Surface & Coatings Technology 229 (2013) 112–119

Contents lists available at SciVerse ScienceDirect

Surface & Coatings Technology

journal homepage: www.elsevier.com/locate/surfcoat

PIII-induced enhancement and inhibition of human cell attachment on chitosan membranes

K. Inthanon [a,1], N. Saranwong [b,1], W. Wongkham [a,*], P. Wanichapichart [c], K. Prakrajang [b], D. Suwannakachorn [b], L.D. Yu [b,d,*]

[a] Human and Animal Cell Technology Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Plasma and Beam Physics Research Facility, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[c] NANOTEC Center of Excellence at Prince of Songkla University, Hat Yai, Songkla 90112, Thailand
[d] Thailand Center of Excellence in Physics, Commission on Higher Education, 328 Si Ayutthaya Road, Bangkok 10400, Thailand

ARTICLE INFO

Available online 26 October 2012

Keywords:
Plasma immersion ion implantation (PIII)
Cell attachment
Chitosan membrane
Enhancement
Inhibition

ABSTRACT

Chitosan membranes are good biodegradable materials for artificial organ applications. Treatment of the membrane surface with energetic ions can promote the application potential. Particular applications may require different properties of the material surface for cell attachment, either promoted or reduced. In this study, nitrogen and argon ions from plasma immersion ion implantation with bias of 5–10 kV were used to bombard chitosan membranes. Subsequent cell attachment using human cancer cells and normal fibroblast cells was investigated on the ion-treated membrane surfaces. Argon ions were found to have an enhancement effect on the cell attachment with increases in the cell attachment by about 20–30% and in the cell proliferation rate by 25% at most, whereas nitrogen ions had an inhibition effect on the cell growth with decreases in the cell attachment by about 5–30% and in the cell proliferation rate by 50–80%. Characterizations of the membranes on the contact angle, chemical bond, surface morphology and filopodia on the surface were carried out for discussion on relevant factors responsible for the cell attachment behavior. Ar-plasma treatment could increase the contact angle by 25% and the roughness by 10% compared with N-plasma treatment so that the cell filopodia migration could be favored. N-plasma treatment could break hydrogen and NH bonds compared with Ar-treatment and hence change the chemistry of the chitosan material.

1. Introduction

Chitosan [(1–4)-2-amono-2-deoxy-β-D-glucan] is an N-deacetylated derivative of chitin which is the main component in shells of crabs, shrimps and krills. Due to its characteristics including biocompatibility, biodegradability, non-toxicity and bioadhesive polymer, chitosan has been an attractive agent for various medical and pharmaceutical applications [1–3]. In addition, chitosan has been reported to have a potential to accelerate the reformation of connective tissues and promote tissue vascularization, as well as provide benefits to wound healing, bone repairs, vascular graft implantations and cell tissue cultures [4]. Depending on the intended medical application, the design of biocompatible synthetic surfaces that are able to control the interaction between a living system and an implanted material remains a major theme for biomaterial applications in medical sciences [5]. For many applications in artificial organs, such as artificial bones, hips, vascular stents, pacemakers, and catheters, excellent biocompatible surfaces are required to promote cell attachment. On the other hand, in certain applications, for instance, the inner surface of artificial blood vessels, and the surface of medical tools and accessories, cell attachment should be inhibited. As many as more than ten material variables can affect the biocompatibility [6] and many are related to the surface properties such as surface topography and morphology, hydrophobicity/hydrophilicity, surface chemical composition, surface energy, surface electrical/electronic properties, etc. But in the final analysis, the basic mechanism comes to the amount of functional or polar groups on the surface. The modifications of biopolymers by means of polymer blending, cross-linking, and surface chemical modification have been recently carried out [7]. The surface-modification technologies allow the development of biologically compatible surfaces of synthetic polymer membranes. These methods include plasma treatments and ion implantation but many unknowns related to the new techniques emerging [8,9]. Particularly, knowledge on appropriate ion beam and plasma parameters to enhance or reduce cell attachment and different mechanisms which are involved in biocompatibility modification for various types of human tissue cells is not yet well gained. The purpose of this work is to investigate the effects of plasma immersion ion implantation (PIII) treatments on the surface characteristics, chemistry and cytocompatibility of chitosan membranes. Biased plasma is composed of highly excited atomic, molecular, ionic, and radical species and thus considered to be more effective in polymeric material surface modification than those

* Corresponding authors.
E-mail address: yuld@fnrf.science.cmu.ac.th (L.D. Yu).
[1] The authors contributed equally.

0257-8972/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.surfcoat.2012.10.042

Table 1
Summary of the treatment conditions of the chitosan membrane samples. Natural control: the chitosan membrane in the natural state, not subjected to any treatment including vacuum and plasma.

Sample no.	Condition		
	Plasma species	Bias (−kV)	Fluence (ions/cm^2)
1	Natural control		
2	Ar	Ar-plasma control, no bias	
3		5	10^{13}
4			10^{14}
5			10^{15}
6		10	10^{13}
7			10^{14}
8			10^{15}
9	N_2	N-plasma control, no bias	
10		5	10^{13}
11			10^{14}
12			10^{15}
13		10	10^{13}
14			10^{14}
15			10^{15}

conventional methods mentioned above. Two different plasma species, argon (Ar) and nitrogen (N), were used. Ar is inert and relatively heavier while N is relatively active and lighter. Hence, the former was supposed to have pure and stronger physical interaction with the target material, while the latter might involve chemical interactions besides physical interactions. It was reported that Ar plasma led to incorporation of polar groups on the exposed polymer surface [10]. While it was supposed that a treatment using nitrogen could induce polar neutralization to reduce the groups. Therefore, Ar and N plasma treatments might result in different modifications of the chitosan material. Normal human fibroblasts (F1544) and amelanotic melanoma cell line (C32) were used for the study of cell attachment as with the former normal cell behavior can be checked and with the latter experiments can be operated quickly and relevant applications can be looked for. The test of using normal cells was aimed at investigating effect on cell attachment improvement, while using the cancer cells was for studying effect on inhibition of cancer cell attachment.

2. Experiment

Chitosan membranes [MW (molecular weight) — 400 kDa, 76% DDA (degree of deacetylation), NANOTEC Center of Excellence, Prince of Songkla University, Hat Yai, Thailand] were prepared by the oven-dry method [11]. The membranes were beforehand cut into pieces of 3×3 cm^2 and cleaned with 70% ethanol. The samples were bombarded with ions from argon (Ar) or nitrogen (N_2) plasma using our PIII facility [12]. Ar or N_2 gas was fed into the chamber and discharged by 13.56-MHz radiofrequency (RF) power. The PIII process was operated using biases of 0, −5 and −10 kV. The bias was related to the ion energy, the higher bias, the higher the energy. The treatment time lengths were corresponding to ion fluences of 10^{13}, 10^{14} and 10^{15} ions/cm^2,

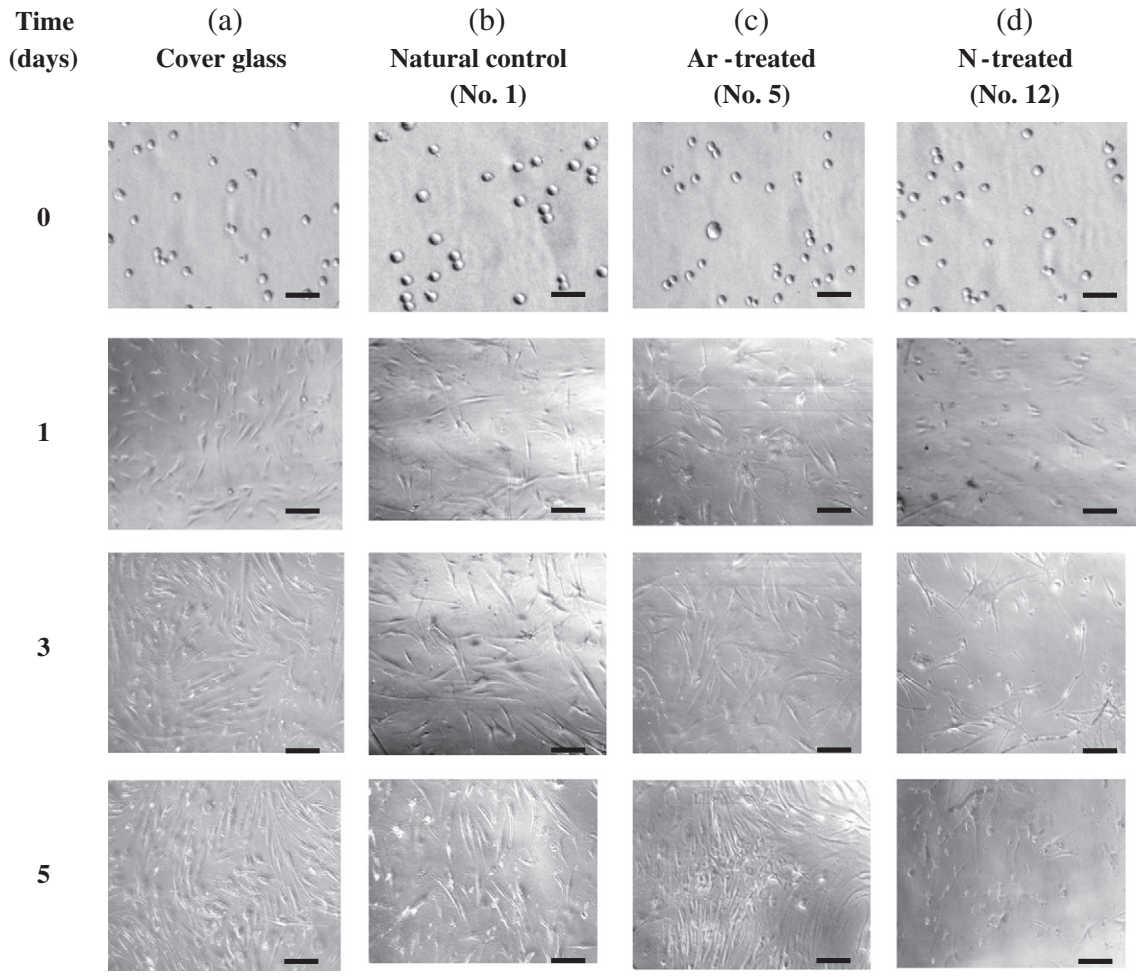

Fig. 1. Examples of the morphology of F1544 on various substrates at 0, 24, 72 and 120 h, or 0, 1, 3, and 5 days, respectively (scale bar = 200 μm). The sample no. refers to Table 1. Cover glass: cells attached on cover glass.