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ScienceDirect
Surface & Coatings Technology 201 (2007) 8055–8061
SURFACE & COATINGS TECHNOLOGY
www.elsevier.com/locate/surfcoat

Effects of low-energy ion beam bombardment on biological cell envelopes

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Available online 12 March 2007

Abstract

Low-energy ion beam biotechnology has seen rapid development worldwide in recent years. This is an important expansion of studies on ion beam modification of solid materials, vastly different from and more complex than the latter. Among other novelties, we have focused our interest on ion beam bombardment effects on plant and bacterial cell envelopes and tried to understand mechanisms involved in ion beam-induced gene transfer. Through a comprehensive investigation, we have discovered ion beam-induced formation of nanocrater-like structures in the cell envelope, a general phenomenon of ion beam bombardment of cells; these structures may act as pathways for exogenous macromolecule transfer. We have also quantitatively obtained abnormally great penetration depth and sputtering of ions in the cell envelope. All these results are significantly advantageous for ion beam processing of biological cells.

Keywords: Ion beam bombardment; Nanocrater; Cell envelope; Gene transfer

1. Introduction

Ion beam technology applied to the modification of materials has been successfully developed for about three decades [1]. Since the mid-1980s when biological effects of low-energy ion beam implantation in plant cells were discovered [2], ion beam material modification has been expanded to biology and a highly interdisciplinary new subject, ion beam biotechnology [3], has been established and rapidly developed worldwide. The technology uses low-energy (of order $10–10^3$ keV) ion beams to bombard biological organisms for broad applications on mutation breeding, gene transfer, heavy ion therapy, life origin study, radiation hazard evaluation and biological structure analysis. Compared with ion beam interaction with conventional solids, ion beam bombardment of living materials has significant differences and complexities:

- Biological organisms are living, and ion beam treatment should not kill them.
- Fresh cells contain a large amount of water, which essentially evaporates in vacuum, and the evaporation causes differences in the target status from that in normal atmosphere.
- Biological material structures are highly porous and inhomogeneous, and ions penetrate and sputter abnormally more than for normal condensed materials.
- The functioning structures of organisms are very complicated and different ion beam-treated locations have different responses, and hence in order to get a certain response, ion beam should be precisely controlled to target the location.
- Biological organisms which are extremely sensitive to ion irradiation, will actively respond to the irradiation and thus highly produce secondary effects, which can greatly affect consequences of ion beam bombardment.
- Organisms have recovery ability, and ion beam radiation damage may be repaired and thus ion beam effects may disappear in a certain time period.

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doi:10.1016/j.surfcoat.2005.12.051

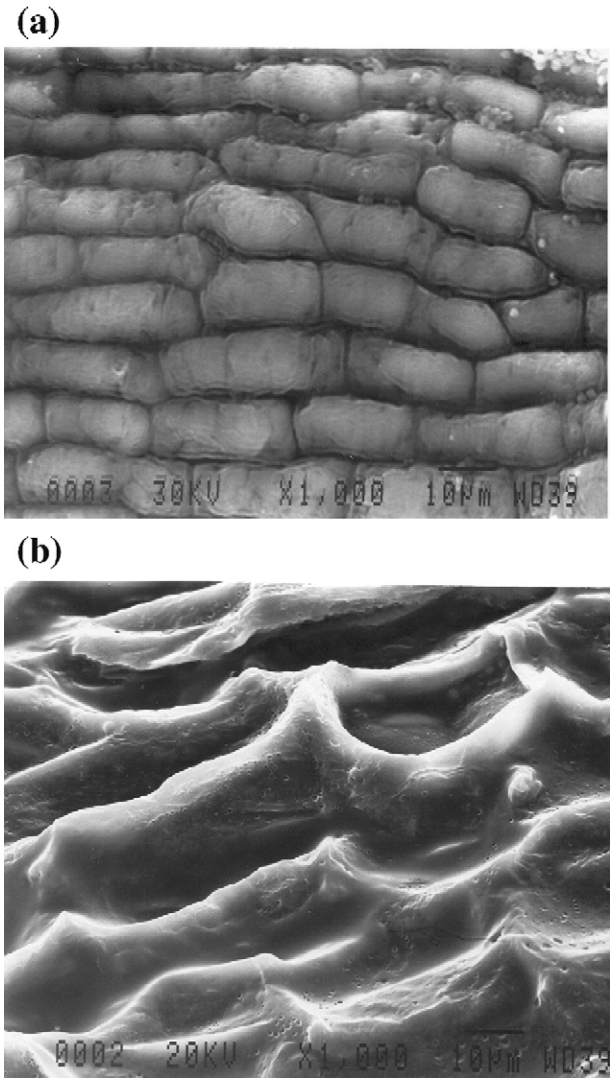

Fig. 1. SEM micrographs of corn embryo cells. (a) Fresh, and (b) in vacuum.

- Different parts of an organism may have intercommunication and an ion beam-treated location may produce unexpected effects.

All of these are great challenges but also attractions to scientists. In developing ion beam biotechnology, we have focused our interest on ion interaction with biological cell envelopes. The cell envelope here means the total outer cell structural material that encases the cell, consisting of the cell wall, plasma membrane and outermost coat for plant and bacterial cells. The cell envelope is the first and also the main material for ions to interact with when they are bombarding biological organisms in vacuum, and the interaction is able to lead to important applications such as induction of gene transfer in the cells [4,5] and mutation of plants [6]. From a physics point of view, the mechanisms involved in the transfer and physical parameters dominating the mutation are still fairly unclear. In this report we summarize the progress that we have recently made in the study of ion beam bombardment effects on the cell envelopes and answers to the unclear questions that we have obtained up to now.

2. Experiments

Ions of gaseous species such as N, Ar, Cl and Xe and metals such as Mg, Al, Ti, Fe, Ni, Cu and Au at an average ion energy about 15–30 keV with fluences typically around 1 to 2×10^{15} ions/cm^2 were used to bombard biological samples such as plant cells of onion skin, corn embryo, *Curcuma* embryo and bacterial cells of *Escherichia coli* (*E. coli*) in vacuum (typical pressures of 10^{-4}–10^{-3} Pa). The ion implanters used were a special bioengineering ion beam facility at Chiang Mai University and a MEVVA (metal vapor vacuum arc)-source ion implanter at Berkeley. Post-ion bombardment biological and physical analyses were carried out to observe features of the cells using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM), and survival of the cells using vital dye staining, and to measure the ion range using Rutherford backscattering spectrometry (RBS). The experimental details refer to our previous publications [4,7–10]. Molecular dynamics simulation (MDS) was also carried out using HyperChem [11] and AMBER8 [12] to simulate ion bombardment of cellulose, the basic substance of the plant cell wall.

3. Results and discussion

3.1. Vacuum effect

It was observed and measured that the fresh cells almost totally lost their contained water after they were exposed to vacuum for a short time period [5]. The water loss was due to low-pressure-accelerated rapid evaporation, which also caused a rapid temperature decrease of the cells. Therefore, the cells in the vacuum chamber suffered severe shrinkage and freezing (Fig. 1). All these harsh conditions affected the cell survival but a part of the cells could still be alive

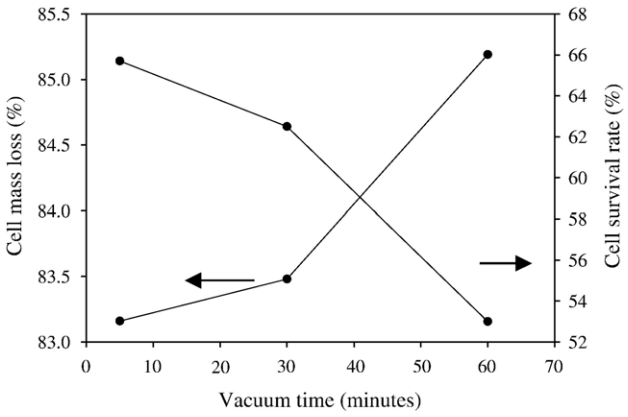

Fig. 2. Measured mass loss and survival rate of onion cells exposed to vacuum.