**Fabrication of Honey-based Biodegradable Scaffold for Ex-vivo Expansion of Mesenchymal Stem Cells**

<table>
<thead>
<tr>
<th>Funding Agency</th>
<th>DST</th>
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<tr>
<td>Sanctioned Amount</td>
<td>Rs. 25 Lakhs</td>
</tr>
<tr>
<td>Project Duration</td>
<td>3 years</td>
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<td>Project Status</td>
<td>Continuing since July 2013</td>
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PI: Dr. Ananya Barui  
*Department of Centre for Healthcare Science and Technology, IIEST Shibpur*

**Brief Description of the Project**

Cell-based therapy is an emerging approach in regenerative medicine that aims to restore damaged organ and tissue functions. In this approach, application of umbilical cord derived mesenchymal stem cells (MSCs) with self-renewal capacity, low immunogenecity as well as immuno-suppressive properties, draws huge attention. However, current reports indicate rapid aging of MSCs under *ex vivo* culture accompanied by significant changes in cell properties. To harness their potential in regenerative medicine, *ex vivo* expansions of MSCs without compromising genetic stability with respect to cellular attachment, proliferation, differentiation and aging are important. However, role of varied extra cellular matrix (ECM) *vis-à-vis* physico-chemical ambience and molecular mechanisms underlying phenotypical changes of MSCs under culture conditions is yet elusive. It is reported that different mechanical forces exerted by ECM influence the differentiation as well as self-renewal properties of stem cells. Selective differentiation of MSCs can be achieved through applying mechanical forces by modulating the stiffness of scaffold. Conversely, concept of cellular bio-electrical signals in regenerative process along with cellular communication and development is a cornerstone aspect in regenerative biology. At this juncture, mimicking micro-environment of stem cell niche with suitable biomaterial matrix is challenging for sustainability as well as in inhibition of early aging and in facilitating differentiation of MSCs towards desired cellular lineage. To address this issue, there is an enormous search for appropriate biomaterials and suitable bio-matrix fabrication processes. Honey, as a natural healing material with fascinating regenerative potential has been explored in fabricating biomaterial scaffold. However, more optimization is required for *ex vivo* harboring of MSCs. To bring specific development of biological rule-bases for matrix bio-compatibility are also very important. Hence, this study aims at developing suitable honey-biomaterial based scaffolds which can provide supportive niche for *ex vivo* expansion of MSCs without concomitant alteration in their phenotype.

*Keywords: Mesenchymal stem cell, differentiation, umbilical cord, scaffold, characterization*

**Methodologies/Approaches Adopted**

1. Isolation of mesenchymal stem cells from umbilical cord  
2. Fabrication of asymmetric scaffold through spinning methods  
3. Characterization of scaffolds- physical, chemical, mechanical  
4. Biocompatibility assessment of scaffolds  
5. Culture of stem cells on scaffold  
6. Characterization of cells cultured on scaffold- biocompatibility, proliferation, live-dead cells assay etc.
Project Highlights

1. Characteristic natural material based scaffold with potential to harbor and facilitate differentiation of MSCs may find applicability in regenerative medicine practices.
2. Multimodal characterization approach for MSCs will be of great value in specific assessment of stem cell integrity in different stages viability and differentiation.
3. The knowledge of stem cell differentiation into desired lineage will provide necessary clue to adopt direction for MSCs application in different disease therapies.

Project Achievements

Culture of MSCs on scaffolds: Scanning Electron microscopy images shows the cell attachment and proliferation on electrospun fibers. Cell growth rate was observed more on 12PH0.5 and 12PH1 in comparison to 12PH0 or 12PH0.2 for nanofibers (Fig. A-D). Moreover, combined scaffolds showed higher growth rate and proliferation in comparison to nanofibers (Fig. E-H).

MTT assays exhibits cellular viability is better in asymmetric combined scaffolds in comparison to nano-fiber formulations. Combined scaffold with 1% honey concentration is showing better viability whereas, in nano-fibre scaffolds, 0.2% honey concentration is showing better viability (Figure 2).

Evaluation of cell cycle progression was understood by quantification of bromodeoxyouridine (BrdU) into newly synthesized DNA of actively proliferating cells. Cells were grown on nanofibers and combined nanofibers within 12 well plates for 10 days and BrdU assay was performed on them. Following result exhibits that scaffolds with 0.2% honey dilution is showing better result in respect to more number of actively proloiferating cells attached with scaffold (Figure 3).
Fig. 3: BrdU Assay of MSCs on Different Fabric Compositions

Publications (Please follow any one specific bibliographic style)


Expression in Lower Limb Wound Healing under Honey Evidence Based Complementary and Alternative Medicine. 2011


Facilities Developed

Instruments Procured

1. Magnetic stirrer with hot plate: LABMAN-LMMS
2. Lyophilizer: TIMC
3. -20 degree fridge: TIMC
4. Electronic analytical balance: Sartorious-BSA 224S-CW
5. Spinning set up: Physics Equipment co. ltd.
6. pH/conductivity meter: Okaton-36510101

Fig. 4: Developed Cell Culture Lab

Project Staff (Designation, number, name, qualification, leading to PhD, etc.)

JRF- Mr. Ripon Sarkar, presently perusing PhD at CHST, IIEST, Shibpur.

Plan of Future Project Proposal based on the Current Project

We plan to develop integrated method for multi-modal characterization of trans-differentiated mesenchymal stem cells for disease specific clinical application.