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

Funding Agency	DST
Sanctioned Amount	Rs. 25 Lakhs
Project Duration	3 years
Project Status	Continuing since July 2013

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 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).

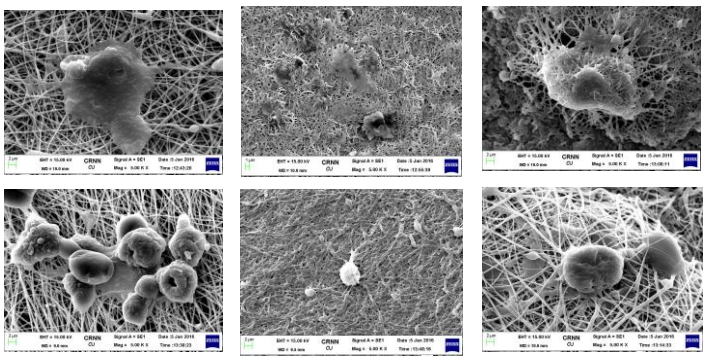

Fig. 1: SEM Micrograph of MSCs attached on Fabricated Scaffold

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).

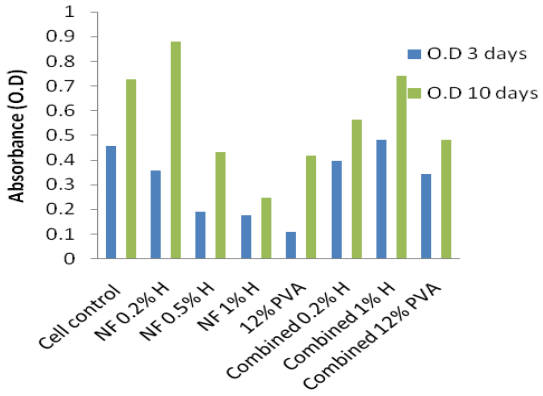

Fig. 2: MTT Assay for Cellular Viability at Different Day of Culture

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).

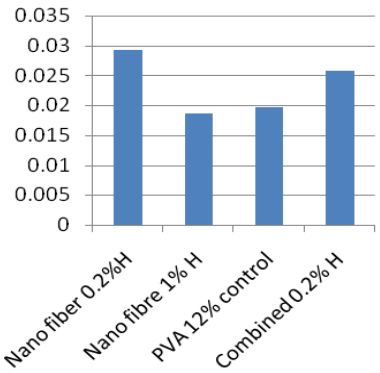

Fig. 3: BrdU Assay of MSCs on Different Fabric Compositions

Publications (Please follow any one specific bibliographic style)

1. Early Cancer Risk Assessment in Smokers through Fuzzy Trend Analysis of AutoFluorescence Shift and Cyto-morphologcal Alteration, Ripon Sarkar, Susmita Dey, Mousumi Pal, RanjanRashmi Pal, Jyotirmoy Chatterjee, Ananya Barui. Medicine. Revision Submitted.
2. Ananya Barui, Provas Banerjee, Amrita Chaudhary, Sailesh Conjeti, Bikash Mondal, Susmita Dey, Jyotirmoy Chatterjee. Evaluation of angiogenesis in diabetic lower limb wound healing using a natural medicine: A quantitative approach. Wound Medicine. 10/2014; 6:26-33. DOI: 10.1016/j.wndm.2014.09.003.
3. Monika Rajput, Nandini Bhandaru, Ananya Barui, Amrita Chaudhary, Ranjan Rashmi Paul, Rabibrata Mukherjee and Jyotirmoy Chatterjee. Nano-patterned honey incorporated silk fibroin membranes for improving cellular compatibility. RSC Adv., 2014, 4, 44674-44688.
4. Amrita Chaudhary, Swarnendu Bag, Ananya Barui, Provas Banerjee, Jyotirmoy Chatterjee. Honey dilution impact on in vitro wound healing: Normoxic and hypoxic condition. Wound Rep Reg (2015), DOI:10.1111/wrr.12297.
5. Amrita Chaudhary, Swarnendu Bag, Mousumi Mandal, Ananya Barui, Monika Rajput, Provas Banerjee, Jyotirmoy Chatterjee. Honey Dilution in Modulating Prime Molecular Expression of Keratinocyte (HaCaT) Population. Journal of Ethnopharmacology, 166(2015)211–219.
6. Taposhree Dutta, Rudra Sarkar, Bholanath Pakhira, Subrata Ghosh, Ripon Sarkar, Ananya Barui and Sabyasachi Sarkar. ROS generation by reduced graphene oxide (rGO) induced by visible light showing antibacterial activity: comparison with graphene oxide (GO). RSC Adv., 2015,5, 80192-80195.
7. Ananya Barui, Naresh Mandal, Subhadipa Majumder, Raunak Kumar Das, Sanghamitra Sengupta, Provas Banerjee, Ajoy Kumar Ray, Chirosree RoyChaudhuri, Jyotirmoy Chatterjee. Assessment of molecular events during in vitro re-epithelialization under honey-alginate matrix ambience. Materials Science and Engineering C-33; 3418–3425, 2013.
8. Ananya Barui, ProvasBanerjee, RushaPatra, Raunak Kumar Das, SantanuDhara, P.K. Dutta, and Jyotirmoy Chatterjee Swept-Source Optical Coherence Tomography of Lower Limb Wound Healing with Histo-pathological Correlation. Journal of Biomedical Optics. 16; 2011.
9. Ananya Barui, Provas Banerjee, Raunak Kumar Das, Shyamal Kumar Basu, S. Dhara and Jyotirmoy Chatterjee. Immunohistochemial Evaluation of p63, E Cadherin, Collagen I and III

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

10. Ananya Barui; Ritesh Khare; Provas Banerjee; Jyotirmoy Chatterjee. Ex vivo Bio-compatibility of Honey-Alginate Fibrous Matrix for HaCaT and 3T3 with Prime Molecular Expressions. Journal of Materials Science: Materials in Medicine, 2011.
11. Raunak Kumar Das, Mousumi Pal, Swarnendu Bag, Subhadipa Majumdar, Ananya Barui, Ranjan Rashmi Paul, Chandan Chakrabortya, Ajoy Kumar Ray, Sanghamitra Sengupta, Jyotirmoy Chatterjee. Epithelio-mesenchymal Transitional Attributes in Oral Sub-mucous Fibrosis. Experimental and Molecular Pathology. 95; 259-269, 2013.
12. Raunak Kumar Das, Mousumi Pal, Ananya Barui, Ranjan Rashmi Paul, Chandan Chakraborty, Ajoy Kumar Ray, Sanghamitra Sengupta, and Jyotirmoy Chatterjee. Assessment of malignant potential of oral submucous fibrosis through evaluation of p63, E-cadherin and CD105 expression. J. Clin. Pathol. 63, 94-899. 2010.
13. Raunak Kumar Das, Mousumi Pal, Ananya Barui, Ranjan Rashmi Paul, Chandan Chakraborty, Ajoy Kumar Ray, Jyotirmoy Chatterjee. ApoTome to visualize E-cadherin and p63 expression in oral pre cancer. Biotechnology Journal, 7, 2012.

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.