Accelerated Neutral Atom Beam processing of medical devices enhances tissue integration while reducing bacterial attachment.

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ABSTRACT

Accelerated Neutral Atom Beam (ANAB) technology is a low energy accelerated particle beam gaining acceptance as a tool for nano-scale surface modification of implantable medical devices. ANAB is created by acceleration of neutral argon (Ar) atoms with very low energies under vacuum which bombard a material surface, modifying it to a shallow depth of 2-3 nm. This is a non-additive technology that results in modifications of surface topography, wettability, and surface chemistry. These modifications are understood to be important in cell-surface interactions on implantable medical devices. Controlling surface properties of biomaterials is vital in improving the biocompatibility of devices by enhancing integration and reducing bacterial attachment.

Keywords: PEEK, polypropylene, titanium, biocompatibility, device-associated infection

1. INTRODUCTION

A common problem with many biomaterials used for implants is their lack of proper tissue integration while also being susceptible to bacterial attachment and subsequent biofilm formation [1]. In many cases, this results in implant failure. Polyetheretherketone (PEEK) and titanium (Ti) used in orthopedic and dental applications, or polypropylene (PP) used in surgical meshes are prime examples of devices that can result in failures.

ANAB technology is gaining acceptance as a method for surface modification of implantable medical devices which creates a nano-scale texture and increased wettability [2, 3]. These modifications in turn result in enhanced cell attachment and integration without modifying the bulk properties of the material. In this study, we characterize the effects of ANAB treatment on several biomaterial surfaces aiming to enhance implantable medical devices. We also look at the possibility of ANAB treatment in reducing bacterial attachment on the implants.

2. METHODS

2.1 Materials and Treatment

For *in vitro* studies, various polymers including PEEK, PP, PVC and metals including Ti were prepared as 1cm diameter, 1mm thick coupons and cleaned in 70% isopropanol for 30 min followed by 3 x 15 min washes in deionized H_2O. Coupons were prepared as control or treated by ANAB using Ar gas on an accelerated particle beam system (nAccel 100, Exogenesis Corp.) with a deflector to remove charged clusters as described in detail previously [4]. Briefly, Ar gas is flowed at 200 SCCM through a 100µm diameter nozzle to create weakly bonded clusters consisting of a few hundred to about a thousand Ar atoms. These clusters are then impact ionized by removing an electron from 1-2 atoms of the cluster resulting in a charged cluster which can then be accelerated by introducing it to a 30 kV electrostatic field. Once accelerated, the cluster is then immediately broken apart by colliding with non-ionized Ar gas atoms in the acceleration chamber. These collisions break the weak van der Waals bonds thus releasing individual neutral atoms along with smaller, charged clusters. The remaining clusters are then pushed away with an electrostatic deflector allowing the neutral atoms to maintain initial momentum until they collide with the material surface. The effective dose of the ANAB was between $1.25x10^{16}$ to $1.0x10^{17}$ Ar atoms per cm^2.

2.2 Atomic Force Microscopy (AFM)

Measurements were taken using a research Atomic Force Microscope (Model XE-70, Park Systems) in non-contact mode. Silicon tips with a resonant frequency of ~330 kHz and a force constant of 42 N/m were used (PointProbe Plus, Nanosensors). Regions of PEEK surface corresponding to 1 μm^2 were imaged and the arithmetical mean roughness (R_a) as well as maximum roughness depth (R_{max}) were measured across this region.

2.3 Contact Angle Evaluation

Contact angle was measured using the sessile drop method on a manual simplified device as described by Lamour et al. [xx] and droplet angles were measured by ImageJ software (NIH) with the contact angle plugin.

2.4 Cell Attachment and Proliferation

Human mesenchymal stem cells (MSC) or primary human osteoblasts were seeded on the treated and untreated coupons at a concentration of $5x10^4$ cells per ml of media and cell attachment and proliferation was measured over a 14-day period using the MTS assay.

2.5 Bacterial Attachment

S.aureus or *P.aeruginosa* bacteria in log-phase growth were seeded on treated and untreated coupons for 4 hours, unbound bacteria were then gently washed off with PBS. Bacteria were fixed in 2.5% glutaraldehyde followed by secondary fixation in 1% OsO_4, dehydrated in a series of ethanol washes, gold plated, and imaged by scanning electron microscopy.

2.6 In vivo Inflammation

Control or ANAB-treated PP hernia mesh were placed bilaterally in a pre-peritoneal location in Yucatan pigs (n=4). After 30 days, animals were euthanized and tissue from the abdominal wall was removed *en bloc*, processed for histology using Masons Trichrome, and visualized by microscopy.

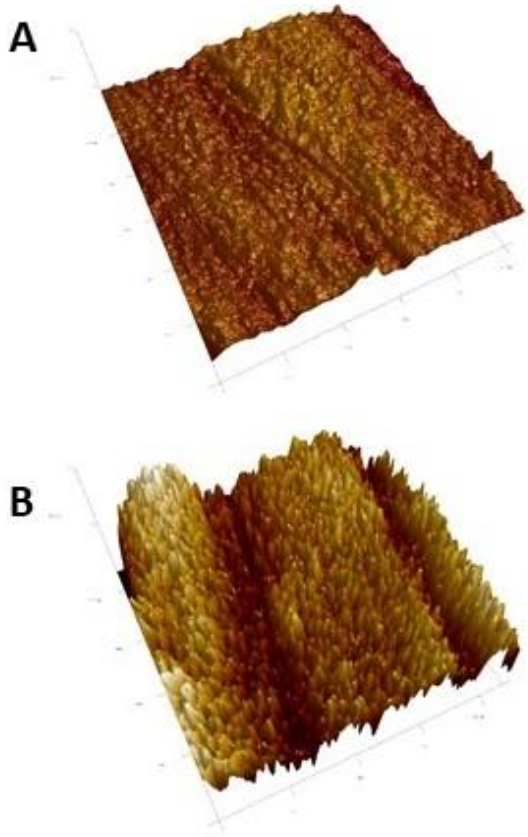

Figure 1. AFM image shows a nano-scale texture on ANAB-treated PP (B) as compared to untreated control (A), 2µm x 2µm scan size.

3. RESULTS AND DISCUSSION

3.1 Material Characterization

AFM analysis of materials after ANAB treatment reveals a nano-textured surface of approximately 20-50 nm from peak to peak with depths of approximately 5-20 nm, controlled depending on applied dose (Fig. 1). The roughness increased on ANAB-treated PP going from an R_a of 1.99nm on control to 6.69nm on treated surfaces; similarly R_{max} increased from 21.2nm to 88.8nm on ANAB-PP. This true nano-scale texture may result in better eukaryotic cell attachment while inhibiting bacterial attachment due to difference of size of the two cell types. Contact angle studies on PP reveal a more wettable surface following ANAB treatment decreasing water contact angle from

99.2±2.7 degrees to 79.8±6.0 degrees (Fig. 2, n=5; p<0.003), similar findings were reported for other polymers and metals. Similarly, it is generally understood that eukaryotic cells prefer a moderately hydrophilic surface, whereas bacteria attach to a more hydrophobic surface [5, 6].

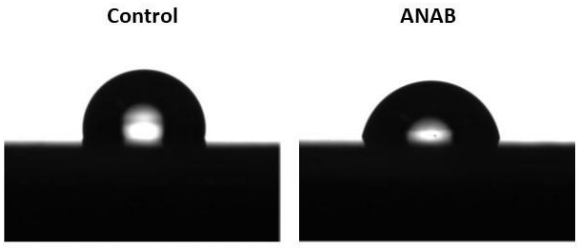

Figure 2. Water contact angle is decreased from 99.2°±2.7 on control PP (left) to 79.8°±6.0 on ANAB-treated PP (right).

3.2 Cell Attachment and Proliferation

MTS assay shows that by day 14, control PEEK has 9,925±1,994 cells while ANAB-treated PEEK has 88,713±6,118 cells (n=3; p<0.0014), similar findings were reported on titanium and polypropylene. Crystal violet staining of osteoblast attachment on PEEK is shown in Fig. 3. This enhanced attachment may lead to improved tissue integration with the device.

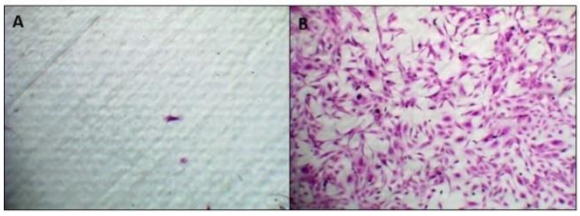

Figure 3. Crystal Violet staining of human osteoblast cell attachment on control PEEK (A) compared to ANAB-treated PEEK (B).

3.3 Bacterial Attachment

By SEM imaging (Fig 4.), we find that numerous colonies have formed on the surface of control PVC coupons and have begun biofilm formation. On ANAB-treated coupons we find a significantly decreased attachment of bacteria without any colonization, similar findings were reported on PP and PEEK.

3.4 In vivo Inflammation

As seen by the Masons Trichrome staining, there is an attenuation of inflammatory cells present around the ANAB-treated PP mesh as compared to the control mesh (Fig. 5).

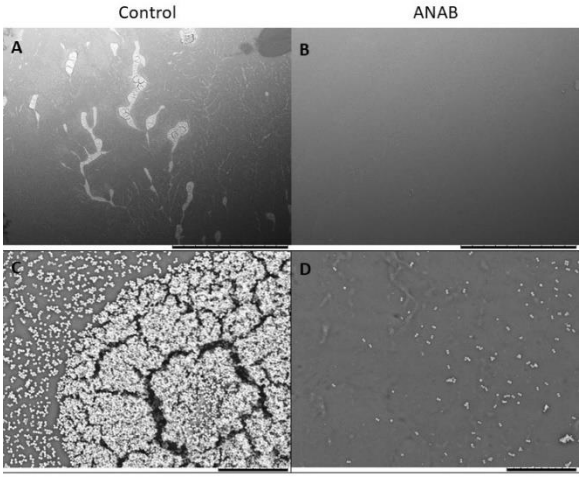

Figure 4. *S.aureus* bacterial attachment (4h) on PVC. Control (A, C) show start of biofilm formation, ANAB-treated PVC (B, D) result in very few bacteria cell attachment. Low magnification (A, B) bar = 1mm; High magnification (C, D) bar = 30µm.

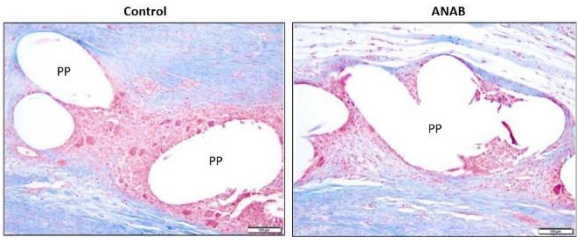

Figure 5. 30 day histology shows reduced inflammation (red-pink) to connective tissue (blue) on ANAB-treated PP as compared to control materials (bar = 100µm).

4. CONCLUSIONS

Two of the most pressing issues with current medical devices, and in the development of new biomaterials, has been the lack of good tissue integration with the device surface as well as an increased ability of bacterial attachment which may lead to biofilm formation and device-associated infections. We have shown that a relatively new technology called Accelerated

Neutral Atom Beam has been gaining acceptance in the biomedical field as a surface modification technique that enhances biomaterials at the nano-scale level. Its effects on surfaces include a nano-topography and increased wettability. These modifications result in better cell attachment and proliferation while at the same time, reduce the ability of bacteria to attach to the surface. FDA approvals have been granted to PEEK interbody spine fusion devices treated by ANAB processing [7]; other devices are currently being evaluated.

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