Nitrogen Ion Beam Enhances Attachment of Collagen on Poly L-Lactic Acid

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1. Introduction

The modern polymer has vast applications in biology and medicine. Many non-electronic medical devices such as stents, catheters are made of polymeric materials. Polymers must have high biocompatible property to be used as biomaterials and must be minimal induced inflammation reaction in the human body. These surface properties can be modified by a variety of methods, including plasma [1] or laser treatment [2], ion implantation [3] and nanoparticle grafting [4].

Poly L-lactic acid (PLLA) has a good biocompatible and bioresorbable property [5], and thus, it has been used as scaffolds for skin reconstruction and restoration of vasomotion [6]. However, despite these properties, the PLLA scaffold has several major problems such as an immune response, prohibition of cell growth and coagulation on materials [7] that must be solved to allow ideal performance in the human body. As a solution to these problems, ion beam irradiation technology can be an alternative. Therefore, we have studied to enhance PLLA surface characteristics by ion beam.

2. Methods and Results

2.1 N^+ beam irradiation for PLLA surface modification.

N^+ beam with 50 keV was irradiated onto the PLLA sheets (goodfellow, Huntingdon, UK) using the gaseous ion beam facility at Korea Multi-purpose Accelerator Complex (KOMAC, Gyeongju, Korea), Korea Atomic Energy Research Institute (KAERI, Daejeon, Korea). A nitrogen dose of $1x10^{15}$ p/cm^2 or $1x10^{16}$ p/cm^2 was delivered to the PLLA surface with a dose rate of $5.5x10^{11}$ p/cm^2 · sec. Fig. 1 shows that PLLA image before and after N^+ beam irradiation.

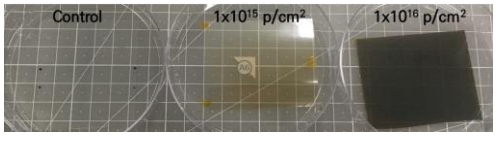

Fig. 1. Picture of PLLA sheet before and after N^+ beam irradiation.

2.2 N^+ beam induced free radical on PLLA surface observed by electron spin resonance (ESR) spectra

All the ESR spectra were measured at room temperature and were performed at the Seoul Western Center of Korean Basic Science Institute (KBSI). As shown in Fig. 2, the ESR intensity of the PLLA surface increases with increasing N^+ concentration.

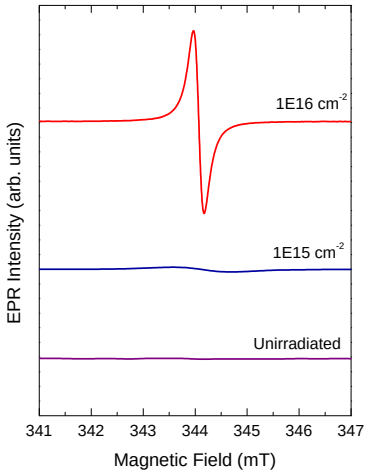

Fig. 2. ESR spectra of N^+ beam-irradiated and unirradiated PLLA as a function of magnetic field

The g-value of the sample with a dose of 1×10^{16} p/cm^{-2} was estimated to be 2.002 as shown in Fig. 3. The g-value is very close to that of the NO_2 radical, thus arising from the N^+ beam irradiation [8]. It is further supported by the markedly increased ESR intensity for the sample with a dose of 1×10^{16} cm^{-2} compared to that with a dose of 1×10^{15} cm^{-2}. In unirradiated sample, the ESR spectrum was not observed, indicating there is no radical in the sample.

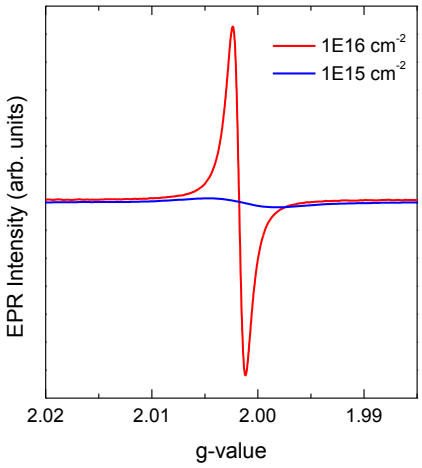

Fig. 3. ESR spectra of N^+ beam-irradiated PLLA as a function of g-value

2.3 Free radicals are involved in the binding between PLLA and collagen.

The FT-IR spectra of untreated PLLA and nitrogen treated PLLA with the highest dose of $1x10^{16}$ p/cm^2 is shown in Fig. 4 and 5, respectively.

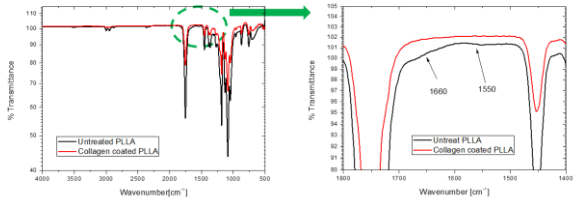

Fig. 4. FT-IR spectra of untreated and collagen coated untreated PLLA

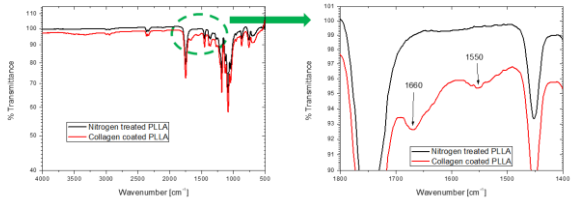

Fig. 5. FT-IR spectra of N^+ beam treated and collagen-coated nitrogen treated PLLA

Fig. 4 shows that IR spectra are observed no significant changed with or without collagen in PLLA. On the other hand, it is observed in the IR spectra that the peaks at 1550 cm^{-1} and 1660 cm^{-1} with collagen-coated nitrogen treated PLLA at the dose of $1x10^{16}$ p/cm^2. According to the reference [9], the peaks at 1550 cm^{-1} (amide I) and 1660 cm^{-1} (Amide II) are represented the collagen secondary structure.

2.4 The adhesion ability of vascular endothelial cells was enhanced by collagen on N^+ beam treated PLLA.

Cultured Human Umbilical Vein Endothelial Cells (HUVECs) were incubated with different conditions for 24 h. Then Cell counting kit-8 (CCK-8) assay was used to investigate the effect of collagen on the adhesion ability of HUVECs. N^+ beam did not significantly enhanced HUVECs adhesion, but HUVEC adhesion ability was significantly increased in the collagen-coated PLLA with N^+ beam irradiation (Fig. 6).

3. Conclusions

As shown in Fig. 1, the N^+ beam effectively generates free electron radicals on the PLLA surface. Therefore, collagen is able to bind to PLLA surface easily and cell adhesion is improved. Although a subsequent study is required, it has been confirmed that the N^+ beam facilitates protein coating on the PLLA surface. The disadvantage of PLLA scaffolds is expected to be solved by the N^+ beam irradiation.

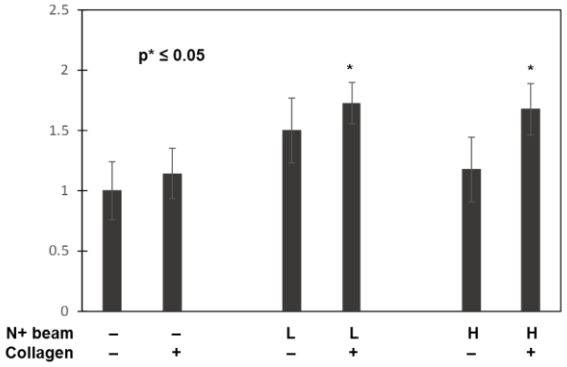

Fig. 6. Collagen on N^+ beam treated PLLA enhance adhesion of HUVEC. L: $1x10^{15}$ p/cm^2, H: $1x10^{16}$ p/cm^2

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