Neural cell functionality modulation and bacterial growth deactivation on patterned carbon substrates

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Statement of Purpose: The failure of various biomedical devices is often caused by prosthetic infection, mediated by bacterial cell attachment, their growth and biofilm formation. The objective of this work is to establish the efficacy of different carbon microstructures towards bactericidal property at the culture conditions, which can simultaneously promote the directional growth of neurite outgrowth of differentiated Neuroblastoma (N2a) cells [1] and the Schwann cells. Several groups found that the nanotextured surfaces can alter bacterial cell adhesion in a cell type dependent manner. Komaromy et al. reported that the squares patterned surfaces was more repellent for live/dead E. coli cells, whereas S. aureus populated on these surfaces very well [2]. It has been well perceived that any implantable biomaterial for majority of the applications should facilitate eukaryotic cell functionality (proliferation, differentiation etc.) and simultaneously inhibit bacterial growth.

Methods: The polyacrylonitrile (PAN, Sigma-Aldrich) derived carbon nanofibers were synthesized by electrospinning (E-spin Nanotech, Kanpur, India) technique and carbon film fabricated with same PAN solution using spin-coater (Apex instruments, Kolkata, India). SU 8 2015 (MicroChem, USA) derived carbon patterns were fabricated using photolithography. The carbon nanofibers and films were characterized by atomic force microscope (AFM) (Agilent, PICOSPM 3000). The tapping mode AFM accompanying Picoscan software was used to measure the roughness of carbon nanofibers and film substrates. The larger carbon patterns (circles, squares and strips) were characterized using optical profilometry (NanoMap-D) and accompanied software SPIP 5.1.11. Escherichia coli (E. coli; ATCC 25922) and Staphylococcus aureus (S. aureus; ATCC 25923), bacterial strains were grown at 37°C and maintained on LB-Agar plates (Sigma-Aldrich). For live/dead assay, E.coli or S. aureus were stained with SYTO 9 and propidium iodide (PI) using Live/dead assay kit, L7012, Invitrogen. For indirect immunostaining, the N2a cells (NCCS, Pune, India) stained with goat anti-mouse IgG-FITC (Invitrogen) [3]. Schwann cells (ATCC) were stained with Alexa Fluor®488 Phylloidin (Invitrogen, USA) and Hoechst 33258 (Invitrogen, USA) dyes.

Results: The roughness of various carbon patterned substrates was indicated as an average roughness (R_a) that describes the typical height variation of the surface [4]. The PAN derived carbon film was porous with average roughness of 372 nm. The roughness of the patterned surface was increased with an increase the height of the patterns. The average diameter of PAN derived electrospun carbon nanofibers was around 200 nm with R_a of 490 nm (Fig. inset). The roughness of SU 8 derived carbon squares, strips and circles was 526 nm, 770 nm and 1200 nm, respectively. The green, green plus orange-red, and red bacterial cells essentially represent the live, damaged, and dead cells, respectively [5]. It is evident from Fig. d that near the boundary of circles, the number of dead (orange-red) E. coli is higher as compared to away from the boundary of circles. In contrast, the live/dead (green/red) S. aureus bacterial cells were evenly distributed throughout the sample (Fig. not shown). The population of live/dead E. coli and S. aureus bacteria on other patterns (squares, strips, film and fibers) does not specifically distribute around the patterns. From MTT assay, it was confirmed that the E. coli bacteria on circles and strips has significantly less viability compared to tissue culture polystyrene (TCPS) (Fig. g). On the other hand, in the case of S. aureus, no significant difference in cell viability could be recorded on circular patterns, when compared to TCPS. The viability of E. coli and S. aureus on carbon film was significantly less when compared to TCPS. Interestingly, the viability of S. aureus on squares is only 50% of that of TCPS, whereas there was no significant difference in E. coli viability on squares, when compared to TCPS.

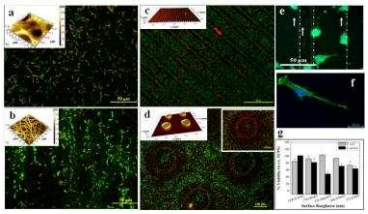

Figure: Live/dead assay of E. coli bacteria on (a) film, (b) nanofibers, (c) strips, (d) circles, (e) Differentiated N2a cells on strips (green: neurofilament-200kD), (f) aligned Schwann cell on nanofibers (Green: actin filament; blue: nucleus) and (g) Bacterial viability (MTT assay) on patterned substrates

Textural features were found to affect neurite outgrowth, over a wide range of length scales: from ~200 nm (carbon nanofibers) to ~60 μm (carbon patterns). Despite their innate randomness, carbon nanofibers promoted preferential differentiation of N2a cells and also alignment of Schwann cells (Fig. e and f), similar to ordered micro-patterns.

Conclusions: The present study confirms that the patterned carbon substrates with comparable height to bacterial cells can potentially reduce the bacterial cell viability and directed the growth of differentiated N2a and Schwann cells.

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