7 Biocompatability (MTT assay) of electrospun PLLA MTAMs with different surface treatment parameters: (1) AAP+G-AAc+ EDC/NHS+chitosan, (2) AAP+G-AAc, (3) AAP+ EDC/NHS+chitosan, (4) AA, (5) untreated+ EDC/NHS+chitosan, and (6) untreated

C. MTT Assay

Fig. 7 shows the MTT assay results of electrospun PLLA MTAMs with different surface treatment parameters. The growth of NIH3T3 cells at the first to the third day is almost the same in different electrospinning process. However, cell growth on chitosan immobilized surface was observed increased. Surface immobilized with chitosan (AAP + G-AAc + EDC/NHS + chitosan) possessed improved biocompatibility, comparing with those of control and other modification parameters.

IV. Conclusions

The results from weight changes and MTT assay reveal that AAP treated electrospun PLLA MTAMs grafted with AAc and, finally, with chitosan immoblized via crosslinking agents showed improved hydrophilicity and biocompatibility, suggesting an effective and potential process to modify PLLA MTAMs for tissue engineering applications.

Acknowledgments

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References

[1] C.C. Chiu, J. Yang, C.H. Lin, C.C. Chen, “Structural tuning of electrospun nano-porous poly-l-lactic acid (PLLA) microtube array

membranes (MTAMs)", 3rd International Conference on Electrospinning 2014
[2] C.C. Chen, C.H. Wu, J.J. Wu, C.C. Chiu, C.H. Wong, M.L. Tsai, and H.T. Lin," Accelerated bioethanol fermentation by using a novel yeast immobilization technique: Microtube array membrane", Process Biochemistry 2015(tba):x-y.
[3] K.L. Ou, et. al., "Membranes of epitaxial-like packed, super aligned electrospun micron hollow poly (l-lactic acid) (PLLA) fibers", European Polymer Journal, Volume 47, Issue 5, Pages 882–892, May 2011
[4] J.C. Yang et. al., "Formation of Highly Aligned, Single-Layered, Hollow Fibrous Assemblies and the Fabrication of Large Pieces of PLLA Membranes": Macromolecular Materials and Engineering, Volume 297, Issue 2, pages 115–122, February 2012
[5] S.B. Rao, and C.P. Sharma, "Use of chitosan as a biomaterial: studies on its safety and hemostatic potential," J. Biomed. Mater., 34: 21–28, 1997
[6] K.S. Chen, S.F. Lin, Y. Ikada, Chinese J. of Medical and Biological Eng., Vol.13 No.3(1993)
[7] Feng-Huei Lin, Jui-Che Tsai, Tim-Mo Chen, Ko-Shau Chen, Jen-Ming Yang, Pei-Leun Kang, Te-Hsing Wu," Fabrication and evaluation of auto-stripped tri-layer wound dressing for extensive burn injury", Materials Chemistry and Physics, Vol.102, 15 April 2007, Pp. 152–158
[8] Hsin-Ming Wu, Yi-Syuan Wei, Ko-Shao Chen* and Yi-Jang Liou, "Plasma Surface Treatment and Grafting of Nano-Gold Surface for Immobilization of Chitosan", Current Nanoscience, 2014, 10, pp.189-193
[9] Hung-Che Chou, Tsong-Rong Yan*, Ko-Shao Chen, "Detecting cells on the surface of a silver electrode quartz crystal microbalance using plasma treatment and graft polymerization", Colloids and Surfaces B: Biointerfaces 73 (2009) 244–249
[10] R. Zeng, Z. Wang, H. Wang, L. Chen, L. Yang, R. Qiao, L. Hu, Z. Li, "Effect of bond linkage on in vitro drug release and anti-HIV activity of chitosan-stavudine conjugates," Macromolecular Research, 20:358-365, 2012
[11] K. S. Chen, C. W. Chou, S. H. Hsu, and H. R. Li, "Surface Grafting Polymerization and Crosslinking of Thermosensitive NIPAAm Hydrogels onto Plasma Pretreated Substrates and Drug Delivery Properties," Materials Sci. Forum, V.:3267-3272, 2003

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