Three-Dimensional Macroscopic All-Carbon Scaffolds: Fabrication, Characterization, and Cytocompatibility

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Statement of Purpose: The assembly of carbon nanomaterials (carbon nanotubes, fullerenes, or graphene) into three-dimensional (3-D) structures is necessary to harness their remarkable physico-chemical properties for a large number of Tissue Engineering applications [1]. Over the last decade, 3-D pristine carbon nanotube (CNT) structures have been fabricated on the sub-micron and micron scale using various techniques such as chemical vapor deposition, substrate patterning, and capillary-induced self-assembly [2, 3]. We have previously reported a facile method to fabricate macroscopic, 3-D, freestanding, all-carbon porous scaffolds using carbon nanotubes, graphene and fullerene as the starting materials, by radical initiated thermal crosslinking of carbon nanostructures [4]. This method can fabricate macroporous (20 nm – 300 μm) all-carbon structures with high porosities (~80-85%). Furthermore, porosity of scaffolds can be controlled by the amount of radical initiator used in the crosslinking process, thereby allowing the fabrication of porous all-carbon scaffolds tailored towards specific applications. As a first step towards their development for tissue engineering applications, in this study, we report the cytocompatibility of all-carbon scaffolds fabricated using single- and multi- walled CNTs and graphene nanoribbons (SWGONRs and MWGONRs). We report cellular attachment, proliferation, infiltration, and ECM formation of human adipose derived stem cells (hADSCs) and MC3T3 pre-osteoblast cells cultured on SWCNT, MWCNT, SWGONR and MWGONR scaffolds for 1, 3, and 5 days.

Methods: Scaffolds using SWCNTs, MWCNTs, SWGONRs and MWGONRs were fabricated using a mass ratio of nanomaterial:BP = 1:0.5 (for maximum porosity), as reported previously [4]. Scaffolds were sterilized by washes with $CHCl_3$ (to remove excess BP), ethanol gradient, PBS, and culture media, and soaked in the media for 24 hours. PLGA scaffolds (85% porosity) and tissue culture plate were used as control groups. hADSCs (cultured in ADSC basal media) and MC3T3 cells (cultured in MEM α) were seeded on the scaffolds (50,000 cells per scaffold) and incubated for 1, 3 and 5 days. LDH assay and LIVE cell imaging (calcein-AM stain) were used to assess cell viability. Cell proliferation was assessed by immunofluorescence staining for Ki-67 gene. Cell attachment was observed via SEM imaging. Furthermore, histology was performed to assess cell infiltration into the porous scaffold and ECM formation.

Results: LDH assay results show negligible cell death (compared to tissue culture polystyrene) for hADSCs and MC3T3 cells cultured on SWGONR and MWGONR scaffolds for all time points (1, 3, and 5 days). LIVE cell imaging using confocal laser scanning microscope confirms the presence of metabolically active live cells, corroborating the results of LDH assay. Cell proliferation on all-carbon scaffolds was observed as the cell number of calcein-AM positive cells increased over time. Immunofluorescence staining showed the expression of Ki-67 gene (proliferation marker) for both the cell types. Formation of focal adhesions was confirmed by fluorescence imaging. SEM imaging of MWCNT scaffolds showed the presence of cellular extensions and protrusions, attaching to the underlying scaffold architecture. 3D stacks of confocal images showed the presence of calcein stained cells inside the scaffolds at depths of ~100-200 μm. Histological analysis of paraffin embedded sections of MWCNT scaffolds showed good cellular infiltration and ECM formation (results not shown).

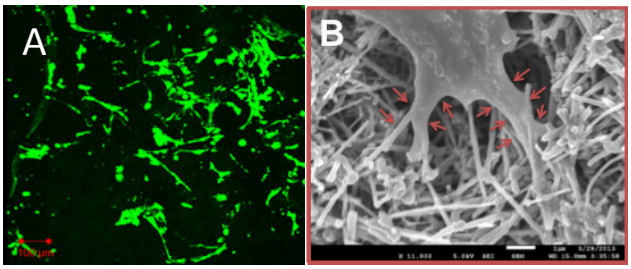

Figure 1: (A) Confocal image showing the presence of calcein stained MC3T3 cells after 5 days of culture on MWCNT scaffolds. (B) SEM image showing the formation of cytoplasmic extensions for cellular cell attachment.

Discussions and Conclusion

hADSCs and MC3T3 cells cultured on all-carbon scaffolds showed good cell attachment (SEM imaging), viability (LDH assay), and proliferation (Ki-67 expression) at all time points. hADSCs appear elongated and stretched on CNT scaffolds and rounded on graphene scaffolds. Formation of focal adhesions was observed by immunofluorescence staining. Furthermore, cellular infiltration inside porous all-carbon scaffolds was observed by confocal 3D reconstructions and histological analysis. Additionally, presence of ECM proteins was observed by histology. These results suggest that all-carbon scaffolds are cytocompatible and can be further used to develop biomedical devices and implants, and for various tissue engineering applications. These scaffolds can also be functionalized with various ligands and functional groups towards specific tissue engineering approaches. Furthermore, results also suggest that cell morphology can be controlled by nanostructured surfaces thereby allowing control of cell processes such as differentiation solely based on nanotopographical cues.

References

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