

# Investigation of cytocompatibility of surface-treated cellulose nitrate films by using plasma immersion ion implantation

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## Abstract

The alpha-particle sensitive colorless cellulose nitrate films (commercially available as LR 115 films from DOSIRAD, France) have been proposed as cell-culture substrates for alpha-particle radiobiological experiments. Cytocompatibility of the substrate is a key factor to the success of such experiments. The present work aims to investigate the cytocompatibility of surface-treated cellulose nitrate films by using plasma immersion ion implantation–deposition. The films were placed in a vacuum chamber, into which nitrogen gas was continuously bled and where the pressure was kept at  $2 \times 10^{-3}$  Torr. Implantation was carried out by igniting the nitrogen plasma at 100 W radio-frequency and applying high bias voltage in pulse with 20  $\mu$ s pulse width and 50 Hz (with 20 kV or no voltage). HeLa cervix cancer cells were then cultured on both the plasma-treated and untreated cellulose nitrate films. Our tests showed that the plasma-treated films are in general more cytologically compatible. © 2006 Elsevier B.V. All rights reserved.

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## 1. Introduction

The alpha-particle sensitive colorless cellulose nitrate films (commercially available as LR 115 films from DOSIRAD, France) have been proposed as cell-culture substrates for alpha-particle radiobiological experiments [1] because the films are cytocompatible, transparent, sufficiently thin to allow alpha particles to go through and can record the path of alpha particle to indicate the positions of alpha-particle traversals. A setup for irradiation of the cell monolayer through an LR 115 film used as substrate for cell culture is depicted in Fig. 1. The LR 115 film is in fact a commonly used solid-state nuclear track detector (SSNTD). A most recent review of SSNTDs can be found in Ref. [2]. More recently, we have studied the properties of the colorless LR 115 films in greater details, including bulk etch characteristics and methodology in studying the bulk etch rate [3]. A key factor to the success of such radiobiological

experiments relies on the cytocompatibility of the substrate. If the substrate is more cytocompatible, the cultured cells will spread on and adhere to the substrate better, and the number of cultured cells will be larger. These will enable the radiobiological effects of alpha particles on the cells to be better observed. The present work aims to investigate the cytocompatibility of untreated and plasma-treated cellulose nitrate films. These films are also characterized through measurements of the contact angles, as well as the ratio between the polar to dispersion component of the surface tension.

## 2. Methodology

All the colorless LR 115 detectors used in this project were purchased from DOSIRAD. The detectors consist of a 14  $\mu$ m active layer of cellulose nitrate on a 100  $\mu$ m polyester base.

The schematic diagram of the plasma immersion ion implantation–deposition (PIII-D) machine used in the present investigation is shown in Fig. 2. The films were placed in a vacuum chamber, into which nitrogen gas was continuously

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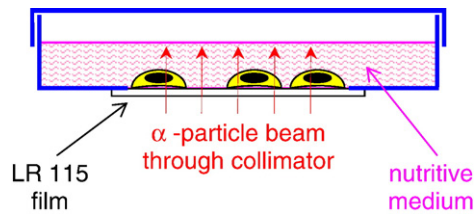


Fig. 1. The irradiation of the cell monolayer through an LR 115 film used as substrate for cell culture.

bled and where the pressure was kept at  $2 \times 10^{-3}$  Torr. Implantation was carried out by igniting the nitrogen plasma at 100 W radio-frequency and applying high bias voltage in pulse with 20  $\mu$ s pulse width and 50 Hz (with or without 20 kV). A review on plasma-surface modification of biomaterials can be found in Ref. [4].

HeLa cells are chosen for our study for a number of reasons. HeLa cells are the classic example of an immortalized cell line. These are human epithelial cells from a fatal cervical carcinoma transformed by HPV18. Moreover, HeLa cells are adherent cells which maintain contact inhibition *in vitro*, so that the cells are spread in a monolayer and the effects can be more clearly shown. The HeLa cells were cultured on both the plasma-treated and untreated cellulose nitrate films. The SSNTDs with similar areas (about  $1 \times 1.5$  cm<sup>2</sup>) were cultured with HeLa cells which were obtained from American Type Culture Collection. The cell line was maintained as exponentially growing monolayers in minimal essential medium supplemented with 10% fetal bovine serum, 1% (v/v) penicillin/streptomycin. The cells were cultured at 37 °C in humidified atmosphere containing 5% CO<sub>2</sub>. Penicillin/streptomycin was produced by Gibco (Karlsruhe, Germany). All other substances were purchased from Biochrom (Berlin, Germany). The cells were trypsinized for 4 min with 0.5/0.2% (v/v) trypsin/EDTA (ethylenediaminetetra-acetic acid; Biochrom), adjusted to a number of about  $2 \times 10^4$  cells/ml (and totally 10 ml) in 60 mm diameter Petri dish for 3 days of culture and plated out on the SSNTDs.

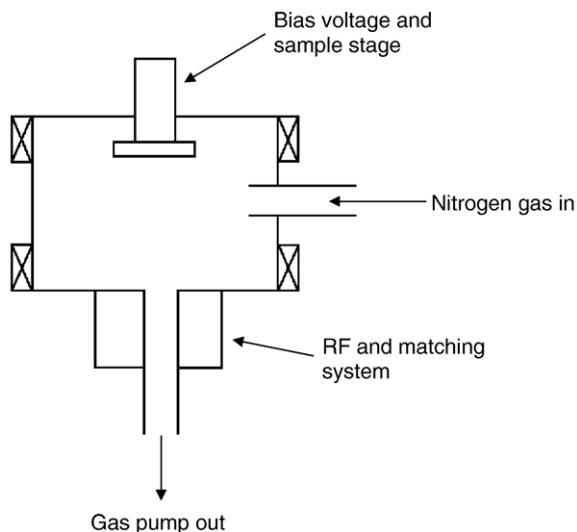


Fig. 2. Schematic diagram of plasma immersion ion implantation equipment used in the present investigation.

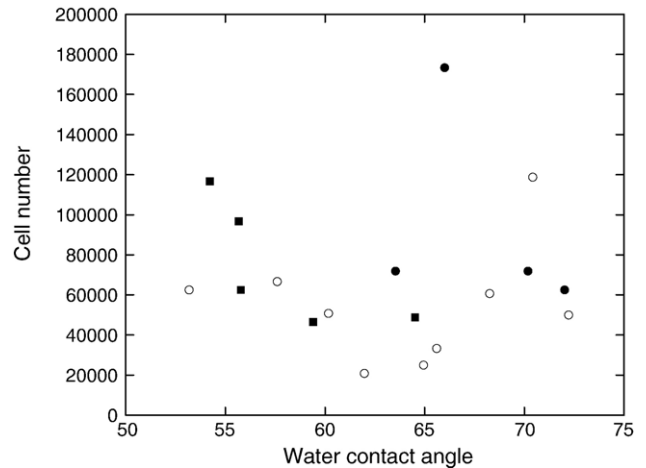


Fig. 3. The relationship between the cell number cultured on the films and the water contact angle. Open circles: untreated films; Solid circles: films treated with plasma with high voltage (20 kV); Solid squares: films treated with plasma without high voltage.

Cell attachment was examined after 3 days of culture. In order to count the cell number on different SSNTDs, the attached cells on the various detectors were released by digestion with trypsin-ethylenediaminetetraacetic acid (Invitrogen) and counted using a hemocytometer (Tiefe Depth Profondeur, Marienfeld, Germany). Cell viability was assessed by staining with 0.2% Trypan blue (Sigma) which only enter across the membranes of dead/non-viable cells.

### 3. Results and discussion

We have obtained results for a total of (a) 9 untreated films, (b) 4 films treated with plasma for a high voltage of 20 kV and (c) 5 films treated with plasma without high voltage. The cultured numbers of cells on the films ( $\pm 1$  S.D.) at the end of 3 days of culture were (a)  $54,300 \pm 29,300$ , (b)  $74,200 \pm 31,100$  and (c)  $94,900 \pm 52,500$ , respectively. Despite the relatively

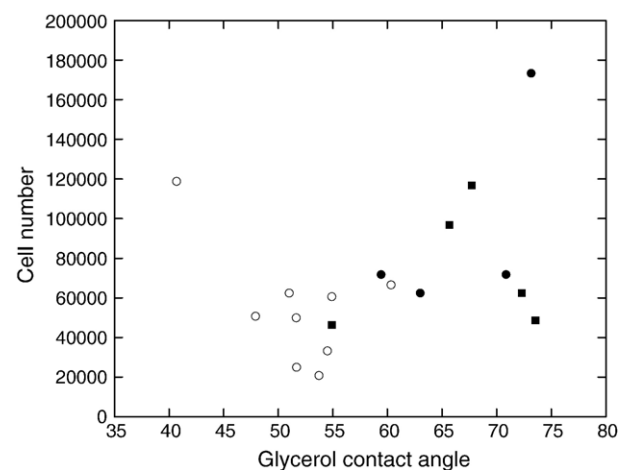


Fig. 4. The relationship between the cell number cultured on the films and the glycerol contact angle. Open circles: untreated films; Solid circles: films treated with plasma with high voltage (20 kV); Solid squares: films treated with plasma without high voltage.

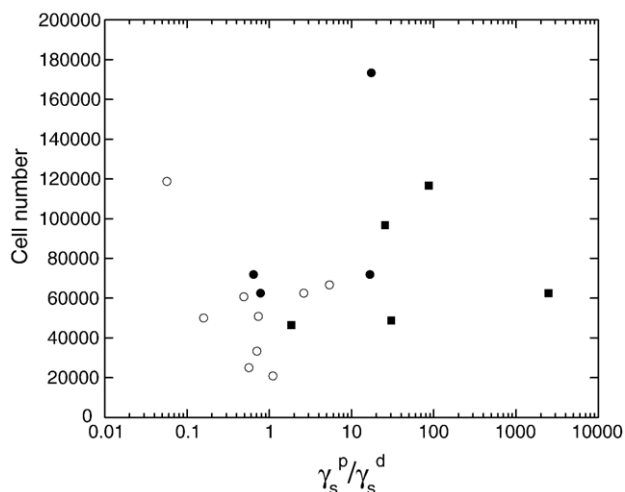


Fig. 5. The relationship between the cell numbers cultured on the films and the ratio of polar to dispersion component of the surface tension ( $\gamma_s^p/\gamma_s^d$ ). Open circles: untreated films; Solid circles: films treated with plasma with high voltage (20 kV); Solid squares: films treated with plasma without high voltage.

small number of samples, our tests showed that the plasma-treated films are in general more cytologically compatible.

The wettability of different surfaces can affect the adhesion, spread and growth of cells [5–9]. This phenomenon can be explained by the preferential adsorption of some serum proteins like fibronectin and vitronectin from the culture medium onto the surfaces; these proteins play an important role for cell attachment onto substrates. Polymer surfaces typically have low surface tension and high chemical inertness and so they usually have poor wetting and adhesion properties, but the surface properties can be changed through modifications of the molecular structure using plasma immersion ion implantation (PIII) [10]. For example, the wettability of Nylon-6 can be improved using oxygen/nitrogen PIII due to the oxygenated and nitrogen (amine) functional groups created on the polymer surface by the plasma treatment. The nitrogen and oxygen plasma implantation can result in C–C bond breaking to form the imine and amine groups as well as alcohol and/or carbonyl groups on the surface [10]. It is expected that nitrogen plasma has similar effects on the cellulose nitrate and will therefore change the wettability of the cellulose nitrate films.

The wettability of the untreated and plasma treated cellulose nitrate films were investigated using the sessile drop technique using a contact angle goniometer (JY-82, China). The accuracy of this technique is typically  $\pm 2^\circ$ . The test liquids employed were doubly distilled water and glycerol. For water (or liquid phase), the surface tension  $\gamma_l$ , its polar component  $\gamma_l^p$  and its dispersive component  $\gamma_l^d$  are given by  $\gamma_l=72.8$ ,  $\gamma_l^p=51.0$  and  $\gamma_l^d=21.8$  mJ/m<sup>2</sup>. For glycerol,  $\gamma_l=63.4$ ,  $\gamma_l^p=26.4$  and  $\gamma_l^d=37$  mJ/m<sup>2</sup>. The reported results are the mean of five measurements made on different positions on the same film surface. To avoid cross-contamination, a dedicated microsyringe was used for each liquid. The relationship between the cell numbers cultured on the films and the contact angles determined using distilled water and glycerol are presented in Figs. 3 and 4, respectively. From these two figures, the cultured cell number tends to increase with the

glycerol contact angle (although it seems to drop again at very large glycerol contact angles) and there is no observable difference between plasma treatments with or without the high voltage. The dependence on the water contact angle seems to be weak, which might be due to the relatively narrow range of the contact angles. As can be seen in Fig. 4, the plasma treatment can increase the glycerol contact angle and enhances the cytocompatibility of cellulose nitrate films.

The work of adhesion ( $W_a$ ) between a liquid and solid as given by Young [11] and Eq. (1) and the Van Oss [12] equation (Eq. (2)):

$$W_a = \gamma_l(1 + \cos\theta) \quad (1)$$

$$W_a = (\gamma_l^p\gamma_s^p)^{1/2} + (\gamma_l^d\gamma_s^d)^{1/2} \quad (2)$$

where  $\theta$  is the contact angle,  $\gamma_s^p$  and  $\gamma_s^d$  are the polar and dispersive components of the solid phase. From Eqs. (1) and (2), we obtain

$$(\gamma_l^p\gamma_s^p)^{1/2} + (\gamma_l^d\gamma_s^d)^{1/2} = \gamma_l(1 + \cos\theta) \quad (3)$$

In this way, measurements with two different liquids with known polar and dispersive components can solve for  $\gamma_s^p$  and  $\gamma_s^d$ . Fig. 5 shows the relationship between the cell numbers cultured on the films and the ratio  $\gamma_s^p/\gamma_s^d$  for the films. From this figure, we see that the cultured cell number tends to increase with the ratio and at the same time the ratio is enhanced with plasma treatment. Therefore, plasma-treated films are again more cytologically compatible.

#### 4. Conclusions

By counting the number of cultured HeLa cells at the end of 3 days of culture, we have found that plasma-treated colorless cellulose nitrate films are in general more cytologically compatible than the untreated films. We have also found that the plasma treatment increases the glycerol contact angle and also increases the ratio of polar to dispersion component of the surface tension. Therefore, for the employed cellulose nitrate films, these two parameters are related to the enhancement in the cytocompatibility. In conclusion, plasma treatment of the colorless cellulose nitrate films can be beneficial for alpha-particle radiobiological experiments since cytocompatibility of the substrate is a key factor to the success of such experiments.

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