Biosynthetic hydrogel poly(mannitol fumarate-co-sebacate)-co-alginate forms favorable micro niche for the differentiation of mesenchymal stem cells to cardiac lineage

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Introduction:

Cardiovascular diseases (CVD), especially myocardial infarction (MI), create major health crises contributing more than 30% of the global death rate. According to a recent report by American Heart association, on every 25 seconds one man will have a coronary event and a death/min due to the same. The regeneration of infarcted heart muscle is a challenge due to the absence of cell division in cardiomyocytes. The tissue engineering approach for developing a suitable microniche scaffoldings for 3D cell growth have been evolved due to the increasing clinical demand for tissue/organ for repair and replacement. Cardiac tissue regeneration involves both cells and supporting matrix. Stem cells form an excellent choice for cardiac tissue regeneration. But for the better performance of stem cells a proper supporting matrix is required. The physiochemical and biological properties of the supporting matrices largely influences stem cell growth and differentiation. Hydrogels have been used as supporting matrices to deliver cells into infarcted cardiac muscle due to their biomimetic characters. In the present study we intended to check the efficiency of our biosynthetic hydrogel scaffolds to favor the differentiation of MSC to cardiac cells.

Methods.

Hydroxyl terminated poly(mannitol fumarate-co-sebacate) (HT-MFS) polymer was synthesized by the condensation reaction of 0.72 M Mannitol, 0.67 M maleic anhydride and 0.42 M Sebacic acid. The polymer was dissolved in DMSO and stored at room temperature for further studies. From this HT-MFS-Alginate co-polymer (MFSA) was synthesized by the acid catalyzed condensation of alginate. From MFSA two hydrogel scaffolds MFSA-PEGDA and MFSA-DEGDMA were synthesized by cross linking with poly (ethylene glycol) diacrylate (PEGDA) and diethylene glycol dimethacrylate (DEGDMA) respectively. Ca^{2+} was used to cross link the alginate fraction of both the hydrogels. The physiochemical characterization of the hydrogels for surface functionality, pore diameter, hydrophilicity, water status, water content and water holding capacity were carried out. The cytocompatibility of the hydrogels were determined by MTT and direct contact assay [1]. In order to determine the capability of our hydrogels to support the differentiation of bone marrow derived mesenchymal stem cells (MSC), the MSC were allowed to grow on to the hydrogels for attachment. After attachment the differentiation medium containing 5uM azacytidine was added and incubated for 24h and then for 14 days in normal DMEM. Then the cardiac specific differentiation markers were analyzed microscopically. The gene expression profile of differentiated cells of cardiac lineage was analyzed by PCR analysis.

Results:

Both the MFSA-PEGDA and MFSA-DEGDMA hydrogels were synthesized using the biological and synthetic molecules. The ATR spectrum of the hydrogels showed a characteristic reduction in the peak intensities corresponding to -C=C stretching of fumarate (1640 cm^{-1}) and - C-H bending of cis –CH=CH- (770 cm^{-1}) due to cross linking by the vinyl monomers. The pore diameter of MFSA-PEGDA (7.50 ± 1.95) hydrogel was found to be greater when compared to MFSA-DEGDMA (5.06 ± 0.73). The contact angle measurements of both the hydrogels fall around 60^{o} indicating their amphiphilic character. The equilibrium water content and water holding capacity of MFSA-PEGDA were 400.5 ± 28.5 and 79.95 ± 1.14 respectively; for MFSA-DEGDMA it was 241.49 ± 41.35 and 70.34 ± 3.52. MFSA-PEGDA possessed higher freezable water content (34.64%); for MFSA-DEGDMA it was26.95% . These hydrogels were cytocompatible with viability greater than 95%.

The grown MSC were healthy on both the hydrogels. The expression of cardiac specific transcription factors like NKX2-5, GATA-4, MEF-2 and the cardiac specific biomarkers like troponin-T and troponin-I revealed the effective differentiation of MSC to that of cardiac lineage. The intensities of the bands were found to be greater in MFSA-PEGDA hydrogel which implies better differentiation with MFSA-PEGDA in comparison with MFSA-DEGDMA. This may be attributed to the higher freezing water content in MFSA-PEGDA..

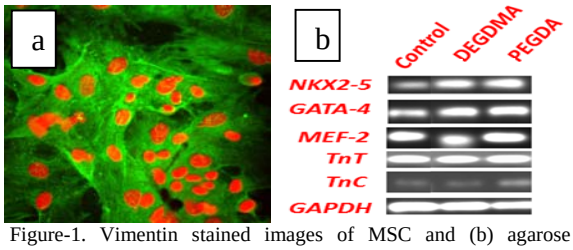

Figure-1. Vimentin stained images of MSC and (b) agarose gel electrophoresis of cardiac specific genes from differentiated MSC grown on hydrogels after amplification.

Conclusions:

Two biosynthetic hydrogels were synthesized from alginate and the polyester HT-MFS. Both the hydrogels favor the MSC differentiation; still MFSA-PEGDA predominates in this aspect. This may be attributed to the higher freezing water content of MFSA-PEGDA. Of the two hydrogels MFSA-PEGDA is a better candidate for the differentiation of MSC to cardiac lineage.

Reference:

[1] Finosh. Colloids Surf., B, 107;2013**:**137.