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Effect of oxygen plasma treatment on surface charge and wettability of PVC blood bag—In vitro assay

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Abstract

Wettability and zeta potential studies were performed to characterize the hydrophobicity and surface charge of PVC blood bag samples and evaluate the effect of these properties on fibroblast cells growth. The surface properties of PVC and plasma treated PVC were compared by water drop contact angle and zeta potential measurement. Light microscopy was used to study the behavior of cell attachment and growth on these surfaces. Water drop contact angle measurement shows that the plasma treated PVC becomes more hydrophilic and wettability increased. Zeta potential and in vitro cell culture measurements noticed that the plasma treated PVC surface is more negatively charge and consequently attachment of the L929 fibroblast cells decreased on this surface.

Keywords: PVC; Wettability; Zeta potential; Cell attachment; Plasma treatment

1. Introduction

Surface properties such as surface chemistry, surface energy, and surface topography could be critical for biomaterial biocompatibility and must be considered in their selection for medical application, together with bulk properties characteristics (Ratner, 1987; Grinnell et al., 1972; Khorasani et al., 2005). In fact, several studies have investigated the relationship between hydrophobicity and electrical surface charge with the complex process of cell adhesion onto biomaterials, and their subsequent influence on the attachment and spreading of cells that ultimately determine the success or failure of the implant in service (Bruck, 1967; Williams and Bagnall, 1998; Khorasani and Mirzadeh, 2004).

Blood bag system is a disposable bio-medical device used for collection, storage, transportation and transfusion of human blood and blood components (Carmen, 1993). Until now, the most widely used blood bag material has been plasticized polyvinyl chloride (PVC) (Lee and Kim, 1999). PVC is a relatively rigid and brittle polymer. Plasticizers are added to the PVC to facilitate processing and increase flexibility and toughness in the final product by internal modification of the polymer molecule (Tickner et al., 2001). The main plasticizer used in blood bags is di-2-ethylhexyl phthalate (DEHP). It is a lipophilic or fat-loving compound and so it tends to concentrate in fatty tissues (Bruck, 1967).

Studies have suggested that the interactions between the biological environment and artificial materials are most likely dominated by the materials' surface properties including wettability, morphology, surface charge and crystallinity (Che Lin and Cooper, 1995; Khorasani et al., 1996, 1999; Lelah and Cooper, 1986). Hence, surface modification of existing biomaterials with an aim towards improving a materials' biocompatibility has been a major focus of biomaterials research in recent years. The synthetic modification methods often lead to alterations of the original materials' physical properties. In contrast, the plasma surface modification process has been shown to be able to modify the surface properties of a biomaterial without affecting its bulk physical properties (Yasuda, 1985).

Electrically charged particles in water form an electrical double layer. They are surrounded by a charge cloud which

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is sheared off during relative motion. In this way, electrokinetic phenomena are generating. They yield the charge of the particle in a given surrounding as a zeta potential (Schurz et al., 1990).

Zeta potential measurements provide a unique method to quantify in situ the actual state of the interface between the material and the solution, depending on the polarity of the absorbed ions, the material surface charge and the ionic concentration in the fluid (Schurz et al., 1990). Recent work has clearly demonstrated that surface charge of polymer surfaces interferes with the adhesion of cells (Suzuki et al., 1996; Sanders et al., 2005).

Probably the most important systems for electrokinetic investigations with flat surfaces are the polymeric materials. Electrical surface charge has been found to be relevant for the biocompatibility of polymers applied in medical devices as well as for polymer adhesion in technical systems and numerous other areas. An automated streaming potential device for the electrokinetic surface characterization of materials of different shapes—including flat surfaces—has been developed a few years ago (Werner et al., 1998). By means of this device valuable information on surface properties of many polymer materials and adsorbed layers on polymer materials has been collected.

Hydrophobic biomaterials are considered as the initiation of the foreign body reaction. Ions present on material surfaces may interact with cell receptors to cause cell adhesion and growth, and to facilitate cell proliferation, leading to the foreign body reaction (DiCosmo et al., 1989; Hattori et al., 1985). Ion presence may also affect endothelial cell growth and migration (Van Wachem et al., 1987; Klein-Soyer et al., 1989). Thus, for an implanted biomaterial, both the local cell density and the vascular ingrowths are expected to be affected by biomaterial surface charge.

If a dependence of cell growth on surface charge were demonstrated for materials test sample in vitro, then useful information towards biomaterial device design would be obtained (Bet et al., 2003).

The purpose of this study was to evaluate cell attachment and growth on plasticized polyvinylchloride (P-PVC) and plasma treated P-PVC with different wettability and surface charge.

2. Materials and methods

2.1. Material

Blood bag samples made from P-PVC were purchased from the Green Cross Medical Co. (Korea). Samples were prepared by cutting the blood bag ($10 \times 5 \times 0.1$ cm) and washed with two time distilled water and then dried in a vacuum oven at ambient temperature.

2.2. Plasma surface treatment

P-PVC film (10 cm × 5 cm × 0.1 mm) was placed inside a Pyrex tubular reactor of plasma EMITEC K 1050X instrument for treatment. The P-PVC samples surfaces were placed above the inner electrode. The gas used was oxygen (>99%). The pressure inside the plasma chamber was kept at 6×10^{-1} mbar. The electrode power was 50 W for a plasma treatment time of 5 min. The gas flow was chosen in such a way that the above mentioned plasma pressure was realized. Treated samples are transparent and seem that only surfaces were treated. Visual observation shows that there are no drastic differences between untreated and plasma treated samples.

2.3. Zeta potential measurement

Streaming potential was used to measure zeta potential (ζ) in P-PVC capillaries with and without plasma treatment using EKA, Anton Paar instrument. Conductivity was measured with a conductivity meter. KCl solution of 0.002 M was used as an electrolyte. Pressure (zero to 750 mbar) was applied to one end of the test capillary using a syringe pump. Fig. 1 shows the correlation of applied pressure versus voltage. Electrodes measured the generated voltage across the capillary and a strain-gage type transducer measured the pressure at the inlet. The forcing pressure, capillary diameter, and capillary length were chosen such that the flow was laminar; surface conductivity was negligible.

2.4. Contact angle determination

Hydrophilicity was evaluated by measuring the contact angle formed between water drops and the surface of the modified samples using contact angle measuring system G 10 (KRUSS). For this purpose, the drops of water were mounted on three different areas of the surface with a microsyringe. The results are mean values of three measurements on different parts of the film.

2.5. Hydrophobic recovery test

In order to quantify the effect of the preserving conditions on the hydrophobic recovery, one group of samples was stored in air after plasma treatment. Water drop contact angles were measured in different time intervals after plasma treatment.

2.6. SEM analysis

Scanning electron microscopy (SEM) was performed on gold coated samples using a polaron sputter coater. A Cambridge S-360 SEM operating typically at 10 kV was employed for morphology study. Samples were mounted onto the sample holder, sputter coated with gold and were studied with SEM.