**NANOSCALE CHARACTERIZATION OF COLD PLASMA TREATED COLLAGEN FILM AND ITS INFLAMMATORY RESPONSE IN VITRO**

Rui Chen1, John Hunt2, Jude Curran1

1School of Engineering, University of Liverpool, 2School of Science and Technology, Nottingham Trent University

Corresponding author: [ruichen@](mailto:ruichen@)liverpool.ac.uk

**Introduction**

Cold gas plasma treatments have been widely used to clean, sterilize and/or improve biocompatibility for biomedical devices [1]. The implantation of biomedical devices including collagen-based implants evokes an inflammatory response [2]. Despite inflammation playing an important role in the early stages of wound healing, excessive and nonresolving inflammation is one of major causes which may lead to the poor performance of biomaterial implants in some patients. In this study, effects of cold plasma treatment on the surface morphology and elastic modulus were investigated through PeakForce Quantitative Nanoscale Mechanical Characterization (PeakForce QNM); And the effects of cold plasma treatment on the non-specific inflammatory response were also investigated in vitro by the measurement of protein expression and cytokine production after one and four days of U937 cell line cultured on the collagen films.

**Materials and Methods**

After spin-coating and dried in the fume hood for 24 hours, collagen coated coverslips were then placed in 24-well plates within a plasma asher (Emitech K1050X, Quorum Technologies, UK) and treated with either nitrogen or oxygen plasma at a pressure of 0.6 mbar with a flow rate of 15ml/min at 20W or 80W for 2 minutes.

AFM analysis was carried out using a commercial AFM (NanoScope VIII MultiMode AFM, Bruker Co., Santa Barbara, CA, USA). The PeakForce QNM was conducted in ambient conditions with a silicon nitride probe (Bruker RTESPA-150A, nominal frequency of 150 kHz, nominal spring constant of 5N/m) with a scan resolution of 256 pixels/line. The data were fitted with the Derjaguin–Muller–Toporov (DMT) model to extract the elastic modulus by fitting the contact region of the retract curve close to the contact point. All post-image processing was carried out using IMAGESXM v1.99 and Nanoscope Analysis 1.7 (Bruker, USA).

For the in vitro experiment, U937 cells were induced to differentiate by exposing the cells (5 × 105 cells/ml) to 5 ng/ml of phorbol 12-myristate 13-acetate (PMA) for 24 hr. Differentiated U937 were seeded at 3 × 105 cells/ml on control and plasma treated collagen films for 1 and 3 days. Quantification of human IL-1β, TNF-α and IL-10 in supernatant were achieved using commercially available kits (Invitrogen™). A total of 4 separate repeats were carried out per single culture system.

**Results and Discussion**

Table 1 showed the surface roughness (Rq) and elastic modulus of collagen films before and after plasma treatment. The results showed that plasma treatment changed the morphology and elastic modulus of collagen film at nanoscale.

Table 1. Surface roughness (Rq) and elastic modulus of Collagen films before and after plasma treatment

|  |  |  |
| --- | --- | --- |
|  | Rq (nm) | DMT modulus (MPa) |
| Control | 2.73 ± 1.47 | 0.75 ± 0.07 |
| N2 plasma treated 20w | 0.62 ± 0.11 | 0.96 ± 0.53 |
| 80w | 0.59 ± 0.15 | 28.44 ± 2.08 |
| O2 plasma treated 20w | 1.56 ± 0.79 | 171.58 ± 15.67 |
| 80w | 1.83 ± 0.03 | 105.57 ± 30.17 |

The results of in vitro U937 cell culture showed that nitrogen plasma treatment may impart an anti-inflammatory effect on collagen film by reducing initial activation of monocytes and macrophages, which led a lower production pro-inflammatory cytokines IL-1β, TNFα and higher production of anti-inflammatory cytokine IL-10.

**Conclusions**

Oxygen and nitrogen cold plasma treatment on collagen films, modified material surfaces not only their chemical composition and surface energy, but also their surface morphology and elastic modulus at nanocale. Oxygen plasma treatment decomposed the 3D network structure and made the surface rougher and stiffer; nitrogen plasma treatment maintained the 3D network structure of collagen films and made the surface smoother at the nanoscale. The results indicated that compared to oxygen plasma, nitrogen plasma treatment may have produced an anti-inflammatory effect. This could have been due to the combination of the amino chemical group and the smoother of the collagen film, introduced by nitrogen plasma treatment.

**References**

1. Chen R et al. Colloids and Surfaces B: Biointerfaces. 96:62-68, 2012.

2. Curran JM et al. Comprehensive Biomaterials. 4: 49-52, 2011.

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