Development of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibrous scaffold for esophageal tissue engineering

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Statement of Purpose: Congenital **(**Esophageal atresia and Tracheoesophageal fistula) as well as other esophageal disorders (cancer, Barrett's esophagus, stricture and gastro esophageal reflux disease (GERD)) severely affects the esophageal transportation functions.[1] Esophageal disorders both congenital and acquired diseases are repaired either by autologous or allogenic or xenogenic grafts.[2-3]However, the post operative obstruction such as infection, stenosis, and morbidity limits their potential applications.[1] Decellularized scaffolds have been used alternatively to the auto/allo/xenografts. Again immunogenicity and disease transmissions restricted its function. Tissue engineering is a promising alternative strategy for conventional grafts. The objective of this work is to fabricate and evaluate the *in vitro* potential of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanofibrous scaffold using Human esophageal epithelial cells (HEEpiC).

Materials: Defect-free PHBV nanofibrous scaffold was fabricated through electrospinning by optimizing the solution and process parameters. The surface morphology of the nanofibrous scaffold was studied by field emission scanning electron microscopy (FE-SEM, JSM 6701F, JEOL, Japan). The *in vitro* potential application of the PHBV nanofibrous scaffold was studied by HEEpiC for adhesion, viability, cytoskeletal, cytokeratin morphology and proliferation.

Results: The surface morphology of PHBV nanofibrous scaffold is shown in Figure 1. Defect-free PHBV nanofibrous scaffold was fabricated and the average fiber diameters were found to be 583±90 nm.

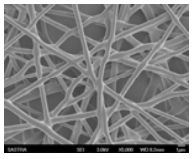

Figure 1. Surface morphology of PHBV nanofiber.

HEEpiC adhered and proliferated well on the PHBV nanofibrous scaffold (Figure 2). MTS results showed that the cell number was increased with time. However, after 7 days of culture HEEpiC proliferation was significantly higher in TCPS than PHBV nanofibrous scaffold due to hydrophobic nature of PHBV.

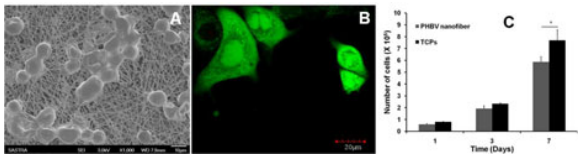

Figure 2. HEEpiC [A] adhesion; [B] viability; [C] proliferation on the PHBV nanofibers

Further, HEEpiC was stained by cytoskeletal protein actin and focal adhesion protein vinculin to determine the cell-matrix interactions (Figure 3). HEEpiC cultured on the PHBV scaffold exhibited the characteristic epithelial cobblestone morphology which demonstrates the favorable nature of the scaffold for epithelial culture.

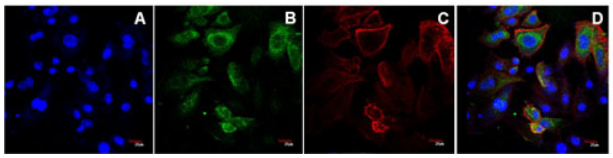

Figure 3. Cytoskeletal morphology of HEEpiC on the PHBV nanofiber [A]-Nucleus; [B]-Vinculin; [C]-Actin & [D]-Merged image of [A-C].

Expression of cytokeratin 14 a phenotypic marker protein for esophageal epithelial cells was qualitatively studied by immunostaining (Figure 4). HEEpiC cultured on the PHBV nanofiber express the cytokeratin 14 which confirms that HEEpiC didn't lose its phenotype.

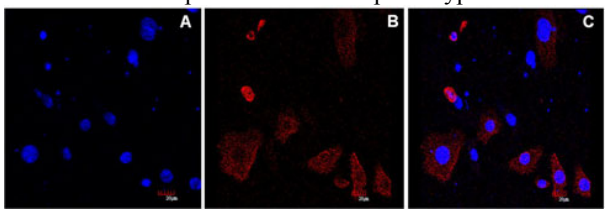

Figure 5. Cytokeratin 14 expression of HEEpiC on the PHBV nanofibers. [A]-Nucleus; [B]-Cytokeratin 14; [C]-Merged image of [A & B].

Conclusions: Defect-free PHBV nanofibers were fabricated and the average nanofiber diameter was found to be 583±90 nm. PHBV nanofibrous scaffold support the adhesion and proliferation of HEEpiC. Actin and vinculin staining of HEEpiC demonstrate the better cell-matrix interactions. Further, phenotypic cytokeratin 14 expression confirms the normal epithelial phenotype. HEEpiC exhibited the characteristic epithelial cobblestone morphology which shows the suitability of the scaffold. Therefore, PHBV nanofibers can be potential scaffold for esophageal tissue engineering.

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References:

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