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In vitro and in vivo study of He^+ ion irradiated collagen for development of small diameter stent graft material

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Abstract

Recently, stent graft technology for endovascular treatment of aortic dissections has made tremendous advances. It is now possible to apply this minimally invasive technique to a wider range of pathology. The aim of this study was to develop anti-thrombogenic coronary stent and graft materials using ion-beam technology. Our previous study indicted that collagen surfaces irradiated with He^+ ion at a fluence of 1×10^{14} ions/cm^2 have excellent blood compatibility. The ion-beam-irradiated collagen grafts demonstrated a high anti-thrombogenicity and graft patency. 150 keV-He^+-irradiated collagen with a fluence of 1×10^{14} ions/cm^2 has the properties of anti-thrombogenicity and cell attachment. In vitro plasma protein adsorption was evaluated to investigate the mechanisms of anti-thrombogenicity of these surfaces. From these results, anti-thrombogenicity of the He^+-irradiated collagen was caused by the reduction of the plasma protein adsorption, such as fibrinogen or von Willebrand factor, by ion-beam irradiation. Japanese white rabbits weighing 3–4.5 kg were used in this animal study. Collagen-coated graft material implanted with He^+ ions at a fluence of 1×10^{14} ions/cm^2 exhibited excellent anti-thrombogenicity and demonstrated patency for one year.

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1. Introduction

Ion implantation has been shown to be a useful technique for improving the surface properties of metals [1] and polymers [2]. It has also been used recently for surface modification of polymers to improve blood compatibility [3–5] and tissue compatibility [6].

Recently, minimally invasive procedures using stent devices have attracted much attention in the treatment of severe coronary diseases like myocardial infarction. A stent is a medical device that keeps occluded arteries open for an extended period of time. It is a twisted coil or metal mesh tube that is inserted into a catheter. The process involves inserting the catheter (containing the stent) intravascularly from a large artery, such as the femoral artery; moving the catheter intravascularly under angiographic monitoring, and then expelling the stent from the catheter in the affected area and expanding it intravenously. When an occluded coronary artery is opened during stent therapy, the ratio of re-occlusion of the coronary is extremely high (40–45%), despite the short-term preservation of blood flow. This is mainly caused by anti-thrombogenicity of stent materials that are currently used. The objective of this work is to fabricate biocompatible graft material with improved anti-thrombogenicity, and to promote cell attachment by He^+ ion implantation into the collagen-coated ePTFE.

The first event in the blood–biomaterial interaction is the adsorption of proteins onto the surface of the synthetic materials. When a foreign material comes into contact with blood, proteins such as fibrinogen, von Willebrand factor (vWF), albumin and fibronectin will rapidly adsorb onto the surface. The adsorbed protein layer will then determine all further events, like platelet adhesion, aggregation and coagulation. Biomaterials adsorbing the least amount of the plasma protein fibrinogen following exposure to blood will support less platelet adhesion and therefore exhibit less thrombogenicity. Furthermore, the adhesion of platelets is promoted when proteins such as fibrinogen, vWF and fibronectin have been adsorbed to a material surface. The adhesive protein that contacts the ion-implanted collagen surfaces was examined in relation to anti-thrombogenic properties.

2. Materials and methods

2.1. Ion implantation

The graft materials used were ePTFE sheets (PSM-01200, W.L. Gore and Associates, United States). The graft materials were coated with type I collagen (CELLGEN, Bovine dermis collagen, KOKEN Co., Japan). The surplus collagen was removed, and the specimens were dried under ambient conditions. He^+ ions were then implanted into collagen-coated substrates at 150 keV with fluences between 1×10^{13} and 1×10^{15} ions/cm^2. The ion-beam current density was kept below 0.5 μA/cm^2 to prevent the substrates from heating.

2.2. In vivo animal study

Twenty Japanese white rabbits weighing 3–4.5 kg were used in this study. The common carotid artery (about 3 cm in length) was exposed under anesthesia with Nembutal (30 mg/kg, intravenous injection; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). Part of the vessel wall was selectively removed by a meticulous microscopic technique to produce a small damage hole on the vessel wall (Fig. 1(a)). Non-implanted ePTFE and collagen-coated ePTFE implanted with He^+ ions at an energy of 150 keV and a fluence of 1×10^{14} ions/cm^2 were used for the test specimens. These specimens were wrapped around the rabbit's damaged carotid arteries and fixed with surgical clips and fibrin glue as depicted in Fig. 1(b). All the procedures were conducted under sterile conditions. The animals were then killed by an intravenous overdose of KCl at different times, and the specimens were surgically removed for histopathological examination and scanning electron microscopic (SEM) study. The tissue for histopathological examination was fixed in a 10% formalin solution, decalcified with formic acid and stained with hematoxylin and eosin. The samples for SEM observation were then washed twice with PBS(−), fixed with 2 vol.% of glutaraldehyde solu-