Abstract:

Bone marrow niche is composed of cellular and non-cellular compartments. Cellular environment includes hematopoietic stem cells (HSCs), non-hematopoietic stem cells and their derivatives. HSCs maintain the blood cell development. HSCs fate is determined by endosteal niche made of osteoblasts and stromal cells, and sinusoidal niche made of endothelial cells. HSCs proliferation and differentiation is regulated by interaction between the cells directly or indirectly. Stromal cells, their derivatives and secretory factors like growth factors, cytokines regulate the stem cells present in the bone marrow. It is a highly regulated process, imbalance in regulation leads to pathological conditions. In addition, it is composed of network like structure known as extra cellular matrix (ECM) which is made of collagen, fibronectin and proteoglycans, also known to regulate the stem cell properties. Mesenchymal stem cells (MSCs) from the non-hematopoietic stem cell compartment known to regulate the HSCs maintenance, proliferation and differentiation. The MSCs have self-renewal capacity to produce same type of stem cells and undergo differentiation to give rise to specialized mature cells like adipocytes, osteocytes, and chondrocytes. MSCs have role in wound healing, immune suppression, immune modulation, and angiogenesis. MSCs were first isolated from the bone marrow and also isolated from the various other sources like cord blood, peripheral blood, adipose tissue, placenta, cartilage etc. MSCs can be identified by expression of cell surface markers CD73, CD90 and CD105 and they are negative for hematopoietic markers CD34, CD45 and CD14. MSCs can be used to treat diseases such as neurodegenerative diseases, heart diseases, leukemia, muscular dystrophy, bone diseases and diabetes.

MSCs were used for cell therapy to treat several osteogenic disorders and have potential to be used for treatment of spinal cord injury, cardiac diseases. Hematopoietic engraftment has been facilitated in patients when MSCs was co-injected with the bone marrow cells (Jaganathan BG et, al. 2010; Chen S et, al. 2006; Hare JM et, al. 2009; Li J et, al. 2013; Park JH et, al. 2012). The clinical use of MSCs and its therapeutic outcomes were limited due to its low survival rate during *in vivo* injection. Rapid proliferation is also essential if these cells have to be used for autologous transplantation soon after the injury to achieve high clinical benefit. *In vitro* cell expansion is performed prior to *in vivo* administration where a large number of cells are required ($1x10^6$ cells/Kg body weight) which needs a rapid expansion system (Jaganathan BG et, al. 2010). To facilitate tissue repair, during

tissue engineering, the cells are directly injected into the injury site or seeded on to scaffold and implanted. (Kim HJ et, al. 2012). There are several reports which show that MSCs underwent rapid cell death soon after injection intravenously and the cells could not be identified when tracked after few months. In the present study, analysis of the cell characteristics of MSCs such as proliferation, survival, differentiation and migration was performed to understand the suitable extracellular matrix that could be utilized for MSCs expansion as well as coating on scaffold for tissue engineering.

In the current study, it was found that when MSCs were cultured on collagen (COL) fibers, they promoted high cell proliferation compared to other matrices such as fibronectin (FBN), poly-l-lysine (PLL) or normal tissue culture treated surface. In addition, cells cultured on COL showed higher resistance to apoptosis induced by oxidative and nutrient stress which might occur *in vivo* during ischemia (Rodrigo R et, al. 2013). COL also promoted faster cell adhesion to the culture surface, within 2hr and ROS production on COL surface was significantly low. These results suggest that COL could be utilized as a suitable coating on cell culture plates to promote higher proliferation or on scaffold for greater survival *in vivo*.

MSCs cultured on COL also showed higher osteogenic differentiation compared to other matrices. Interestingly, cells that were cultured on other matrices, when induced to differentiate on COL surface showed higher osteogenic differentiation. Importantly, culturing on COL surface was sufficient to promote higher osteogenesis even when differentiated on non-COL matrices. High osteogenic differentiation on COL also correlated with increased RHOA GTPase activity. Although chondrogenic differentiation is an important property of MSCs, it was not tested in the current study as chondrogenic differentiation *in vitro* was usually performed as a pellet culture where the cells do not have high contact with the culture surface during differentiation. Conversely, MSCs differentiated into adipocytes on control tissue culture treated (CON) surface showed significantly higher percentage of adipocytes, which correlated with the high expression of linage marker gene *PPAR gamma* whereas no difference in *Osteocalcin* levels were observed on different ECM. Since COL promotes higher osteogenic potential, we identified the role of ECM switching on MSCs differentiation. MSCs were expanded on different ECM and differentiated on different ECM. Our analysis showed that whenever cells were cultured or differentiated on COL, they showed high osteogenic potential. This

indicates that switching of cells from one ECM to other help to achieve better differentiation potential for therapeutic applications.

The actin cytoskeleton plays an important role in regulating cellular properties. To determine the actin modification and cell shape, MSCs were cultured on different matrices and stained for F-actin. MSCs cultured on PLL showed polygonal arrangement of F-actin, whereas MSCs cultured on COL, FBN or CON surfaces acquired parallel F-actin arrangement. Actin also mediates the attachment of cells with the cell culture surface through integrin and other proteins. To identify the cell contact points, MSCs were cultured on ECM proteins, stained for F-actin and examined with total internal reflection fluoroscence (TIRF) microscope. MSCs cultured on FBN, COL or PLL showed high number of contact poins, whereas cells cultured on CON surface showed very few contact points. Another important feature that is required during tissue remodeling and repair is active migration of cells. COL surface promoted higher cell migration, cell attachment and significantly high cell-surface contact points were observed in MSCs cultured on COL. Culturing MSCs on COL resulted in significantly high CD49e cell surface expression and CD49e was shown to mediate migration of hematopoietic stem cells (Sahin AO et, al. 2012) and an earlier study from our group also showed that CD49e cell surface expression increased during osteogenesis (Sonowal H et, al. 2013). This increase in CD49e expression on COL might be associated with its increased osteogenic differentiation potential. However, no significant difference in CD29 and CD49D expression level were observed in all the matrices. Furthermore, MSCs cultured on collagen showed mitochondrial distribution pattern similar to that observed in stem cells, where it was predominantly peri-nuclear mitochondrial distribution suggesting that COL might promote or maintain more stem cell-like state in MSCs.

In our earlier study, high RHOA levels were observed on COL grown MSCs. RHOA is essential for proliferation, differentiation, migration and other cellular functions. Inorder to understand the RHOA signaling pathway in MSCs, we expressed constitutively active (RHOAV14) or dominant negative inactive (RHOAN19) forms of RHOA in MSCs. In our study, we transduced the MSCs with lentiviral vector for stable expression and after 48 hours of transduction, morphology change was observed. Control MSCs showed spindle shape with normal fibroblastic morphology but MSCs expressing RHOAV14 lost their normal spindle shape and showed stellate like morphology. To demonstrate the role of RHOA in MSCs proliferation, MTT cell viability assay was performed. The results of

MTT assay showed that there was a significant reduction in proliferation in MSCs expressing RHOAV14 compared to control. Furthermore, we elucidated the role of RHOA in actin modification. Parallel F-actin arrangement was seen in control MSCs, whereas crisscross arrangement and increased stress fiber formation was observed in MSCs transduced with RHOAV14. The MSCs transduced with RHOAV14 showed significant less migration potential than others. For evaluation of differentiation potential, RHOA transduced MSCs were subjected to osteo and adipogenic differentiation for 21 days and stained with alizarin red (ARS) for osteocytes and Oil red O (ORO) for adipocytes. There was an increase in osteogenesis and decrease in adipogenic potential of MSCs transduced with RHOAV14. However, no difference was seen in MSCs transduced with dominant negative RHOAN19.

Leukemic stem cells modify or manipulate the environment by secreting factors required for cancer cells. To study the changes in MSCs due to interaction with leukemic cells, MSCs were co-cultured with primary leukemic cells for 1 week and expression of surface markers CD13, CD29, CD49E, CD73, CD90 and CD95 was determined by flowcytometry. Decrease in CD73, CD90 and CD95 expression was observed in MSCs during co culture with primary leukemic cells. Furthermore, in order to understand the fate of MSCs during leukemic state, MSCs were co cultured with leukemic cells for 1 week and induced to differentiate into adipocytes and osteocytes with respective induction media for 28 days. The MSCs co cultured with primary leukemic cells showed reduced adipogenic potential compared to control, whereas no change was observed in osteogenic differentiation. MSCs were co cultured with THP1 and HL60 leukemic cell lines and screened for the expression of MSCs surface markers. No significant difference was seen in MSCs cell surface marker expression during THP1 or HL60 co-culture.

Understanding the changes in MSCs after chemotherapeutic treatment is essential to determine their potential for cell therapy; we treated MSCs with cytrarabine (CYT), daunorubicine (DAU) and vincristine (VIN) and evaluated their properties. The cells treated with VIN induced a marked change in cell shape, converting spindle shaped cells to round shape, whereas no change in morphology was observed in CYT and DAU treated MSCs after 48hr. We observed following changes in cell surface marker expression profiles a) significant decrease in CD49E expression in CYT treated MSCs, b) MSCs treated with DAU showed reduced levels of CD13, CD29, CD49E, CD90 and CD95 expression, c) MSCs treated with VIN down regulated the expression levels of CD13,

CD49E and CD90. Significant reduction in MSCs proliferation was observed after 48hr of drug treatment. To evaluate the effect of chemo drugs on differentiation potential, we stained post-differentiated MSCs with Oil Red O (ORO) and Alizarin Red S (ARS) after adipocytes and osteocytes differentiation respectively. We observed significantly less adipogenic and osteogenic potential in all the drug treated MSCs.

MSCs treated with chemotherapeutic drugs were allowed to recover and their cell surface antigen expression was determined. DAU treated MSCs showed reduction in CD13 and CD29 expression after 7 days of recovery. However, VIN treated MSCs completely recovered from chemo drugs and showed normal surface marker expression. MSCs recovered from VIN and CYT retained their normal adipo and osteo differentiation potential.

To check if recovered MSCs could potentially support the growth of leukemic cells, MSCs were co cultured with AML cells HL60 and treated with CYT, DAU and VIN. Increased apoptosis was observed in HL60 after treatment with all three drugs, whereas treatment with drugs in the presence of MSCs reduced their apoptotic percentage except during VIN treatment.

We also evaluated the effect of antimitotic drugs, colchicine and nocodazole on MSCs properties, using phenotypic markers CD13, CD29, CD49E, CD90 and CD95. In colchicine treatment we found decreased expression of CD49E and CD90, whereas, CD13 and CD90 expression was reduced by nocodazole treatment. To understand the effect of drug treatment on cell differentiation, MSCs were treated for 24hr and 1 week with colchicine and nocodazole in differentiation media. We observed, reduced adipogenic and osteogenic differentiation in both colchicine and one week nocodazole treated MSCs. However, no significant difference was seen after 24hr nocodazole treatment. After removing the nocodazole, MSCs recovered and regained normal differentiation potential. However colchicine treated MSCs did not achieve their normal differentiation potential after the drug removal.

In conclusion, the present study demonstrates that extracellular matrices control the characteristics of MSCs by altering their cell proliferation, migration and osteogenic differentiation. Our study shows that MSCs could be cultured or pre-differentiated on COL surface to obtain high osteogenic differentiation and culturing them on uncoated tissue culture plastic primed them to adipogenesis. Increased proliferation can be achieved by

coating the cell growth surface with COL and when used in tissue engineering, COL coated scaffolds will promote high cell migration, proliferation, survival and osteogenic differentiation. MSCs treated with chemotherapeutic drugs recovered their cell surface expression and differentiation potential after removal of the drugs and thus might be suitable for cell therapy.