Université de Montréal

Optimization Studies to Improve MSC-based Cardiac Cell Therapy: Cytokine Preconditioning and Nanoparticle Coupling

by

Zhou Wanjiang

Department of Biomedical Science

Faculty of Medicine

Memoire presented to Faculty of Medicine for the Degree of Master of Science (M.Sc)

In Biomedical Science

December, 2012

Université de Montréal
Faculté des études supérieures et postdoctorales

Ce mémoire intitulé:

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Présenté par:
Zhou Wanjiang

évalué par un jury composé des personnes suivantes :

Dr. Marc Jolicoeur, président-rapporteur
Dr. Quoc-Hung Ly, directeur de recherche
Dr.Thierry Charron, membre du jury

I Abstract

Background: Ischemic heart disease (IHD) remains a leading cause of mortality in North America. Cardiac cell therapy (CCT) has emerged as a promising therapy to help heal the damaged heart. Among the various candidates for stem-progenitor cells, Mesenchymal Multipotential Stromal/Stem Cells (MSC) is of great promise. However, there remain unresolved issues and challenges that prevent clinical application of MSC-based CCT in IHD. Among the latter, low cellular retention rate, in vivo cell tracking and post-delivery apoptosis. Here in, growth factor preconditioning and MSC coupling to nanoparticles are investigated as methods to optimize MSC. **Methods**：Lewis Rat MSC (rMSC) and pig MSC (pMSC) were isolated from bone marrow. Rat MSCs were preconditioned with SDF-1a, TSG-6 and PDGF-BB, and then subjected to hypoxia, serum deprivation and oxidative stress. Wound healing assays were also done with preconditioned rat MSCs. In parallel, novel ferromagnetic silicone core-shell nanoparticles (NP) were synthesized. Pig MSCs were coupled to NPs following functionalization of the NPs with an antibody to a well-recognized MSC surface antigen, CD44. Subsequently, biocompatibility studies were performed on the pMSC-NP complex and included testing of key cellular processes such as migration, adhesion, proliferation and differentiation properties. **Results:** Of all cytokines used, PDGF-BB showed greatest capacity to improve MSC survival under conditions of hypoxia, serum deprivation and oxidative stress. NP conjugation has mitigated effect on the migration and proliferation of pig MSC, but do not change the differentiation capacity of MSC. Finally, the MSC-NP complex was detectable by MRI. **Conclusion:** Our data suggest that novel strategies, such as PDGF-BB preconditioning and ferromagnetic nanoparticle coupling, can be considered as promising avenues to optimize MSCs for CCT.

Key words: MSC, **CCT**, **Precondition**, **PDGF-BB**, **Magnetic Nano-particles**, **MRI**

Resumé

Contexte: La cardiopathie ischémique (IHD) reste une cause majeure de mortalité en Amérique du Nord. La thérapie cellulaire cardiaque (CCT) a émergé comme une thérapie prometteuse pour aider à guérir certaines malades cardiaques. Parmi les cellulaires avec propriétés pluripotentes, les cellules stromales mésenchymateuses (MSC) sont prometteuses. Cependant, plusieurs questions demeurent non résolues et certaines défis empêchent l'application clinique de la CCT se dans l'IHD, tels que le faible taux de rétention cellulaire in situ, le suivi des cellules in vivo post-implantation et post-acheminements et l`apoptose. Ici, le traitement préliminaire des MSC avec des facteurs de croissance et leur couplage avec des nanoparticules (NP) seront étudiés comme des méthodes pour optimiser MSC. **Méthodes:** Des MSCs provenant du rat (rMSC) et du cochon (pMSC) ont été isolés à partir de moelle osseuse. Les rMSC ont été préconditionnées avec SDF-1a, TSG-6 et PDGF-BB, et ensuite soumises à une hypoxie, une privation de sérum et a un stress oxydatif. Des études de cicatrisation ont également été effectués avec rMSCs préconditionnées. En parallèle, de nouvelles NP ferromagnétiques liées aux silicones ont été synthétisées. Les NPs ont été couplées aux pMSCs suivant leur fonctionnalisation avec l`anticorps, CD44, un antigène de surface du MSC bien connu. Par la suite, les études de biocompatibilité ont été réalisées sur pMSC-NP et en incluant des tests des processus cellulaires tels que la migration, l'adhésion, la prolifération et les propriétés de la différenciation. **Résultats:** Parmi toutes les cytokines testées, PDGF-BB a démontré la plus grande capacité à améliorer la survie de MSC dans des conditions d'hypoxie, de privation de sérum et en reponse au stress oxydatif. La conjugaison de NP a atténué la migration et la prolifération des pMSCs, mais n`a pas changé leur capacité de différenciation. Enfin, la complexe du MSC-NP est détectable par IRM. **Conclusion:** Nos données suggèrent que de nouvelles stratégies, telles que traitement préliminaire de PDGF-BB et le couplage des

nanoparticules ferromagnétiques, peuvent être considérés comme des avenues prometteuse pour optimiser les MSCs pour la CCT.

Mots clés: MSC, TDC, Condition, PDGF-BB, Nanoparticules Magnétiques, l'IRM

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IV List of Abbreviation

AAV: Adeno-Associated Vector

AdV: Adenovirus Vector

AMI: Acute Myocardial Infarction

AT: Adipose Tissue

BM: Bone Marrow

CAD: Coronary Artery Disease

CCT: Cardiac Cell Therapy

CHF: Chronic Heart Failure

CSPC: Cardiac Stem-Progenitor Cell

DMEM: Dulbecco Modified Eagle's Medium

ELISA: Enzyme Likned Immunosorbent Assay

EPC: Endothelial Progenitor Cell

ESC: Embryonic Stem Cell

FBS: Fetal Bovine Serum

FGF: Fibroblast Growth Factor

fPAM: functional Photoacoustic Microscopy

GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor

GVHD: Graft Versus Host Disease

HLA-DR: Human Leukocyte Antigens –DR

HSCT: Hematopoietic Stem Cell Transplantation

Hy: Hypoxia

Hy+SD: Hypoxia + Serum Deprivation

IHD: Ischemic Heart Disease

iPS: induced Pluripotent Stem Cell

ISCT: International Society of Cell Therapy

LV: Lentiviral Vector

LVEF: Left Ventricle Ejection Fraction

MLA: Magnetic Labeling Agent

MSC: Mesenchymal Multipotential Stromal Cell

MTS: 3-*(4,5-dimethylthiazol*-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

NP: Nanoparticle

PDGF-BB: Palette Derived Growth Factor –BB

PET: Pistron Emission Tomography

PMS: Phenazine Methosulfate

QD: Quantum Dot

rhVEGF: recombinant human VEGF

RLA: Radioactive Labeling Agent

SDF: Stromal Derived Factor

SM: Skeletal Myoblast

SPECT: Singe Photon Emission Computational Tomograhpy

SPIO: Supermagnetic Iron Oxide

TGF: Transformation Growth Factor

TSG-6: Tumor Necrosis Factor-induced Gene-6

UCB： Umbilical Cord Blood

VEGF: Vascular Endothelial Growth Factor

VSMC: Vascular Smooth Muscle Cell

V Acknowledgements

The work of my master could not have been accomplished with the help of many others, who also made my experience in Montreal Heart Institute a memorable one.

First I will give my thanks to Dr Ly. Hung. He provided the lab that makes the work possible to happen. During my time in the lab is always ready to help me with my questions in spite of his busy occupation as a cardiologist. Thank you for all your admonishment, encouragement and guidance for the past two years.

Valerie, thank you for preparing all the materials and equipments in the Lab, which made my work convenient and comfortable. Thank you Yassin. Irislimaine for being a good companion for me in the Lab and helping me collect data on NPs. Thank you, Marie. When I had problems with equipments you were always ready to help.

Also thank you Dr Stephanie. Talliez for all the helps you rendered concerning the School. You were always kindly answering the questions I had as a newcomer to Canada and University of Montreal. There are more than I can numerate one by one. Thank you all, without you, I could not have finished this work.

VI INTRODUCTION

Chapter 1 Introduction

1.1. The clinical burden of ischemic heart disease

Ischemic heart disease (IHD) remains one of the leading causes of mortality and morbidity worldwide (Antman, Anbe et al. 2004; Hunt, Abraham et al. 2005; Krum 2005; Choi D 2009). In North America, acute myocardial infarction (AMI) accounts for more than 50% of cardiovascular-related deaths. Annually, approximately one million patients suffer an AMI, which carries a mortality rate of about 25% over 3 years. Less than 20% of patients who survived an ST-elevation myocardial infarction will have achieved normal restoration of epicardial coronary flow and adequate microvascular reperfusion (Gibson, Cannon et al. 2000; Giugliano, Sabatine et al. 2004). The subsequent loss of viable myocytes irreversibly damages the myocardium. With greater numbers of patients surviving AMI as well as an aging population, post-infarction congestive heart failure (CHF) has become a health issue with increasing social impact and economic burden. The latter carries a poor prognosis for symptomatic patients (up to 50% mortality per year)(National Heart 2004).

1.2. Limitations of current therapeutic strategies for IHD:

Over the last decade, despite substantial progress made both in acute reperfusion strategies in AMI and chronic/preventive therapies for post-infarction CHF, current treatment methods are limited as can be evidenced by the aforementioned statistics. Current therapeutic strategies aim to enhance myocardial reperfusion during AMI(Gibson 2003) and limit the untoward effects of activation of the sympathetic/neuroendocrine systems in the hopes to halt the progressive nature of CHF(Jessup and Brozena 2003). At present, orthotropic heart transplantation remains

the only treatment for severe and irreversible post-infarction myocardial damage. Yet, increasing organ donation shortages have blunted its contributions. This discrepancy between demand and supply emphasizes the stark reality that this avenue no longer fills the growing clinical need. Accordingly, numerous novel therapies including molecular, pharmacological and mechanical have been explored (Landmesser and Drexler 2005; Yacoub, Suzuki et al. 2006). Among these strategies, cell-based therapy for cardiac regeneration has captured the attention of the clinicians and scientists alike and has emerged as a controversial but promising and potentially curative therapeutic strategy for IHD(Sanchez, San Roman et al. 2006).

Chapter 2 Cardiac Cell therapy

2.1. Biological basis of a paradigm shift

The human body possesses a varying capacity to repair and recover after injury, which helps to maintain homeostasis throughout life. The latter process is made possible due to a group of specialized cells, stem and progenitor cells that are able to regenerate and replace somatic cells (IL 200) Following a myocardial infarction (either acutely or chronically), a maladaptive process is initiated which results in injury progression and adverse cardiac remodeling. Current therapies have yet to exploit the reparative and/or regenerative potential of the heart.

A long-held scientific belief was that the heart was a post mitotic organ with terminal differentiation. In recent years, a growing body of evidence has challenged this axiom, supporting the notion that the adult mammalian heart can indeed undergo repair by regenerating cardiomyocytes from both endogenous and circulating sources. Reports documenting the existence of immature cardiomyocytes capable of re-entering the cell cycle(Beltrami, Barlucchi et al. 2003) or of cardiac repair by non-cardiac stem cells has further strengthened the heart's regenerative potential (Orlic, Kajstura et al. 2001). Much of the recent excitement and interest garnered by the field of regenerative cardiovascular medicine revolves around the concept of plasticity of adult stem cells (SC) (Blau, Brazelton et al. 2001; Wagers and Weissman 2004; Zipori 2005). SCs are undifferentiated cells that are defined by their capacity for self-renewal, clonogenicity and mulitpotentiality. Residing in "niches", SC become activated under conditions such as tissue injury(Fuchs, Tumbar et al. 2004). Various mechanisms have been put forward to explain the lineage conversion (plasticity) of adult stem cells: 1) multiple, distinct SCs within various organs that are able to contribute to tissue-specific repair; 2) the persistence of a single pluripotent cell giving rise to cells of different lineage; 3) the dedifferentiation, whereby tissue-specific cells revert to a more primitive, multipotent form, which

subsequently redifferentiates along a new lineage; 4) the cell-cell fusion by which heterotypic cells combine to form new, chimerical cells; and 5) the transdifferentiation whereas SCs give rise to various cell lineages by activation of dormant differentiation cellular pathways altering its lineage-specific commitment(Wagers and Weissman 2004).

2.2. Potential source of cells for cardiac regeneration

The pool of stem cells and progenitor cells for myocardial regeneration is quite versatile(Fraser, Schreiber et al. 2004; Caplice, Gersh et al. 2005; Leri, Kajstura et al. 2005; Muller, Beltrami et al. 2005; Fukuda and Yuasa 2006; Hristov and Weber 2006). They can be divided into embryonic vs. adult stem cells; the latter can be further divided into endogenous(cardiac) vs. exogenous stem cells. In brief:

2.2.1. Embryonic SC: are obtained from the inner mass of embryos at the blastocyst stage. They are highly proliferative (can replicate indefinitely in vitro) and are considered totipotent i.e. able to differentiate into cells of all germ lines. Nevertheless, the use of human embryonic stem cells faces several hurdles such as ethical issues, the need for immunosuppression, unresolved culture conditions and the potential for tumorigenicity.

2.2.2 Adult Stem-progenitor Cells:

i. *Skeletal muscle-derived cells:* Skeletal myoblasts are found in a quiescent state in the basal membrane of skeletal muscles. Readily isolated from a muscle biopsy and considered to be more resistant to ischemia than cardiomyocytes, they can be expanded *ex vivo* then transplanted in an autologous fashion. However, their survival rate is low (less than 50% at 48 hours) following transplantation into ischemic myocardium. In addition, the debate remains open whether skeletal myoblasts directly act on injured myocardium. Evidence suggests that MSCs do not form electro-mechanical coupling with the surrounding cardiomyocytes post-implantation. This evidence has led to the cautionary use of these cells. Nevertheless, most studies to date

have used sample from a heterogeneous population of skeletal myoblasts with less efficient engraftment. A distinct subpopulation of more primitive cells, the muscle-derived stem cells, show promise of both enhance regenerative potential and improve graft survival, with possibly less arrhythmogenicity (Jankowski, Deasy et al. 2002; Oshima, Payne et al. 2005).

ii. *Bone Marrow-derived SC:* Recent studies have highlighted the importance of the Bone-Marrow-derived SC(BMSC) in cardiac homeostasis (Vandervelde, van Luyn et al. 2005). It has been hypothesized that the BM not only harbors hematopoietic stem cells but also is a repository of circulating tissue-committed stem cells for various non-hematopoietic organs (liver, muscle, brain and heart)(Kucia, Dawn et al. 2004; Ratajczak, Kucia et al. 2004).The BM is comprised of a heterogeneous population of cells, and the on-going research is to define their contributions to myocardial repair(Dimmeler, Zeiher et al. 2005; Lee 2011). Currently, the most studied BM-SCs or circulating BM-SCs are as follow:

- Hematopoietic SC: Discovered over four decades ago and well known for their capacity to repopulate the BM, these multipotent cells reside within BM niches and give rise to mature blood cells. Of note, no truly specific antigen for identification of these cells have been found so far; the known surface markers allow to recognize more specific subpopulation. In mice, they are characterized by their surface epitopes, c-kit+Sca-1+Thy-1 low; whereas in humans, markers such as CD34, CD 117 and CD 133 allow for their identification. Current efforts in cardiovascular research have focused on the identification of a specific subpopulation of these cells.

- Endothelial progenitor cells (EPCs): Derived from mononuclear cells collected from BM or peripheral blood, these cells are isolated based on antigens found on both hematopoietic and endothelial cells (CD34, VEGF receptor 2 [FLK-1/KDR] or CD133). Well-documented for their in vitro maturation towards endothelial cells and convincingly proven to contribute to angiogenesis,

these cells have been reported to transdifferentiate into cardiomyocytes. A subpopulation of these cells has been associated with minimal expression of surface antigens and a high degree of plasticity. Their low abundance in circulation is a limiting step. Hence to achieve sufficient numbers for either research or clinical purposes, there is a need for additional steps for ex vivo expansion (use of growth factors or cytokines to promote increased mobilization of these cells) for greater yields during harvesting.

- Side-population SC (SPCs): These are a primitive stem cell population, shown to be potent hematopoietic stem cells capable of contributing to BM regeneration. These cells can be easily selected by their capacity to extrude the dye Hoechst 33342. A distinct, characteristic pattern is obtained on fluorescence activated cell sorter (FACS) resulting from this high effluxing dye activity, giving a separate population to the side of the main population on a dot-plot emission spectra in both the blue (450 nm) and far red (>675 nm) emission channels (Goodell, Rosenzweig et al. 1997). This ability is conferred by the ATP-binding cassette (ABC) transporter Abcg2/brcp1, the latter protein being restricted to the SP population. First isolated from the BM (accounting for ~0.05% of the total cell population), these SCs have since been identified within various tissues, including the heart (Hierlihy, Seale et al. 2002; Pfister, Mouquet et al. 2005), with documented multilineage differentiation ability regardless of tissue origin.

- Mesenchymal Multipotential Stromal/Stem Cells (MSCs): These cells will be discussed more in detail in the following section as they represent the main cell population related to my research work.

iii. Adipose-derived SC: Adipose tissue is now increasingly recognized as a reliable and easily accessible source of stem and progenitor cells. Investigators have managed to isolate from this reservoir numerous cell types: hematopoietic stem cells, mesenchymal-like stem cells as well as cells presenting the side-population phenotype. Functional improvement of cardiac function has

been reported with stem cells derived from this particular niche. As with the BM, isolating which cellular fraction of this promising new cell reservoir holds the most potential for myocardial repair remains a challenge for the scientific community.

2.2.3 Adult Endogenous Cardiac SC: Several groundbreaking reports (Quaini, Urbanek et al. 2002; Nadal-Ginard, Kajstura et al. 2003) have paved the way to the discovery of primitive, multipotent resident cardiac stem populations. Cycling cardiomyocytes have been documented in both the normal and pathologic adult heart. Currently, four resident cardiac stem cell populations have been clearly identified (Anversa, Kajstura et al. 2006; Wang and Sjoquist 2006). They can be distinguished either by their surface markers (such as c-kit(Beltrami, Urbanek et al. 2001; Beltrami, Barlucchi et al. 2003) and Sca-1(Oh, Bradfute et al. 2003; Matsuura, Nagai et al. 2004), the presence of the ABCG2 transport protein(Martin, Meeson et al. 2004) and the capacity for Hoechst dye efflux(Hierlihy, Seale et al. 2002; Mouquet, Pfister et al. 2005; Pfister, Mouquet et al. 2005). Although an ideal donor source of cells for cardiac regeneration, extensive, prolonged ex vivo expansion process, the unexplored issue of cross-talk between these resident cells and the highly invasive nature of cardiac tissue-cell harvesting represent current limitations to their clinical use.

2.2.4 Induced Pluripotent Stem cells

A novel alternative is the production of iPS (induced Pluripotent Stem) cells. Recently, Takahashi et al. demonstrated that four key transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) could be used to reprogram adult somatic skin-derived fibroblasts into functionally cells with embryonic-like properties.(Takahashi, Tanabe et al. 2007) The iPS cells have opened the doors to vast new possibilities in therapeutic tissue (cardiac and non-cardiac) regeneration from a translation perspective: a patient's own dermal fibroblasts could be reprogrammed into tissue-specific cells after ex vivo expansion, which can then serve as an autologous source of

therapeutic cells.(Nakagawa, Koyanagi et al. 2008) Initially, generation of iPS cells required retroviral infection of somatic cells with retrovirus encoding for 3 or 4 genes (Oct3/4, Sox2, and Klf4, with or without c-Myc) (Takahashi and Yamanaka 2006; Nakagawa, Koyanagi et al. 2007; Takahashi, Tanabe et al. 2007; Nakagawa, Koyanagi et al. 2008). The possibility of random integration of these genes in the cell genome with retrovirus has raised safety concerns. However, recent reports on the use non-integrative adenoviral vector or transfection with plasmid vectors to introduce the latter key "reprogramming" genes have alleviated the risk and concern regarding potential insertional mutagenesis (Okita, Nakagawa et al. 2008; Stadtfeld, Nagaya et al. 2008).

2.3 Clinical applicability of cardiac cell therapy:

Over the last five years, clinical trials based on the early, dramatic animal studies have rapidly exploited the underlying assumption that repopulation of scarred myocardium is feasible by exogenously supplied cellular surrogates of cardiomyocytes. This recent interest of the scientific community for organ regeneration is not without merit as these trials have shown both structural and functional improvements following SC transplantation. Of the various cell types, skeletal myoblasts(Hagege, Marolleau et al. 2006; Menasche 2006) and BM-derived cells(Assmus, Honold et al. 2006; Lunde, Solheim et al. 2006; Schachinger, Erbs et al. 2006) have been the most extensively investigated. The rapid transition of the cell-based approach to clinical application such as cardiac repair has been fueled in part by the ease of collection and the scalability of these two cell populations. The bulk of the evidence from clinical trials to date has documented only safety and feasibility in two patient populations: those with recent AMI or post-infarction CHF. However, small patient numbers, use of surrogate endpoints and negative long-term data have limited the ability to draw any firm conclusions as to the clinical efficacy of this modality in IHD (Wollert, Meyer et al. 2004; Janssens, Dubois et al. 2006; Oettgen, Boyle et al. 2006). Certain unresolved issues still need to be addressed before cell-based therapy can move

towards more widespread clinical use (Ott, McCue et al. 2005; Gersh and Simari 2006; Rosenzweig 2006). In light of the main focus of my research, an exhaustive review of the clinical cell-based trials published to date is beyond the scope of this writing.

2.4 Current controversies and limitations regarding the cell-based approach to cardiac regeneration and repair

Ongoing debate regarding the role of cell-based therapy in ischemic heart disease has highlighted current controversies and limitations (Stamper and Woodruff 1976; Chien 2004; Caplice, Gersh et al. 2005; Wollert and Drexler 2005; Chien 2006):

i. The most appropriate cell type remains to be defined and, most likely, may vary according to the time from injury as well as the type of myocardial injury.

ii. The survival rate as well as the myocardial retention rate of delivered cells may be low regardless of cell type with possible explanations ranging from cell-dependent (differentiation level, resistance to ischemia, apoptosis) to cell-independent factors (timing of cell therapy, delivery method, interaction with other treatment methods).

iii. There is no clear certainty about which mechanisms underlying the positive clinical benefits of cell-based therapy play a pivotal role in cardiac regeneration/repair: de novo cardiomyocyte formation, pro-angiogenic effect, paracrine effects on surrounding cardiomyocytes, passive grafting with altered remodeling, recruiting and/or activation of resident cardiac stem-progenitor cells.

iv. Conflicting findings on the functional benefits of transferred cells have only highlighted the lack of methodological uniformity in the field.

Despite unresolved issues from a mechanistic point of view, the pressing and largely unmet clinical need has prompted a rapid transition from basic research to clinical trials. For these

reasons, there is now a growing consensus that a greater understanding of basic mechanisms involved in myocardial repair is required to push this promising field forward and exploit its full potential(Rosenzweig 2006).

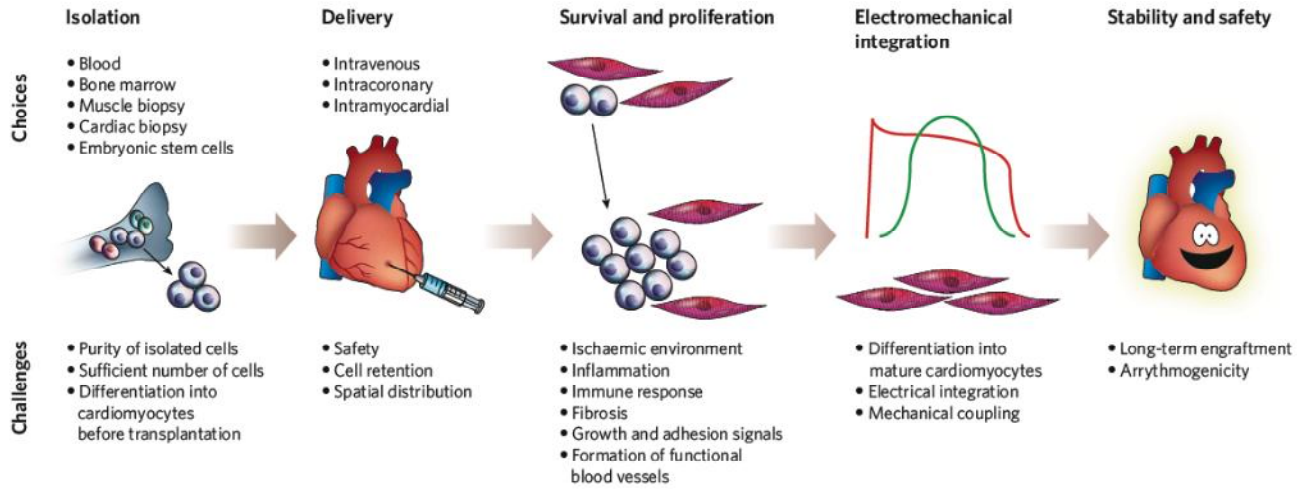


Figure: 1 Cardiac Cell Therapy**:** A general process of stem cell therapy, from isolation and selection of stem cells to the final realization of therapeutic goal. Adapted from Serger etal, (Segers VF 2008).

Chapter 3 Mesenchymal Multipotential Stromal/Stem Cells

MSCs are a rare population of cells residing in the BM with putative cells isolated from embryonic, fetal and postnatal organs(Boyle, Schulman et al. 2006). However, the BM constitutes the main source of MSCs in the adult. These adherent fibroblast-like cells are isolated from the mononuclear cells from the BM. No definitive consensus has been reached on the exact definition of MSCs. While positive for various adhesion proteins, these cells are CD45, CD34 and CD133 negative; CD29, CD44, CD105 and Sca-1(in mice) are widely accepted as surface markers for MSCs. It has been argued that MSCs should not be defined strictly based on surface antigen expression but also by functional properties such as multipotent growth and differentiation behavior. During expansion, MSC have been known to respond to growth factors and cytokines: most notably, addition of 5-aza-cytidine has been well documented to favor cardiomyocyte phenotypic differentiation (Makino, Fukuda et al. 1999; Pittenger and Martin 2004).

MSCs are considered important candidates for regenerative/reparative therapy as they are readily obtained from the BM, can home to and repair injured tissues, are known to have immunomodulatory properties (therefore opening the possibility to allogenic transplantation) and have good survival following transplantation(Zimmet and Hare 2005).

The term MSC is widely used, but still ill-defined in a field with rapid growth and expansion during the last decade (Bianco P 2008). According to the International Society for Cell Therapy (ISCT), there are suggested minimal criteria for the using of the term Mesenchymal Stem Cell, (**figure 2)**, including fibroblast-like morphology, adherence to plastic upon isolation and expression of certain surface markers and differentiation capacity (Dominici M 2006). Instead of being a specified progenitor cells like EPC or Heamtopetic Stem Cells, MSC is more of a multipotent Stem Cells. The most usual examination includes the classic trilineage test, adipo,

oesto and condro. And MSC has been isolated from different parts of the body, bone marrow, fat tissue, blood, placenta and almost every postnatal tissue (da Silva Meirelles L 2006). Thus MSC is more likely to be called the residing stem cells with multipotency. The simplicity of isolation, excellent multipotency and immuno tolerance distinguish MSC from all potential stem cell therapy candidates.

1	Adherence to plastic in standard culture conditions		
2	Phenotype	Positive ($\geq 95\%+$)	Negative ($\leq 2\%+$)
		CD105	CD45
		CD73	CD34
		CD90	CD14 or CD11b
			CD79α or CD19
			HLA-DR
3	*In vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)		

Figure: 2 Minimal Criteria for Mesenchymal Stem Cells: Suggested minimal criteria for the definition of Mesenchymal Stem Cell(MSC), adapted from Dominici etal (Dominici M 2006).

3.1 Sources of MSC

Even though MSC is almost omnipresent in the body, commonly used sources are bone marrow (BM), umbilical cord blood (UCB) and adipose tissue (AT), although MSC is also present in other sources. They are similar in morphology, surface markers, multipotency and other characteristics. Based on the standards of the minimal criteria of the establishment of MSC, comparison of the three main sources is focused on morphology, surface marker, and differentiation capacity. Kern et al carried out an investigation on MSC from the three sources on human; they found that morphologically speaking the cells are of no differences; MSCs from different sources are also uniform in immuno phenotype (Kern S 2006). Upon the confirmation of same morphology and immuno phenotype (22 surface markers were checked), Wagner et al confirmed that they are also same in differentiation capacity, and share 25 overlapping gene

expression traits (Wagner W 2005). Another checkpoint for MSC from different sources is their immunosuppressant capacity. MSCs from either BM or AT or UCB exert immunomodulatory capacity (Le Blanc K 2004; Yañez R 2006; Tipnis S 2010).

And of course, there are differences. The success rate of isolating MSC is near 100% for BM and AT, but the chance drops to 63% with UCB. While BM-MSC is a very rare faction of the BM nucleated cells (around 0.001-0.01%), MSCs are 500 times more abundant in AT (Strioga M 2012). Out of the three, BM-MSC has shortest culture period and lowest proliferation rate, while UCB-MSC has longest culture period and highest proliferation rate. The differentiation capacity also varies, AT-MSC posses higher frequency of oil-positive cells under adipogenic differentiation, while BM-MSC performs better for calcium deposition under osteogenic differentiation (Sakaguchi Y 2005).

In fact, MSC can be procured from almost all tissues. Periosteum (Ringe J 2008), Placenta (Fukuchi Y 2004), Skin (Belicchi M 2004), Synovium (De Bari C 2001; Sakaguchi Y 2005) etc.. are all identified as reliable sources for MSC. Still, more sources are being explored for potential high profile MSCs. A comparison among BM, Synovium, Periosteum, AT and muscle derived MSCs has shown that Synovium-MSC is superior in isolation yield, proliferation profile and chondrogenesis (Yoshimura H 2007), corresponding to the fact that AT-MSC is best for Adipogenesis (Sakaguchi Y 2005). Different sources of isolation may be useful facing different clinical demands. Synovium-MSC is suitable for cartilage repair, while AT-MSC is better for adipose regeneration, which can be used for breast enlargement (Yoshimura K 2008).

3.2 Multipotency of MSC

Compared to other progenitor cells, like EPCs which are lineage-driven towards endothelial cells, MSCs are considered to have multipotency and not to be lineage-driven toward one single cell population. The classic differentiation of MSC, however, is only the tip of the iceberg since,

under proper conditions; MSCs can be coaxed to generate various cell lines. The multi-organ niches of MSCs constitute a likely underlying explanation for this characteristic multipotency. Liechty et al. transplanted human MSC into fetal sheep, which differentiated into chondrocytes, adipocytes, myocytes, cardiomyocytes and thymic stoma in a site-specific manner. This indicates that MSCs are multipotent, and seem to have adaptive differentiation response depending on microenvironmental cues (Liechty KW 2000). Gang et al. incubated umbilical cord blood-MSCs with promyogenic conditions for 6 weeks. Half of the cell population expressed myosin heavy chain, a late marker of myogenic differentiation (Gang EJ 2004); Long etal. cultured BM-MSC in neurogeinc medium for 6 days, 66% of the cells adopted a dendritic morphology and a portion of cells express a wide range of neural markers including Nestin and β-tubulin, indicating mature neuron-phenotype (Long X 2005); Chen et al successfully induced insulin secretion from rat MSCs after generating differentiated cells with a pancreatic phenotype (Lee KD 2004); By a two-step induction protocol using hepatocyte growth factor, Lee etal. acquired cells with hepatocyte function including albumin production from human MSC (Chen LB 2004). For cardiac cell therapy, transdifferentiations of MSCs are of great value. Silva et al. confirmed in a canine chronic ischemia model that MSCs can differentiate into smooth muscle cells and endothelial cells, leading to improved vascularity (Silva GV 2005); In addition, MSC have been induced to differentiate into cardiomyocyte (Liechty KW 2000; Wang T 2003; Kawada H 2004). Thus, MSC hold many advantages for therapeutic use and for regeneration medicine as they can be adapted to many tissue damage settings.

3.3 Paracrine effect of MSCs

As mentioned above, MSCs excrete proteins that influence other cells in their vicinity. MSC secrets a wide range of soluble factors, including PGE-2, VEGF, HGF and SDF-1, which have affect the immune system, apoptosis, angiogenesis, chemoattraction, scarring , growth and differentiation of other progenitor cells (Meirelles Lda S 2009) (**figure 4**). Evidences have shown, the restoration of injured heart by AKT overexpressing MSCs is related to the paracrine effect of MSC. Genecchi et al. found that this restoration is not associated with cardiac differentiation of implanted MSC, but paracrine effect. The injection of AKT-MSC preconditioned with hypoxia also improved cardiac repair in the rat model of myocardium infarction, again indicating that the paracrine secretion of cardioprotection substances by MSCs play a major role in the repair of the injured heart(Gnecchi M 2005). Another study done by Mirutsou et al. revealed the secreted frizzled related protein 2 (sfrp-2) plays an import role in this beneficial paracrine effect on cardiomyocytes by AKT-MSC (Mirotsou M 2007). Beside this seminal study, there are also other interesting facts. MSC conditioned medium is able to activate Cardiac Progenitor Cells (Nakanishi C 2008), a possible reason for the improvement of cardiac function after MSC transplantation. Another interesting fact is that MSC conditioned culture medium downregulates cardiac fibroblast proliferation and collagen synthesis, which can be translated into potential anti-fibrosis effect (Ohnishi S 2007). Tang et al. found that implantation of MSC decreases the expression of proapoptotic protein Bax in the ischemic myocardium (Tang YL 2005). In an experiment done by Uemera et al, culture medium of hypoxia preconditioned BM-MSC significantly reduced the infarct area in a mice model (Uemura R 2006). Li etal demonstrated that the overexpression of GATA4 in MSC significantly increased the secretion of VEGF and IGF, which results in cardiac protection effect (Li H 2010). The diversity and therapeutic potential of the paracrine effect of MSC might provide an answer to the complexity of related to IHD.

3.4 Immunomodulatory property of MSC

The immune response is a major concern in the successful delivery of therapeutic cells in the obstructed heart. MSCs have the unique capacity to induce immunotolerance in their hosts, even in the setting of a xeno-transplantation (Liechty KW 2000). The mechanisms underlying this phenomenon are multifold, as shown in (**figure 5**). Firstly, MSC is of low immunogenicity. Both in human and animal, MSCs express the major histocompatability complex (MHC-1) but not MHC-2 (Barry FP 2005; Ryan JM 2005), which is known to induce tolerance: for example, positivity of MHC I can help avoid NK cells mediation immune response (Ruggeri L 2001). Secondly, MSCs also regulate the immune responses by direct interaction with immune cells or indirectly via a paracrine effect. Chen etal. showed that human UCB-MSCs exerts their immunosuppressive effect by producing Postaglandin E_2, which regulates the production of inflammatory cytokines like IFN-γ by peripheral blood mononuclear cells (Chen K 2010). While IFN-γ elicits the expression of MHC II, the presence of the latter cytokine enhances the immunosuppression of MSC. IFN-γ induces the production by MSCs of Nitrite Oxide (NO) and several other chemokines by MSC. These secreted chemokines attract T-lymphocytes, which are then suppressed by NO (Ren G 2008). MSC also regulates immune reactions by expressing Indoleamine 2,3 dioxygenase (IDO) Which has been found to play a key role in the immune tolerance in murine kidney allograft model (Ge W 2010). These three examples are just part of the many mechanisms displayed figure 4. Full comprehension of MSC's immunomodulatory properties remains incomplete as many of the latter remain under active investigation. Nevertheless, this unique immunomodulation property of MSCs opens the door for the cells in clinic

The immunomodulation of the MSC does not only support MSC as an excellent choice for regenerative cell therapy, but also expand their application for other usages. MSCs are best studied in the GVHD, a Graft Versus Host Disease is one of the fields, that MSC is rigorously

tested. In the case of Hematopoietic Stem Cells Transplantation (HSCT), a routine cure for leukemia, GVHD is a common and serious implication than can lead to death. As reported by Fang et al, the infusion of HLA-mismatch and unrelated AT-MSC successfully treated two children with severe therapy-resistant GVHD after HSCT (Fang B 2007). In various clinical trials, MSCs have been shown successful. And Osiris Therapeutics, a company endeavoring on MSC therapy, has completed phase 2 clinical trials for an off-the-shelf MSC product PROCHYMAL® targeting GVHD.

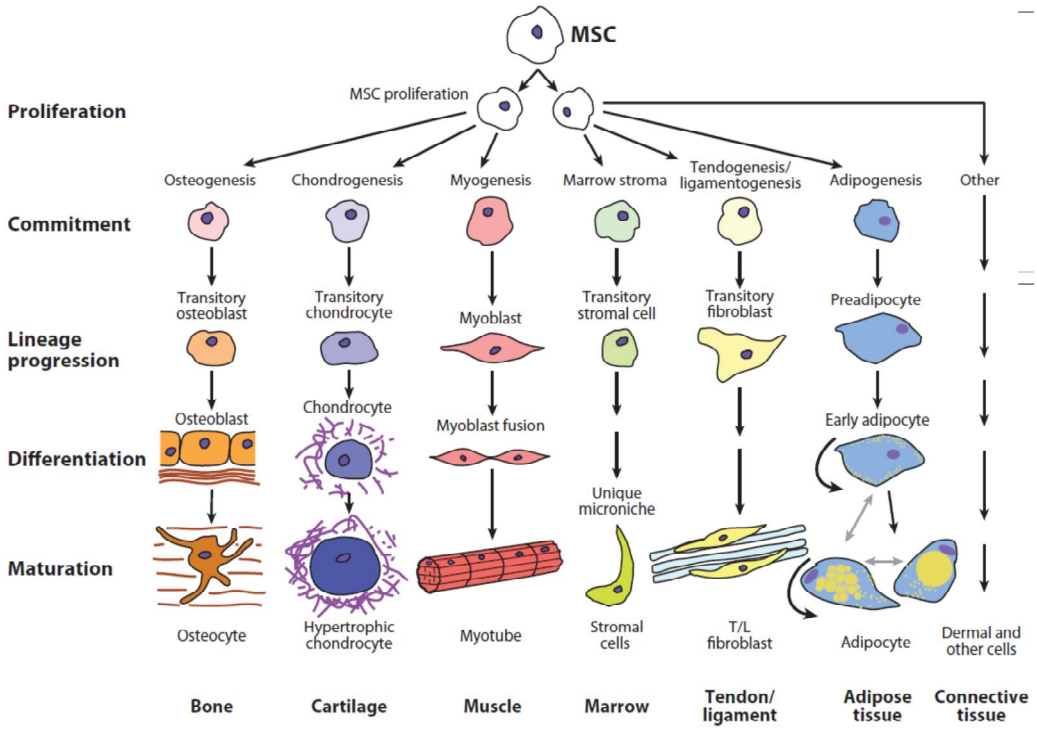


Figure: 3: Multipotency chart of Mesenchymal Stem Cell: MSC give rises to a variety of cell lines.

Photo adapted from Singer etal (Singer NG 2011).

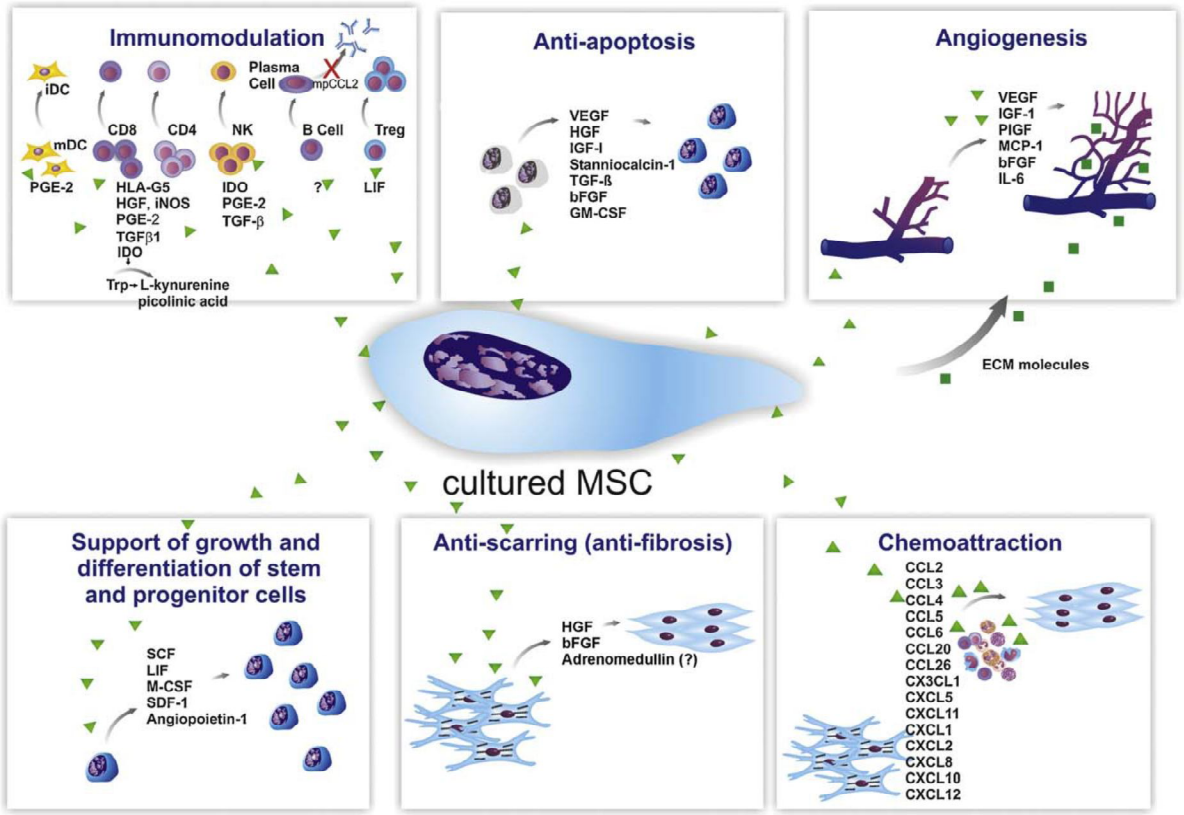


Figure: 4 Paracrine effect of Mesenchymal Stem Cell, Mesenchymal Stem Cell secrets wide range of soluble factors that regulate a number of beneficial physical functions. Photo adapted from Meirelles et al (Meirelles Lda S 2009).

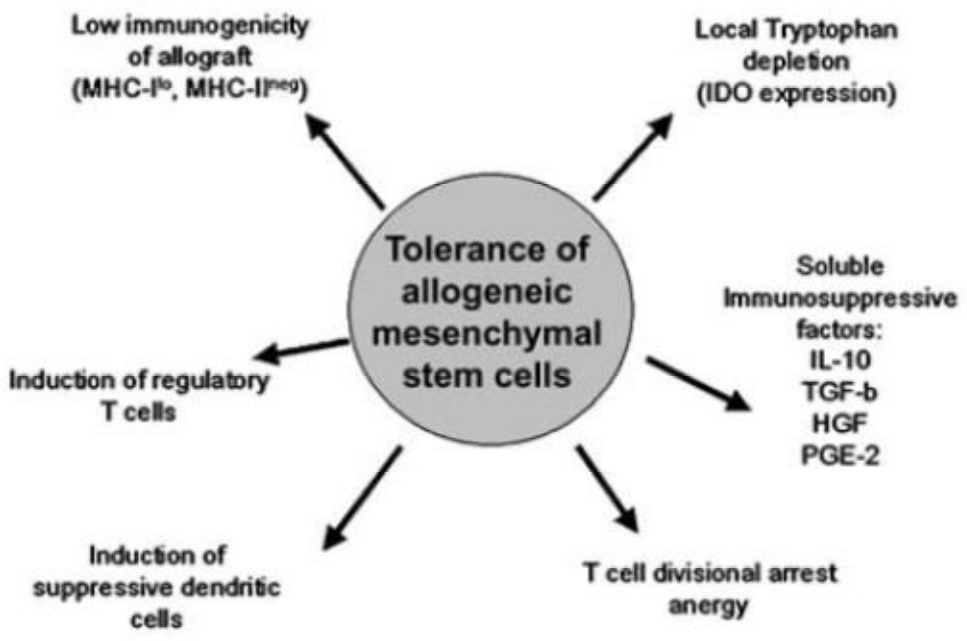


Figure: 5 Immunomodulatory Mechanism of Mesenchymal Stem Cells: A schematic summarization of the immunomodulatory mechanisms of Mesenchymal Stem Cells. Photo adapted from Barry etal (Barry FP 2005).

3.5 In Vivo Distribution and Tracking

Much like any pharmacological therapies, once delivered in a living system, stem-progenitor cells will face problems related to biodistribution. The ideal scenario is that delivered cells would remain in the target tissue or organ. However, the distribution of MSCs after injection (regardless of delivery strategy) is systemic. After IV injection, delivered MSCs can be detected in BM, spleen, lungs, kidneys and heart with quite low engraftment rates (Allers C 2004; Bentzon JF 2005). Frey et al. did a quantitative comparison of three delivery strategies of MSC therapy, transvenous, intracoronary and endocardial, in a porcine model of myocardial infarction. At day 14 after cell injection, 6% and 3% of initial cells could be detected in the target myocardium after intracoronary and endocardial delivery, respectively; no cell could be detected in the transvenous group. The major organs with high cell retention rates were mostly the lungs but also the liver regardless of delivery strategy (Freyman T 2006). The off-target engraftment could undermine the therapeutic potential of these cells and increase their possible deleterious effects by introducing MSCs to unaimed organs.

Thus, better tools are recommended to understand the biodirstribution of cells used for therapy. Various imaging techniques have been developed to allow in vivo cell tracking. Current strategies revolve around the simple concept of cell labeling with a given agent that could be detected with in vivo imaging modalities. Here, three major categories of labeling will be reviewed: Quantum Dots (QD). Radioactive Labeling Agent (RLA), and Magnetic Labeling Agent (MLA).

3.5.1 Quantum Dots

Biofluorescence and bioluminescence represent very interesting tools for imaging in medicine. A potential attractive technology is near-infrared imaging. Fluorescence with longer waves (>700 nm) are less bothered with artefact related to inherent fluorescence of tissues and/scar, and

might therefore provide a possible way for in vivo tracking. Indeed this has been done in small and large animal model mostly in cancer detection (Blum G 2007). However, clinical applicability is limited as it lacks tissue penetration and therefore cannot be detected with normal measure tools. However, near-infrared imaging remains a powerful tool for research and usage in sites closer to the body surface.

Quantum Dots (QDs) refer to semiconductors with special electronic characters related to the crystal size and shape, also known as fluorescent semiconductor crystals. Compared to organic fluorophores, QDs are brighter, more stable in fluorescence and allow for multicolor imaging (Jaiswal JK 2004). A typical QD in use consists of an inorganic fluorophore as the core (such as CdSe), and coating layers (usually ZnSe and other molecules) that allows QDs to label the cells. Interestingly, QDs' emission length is determined by its size, and a wide spectrum is provided by different QDs ranging from UV to Infrared, thus detectable with optical imaging systems (Medintz IL 2005). By conjugating QD with cancer cell specific antibodies, Gao et al successfully labeled prostate cancer cells and obtained sensitive, multicolor in vivo fluorescence imaging(Gao X 2004) .

For cell therapy, QDs are able to enter the MSC efficiently and display a emission of fluorescence consistent with the distribution traits of MSC location in vivo (Lei Y 2008). Rosen et al reconstructed 3D locations image of MSC labeled with QDs 8 weeks after injection, which indicates excellent applicability of QD as an in vivo cell tracking imaging modality (Rosen AB 2007). However, there are also some pitfalls for QDs. For example, the QDs are vulnerable to autophagy, which transfers the fluorescence to non-target cells (Seleverstov O 2006). Another concern is the size of QD probe, usually a conjugated QD is the size of 500-750KDa which could affect cell physiology and function(Jaiswal JK 2004). Nevertheless, QD be a powerful non-invasive tracking method for cell therapy.

3.5.2 Radioactive Labeling Agent

RLAs mediated in vivo tracking enables several high resolution imaging techniques to follow biological fate of the injected cells, including Combined Single-photo Emission CT (SPECT) and X-ray CT (SPECT/CT). In this setting, commercially available labeling agent such as ^{111}In oxine have already been used to label a variety of cells (Kraitchman DL 2005). By labeling MSC with 30 Bq ^{111}In oxine, Bindslev et al were able to label and track injected cells without altering the proliferation and the differentiation of MSCs (Bindslev L 2006). A clinical study done in patients with advanced cirrhosis showed that ^{111}In oxine labeled MSCs (0.21-0.67MBq/10^6) could be successfully tracked and quantified (Gholamrezanezhad A 2011). Radioactive labeling also enables 3D imaging by SPECT/CT imaging (Gildehaus FJ 2011). Besides ^{111}In oxine, there are also other agents. One of them is [18F]-fluoro-deoxy-glucose (18FDG), which has been used successfully and accurately to measure and quantify the distribution of cardiac derived stem cells in vivo in a rat model by PET imaging (Terrovitis J 2009).

Though RLAs are efficient in labeling and visualizing the MSCs in vitro, they posses serious defects. The major problem is the radioactivity itself. While the dosages used are hardly harmful to the patients, it may be detrimental to the cells labeled, which can lead to the failing of the therapy. Moreover, the negative effects of the radioactivity on the patients cannot be fully neglected, thus the potential of using RLAs in the clinic is highly mitigated.

3.5.3 Magnetic Labeling Agent

MLAs are normally iron oxides or Surpermagnetic Iron Oxide (SPIO), which are detectable by Magnetic Resonance Imaging (MRI). The labeling of MSC with SPIOs enables sense in vivo high-resolution imaging. The resolution achieved by MRI of SPIO labeling is adequate enough so that as few as 1,000-labeled cells were detectable even at one month after the implantation (Loebinger MR 2009). The labeling of cells by SPIOs is related to a phagocytic effect as the probe transits inside the cell (Rogers WJ 2006). One advantage of SPIO is that the clinically

available MR scanners can be used to detect them. MSCs labeled with SPIOs show unmitigated differentiation, proliferation ability and viability. Using a porcine myocardium infarction model followed by MSC injection, Kraithchman et al demonstrated that the location of SPIO-labeled MSCs detected by MRI correlates with histological examination. Moreover, MSC labeling allows serial tracking of the injected cells over time (Kraitchman DL 2003). To facilitate clinical usage, Frank et al. reported a combination of commercially available non-viral transfection agents (TA) and SPIOs. The former facilitates the uptake of SPIOs by cells, thus increase the MRI signal by 40 folds, which means less SPIOs can be used for similar outcome (Frank JA 2003). The size of SPIO may also play a role in the labeling process as choosing the optimal SPIOs: cell ratio can contribute to improved labeling efficiency (Lee ES 2009). SPIOs have also been shown to be able to detect low number of cells in conjunction with detailed topographic analysis with documented clinical safety (de Vries IJ 2005). SPIOs may also have additional advantages other than use as a labeling agent. Due to its supermagnetic nature to drag and retain labelled cells in target organs by adding an external magnetic field (Arbab AS 2004). However, SPIOs also have limitations. The study by Amsalem et al described that 4 weeks after the implantation of SPIO-labeled MSC into the infarcted rat heart, the MRI signal did not come from the scar zone but that cardiac macrophages that engulfed the SPIOs. The marker therefore does not mean a true labelling of the labelled cells. Though the implantation still resulted in improved LVEF, it may indicate that SPIOs are not suitable for long term labeling, with "false positive" following phagocytisis of released probes after transplanted cell death (Amsalem Y 2007). Nevertheless, due to their high resolution, and low toxicity profile, SPIOs represent an excellent means to provide non-invasive in vivo tracking. Thus, there is much therapeutic potential for ferromagnetic probes as tools in cardiac cell therapy.

Chapter 4 Optimizing Strategies for MSC Therapy

Even though MSCs represent promising candidate for cardiac cell therapy, certain challenges must be addressed in order to achieve proper clinical translation and efficacy. The pathological process ischemic heart failure post-AMI is chronic and slowly evolving in certain patients. However, injected MSCs are rarely found engrafted after delivery (Zhang M 2001)due to the hostile post-infarction microenvironment, such as hypoxia, lack of serum, inflammatory secretion and increased oxidative stress (Byun CH 2005; Zhu W 2006; Brandl A 2011). In order to maximize the cardioprotective effects of MSCs, enhanced cell survival is necessary. To overcome these challenges, potential strategies are investigated. I will briefly discuss three key options: gene therapy, preconditioning and nanotechnology.

4.1 Gene transfer for MSCs

Genetically modified MSC can potentially improve cell survival and decrease cell apoptosis. Various genes have already been tested for their therapeutic potential in modifying MSCs to be more adaptable to a hostile environment while maintaining their phenotype. Akt was one of the earliest tested genes: In a murine model of cardiac ischemic injury, the overexpression of Akt reduced MSC apoptosis after transplantation, leading to a acceptable post-infarction remodeling process (Mangi AA 2003).Li et al. modified MSC to overexpress Bcl-2, an essential anti-apoptotic gene. Tested under in vitro conditions of hypoxia, the Bcl-2 gene protected MSC from of apoptosis and improved the secretion of vascular endothelial growth factor(Li W 2007). Overexpression of the Heat Shock Protein-20 (HSP-20) on MSCs also resulted in enhanced resistance to oxidative stress and improved the post-transplantation survival. Beneficial paracrine effect was also improved with elevated levels of VEGF, FGF-2 and IGF-1 following HSP-20 overexpression(Wang X 2009). Tsubokawa et al. reported another promising gene tool, Heme oxygenase-1 (HO-1), an anti-oxidant and anti-inflammatory protein. The HO-1 overexpressing MSCs are more resistant to cell apoptosis and cell death; also the VEGF

secretion was detected, which leads to larger capillary density and decreased infarction size 28 days after transplantation (Tsubokawa T 2010). MSCs gene-modified to overexpress CXCR4 (a key chemoreceptor involved cell migration and engraftment) or transglutaminase (a fibronectin receptor involved in cell adherence) were associated with improved cardiac repair in a model of coronary occlusion and reperfusion in rat (Song, Chang et al. 2007; Cheng, Ou et al. 2008) All these results point out that the strategies aiming at rescuing the MSCs from apoptosis and cell death are promising venues for cardiac cell therapy. However, genetically modificated cells are not ready for clinical use because of concerns of insertional mutagenesis associated with the viral vector. Finally, genetic engineering or gene transfer remains to a certain extent limited, as ethical and scientific questions regarding cell phenotype modulation remain unanswered.

4.2 Preconditioning

Compared to gene modification, cell preconditioning represents an attractive avenue. As discussed in the previous chapter, the paracrine effect plays a central role in MSC cell physiology. The pivotal idea underlying preconditioning is to prepare cells by mimicking the hostile microenvironment in which the cells will be engrafted by either a pre-exposure to cytokines, growth factors or other chemical agents. MSCs preconditioned could improve therapeutic effect either by improving their survival or by potentiating their paracrine properties.

Hypoxic precondition (HPC) is a simple and effective strategy. By exposing MSCs to hypoxia, the cells can be programmed to a state of unregulated paracrine secretion, which can be resulted into a better post-transplantations survival, and to improved therapeutic outcomes. The intrinsic reason why HPC improves the prospects of MSC is that it mimics the low oxygen concentration(1%) naturally seen by MSCs in their BM niche(Chow DC 2001). Chacko et al. have shown that hypoxic precondition induces the expression of proangiogenic and prosurvival genes, such as SDF-1a, VEGF, HIF-1a and AKT (Chacko SM 2010). The preconditioned cells were also rendered more resistant to different hostile microenvriroments. Leroux et al. used

HPC MSCs (0.5% Oxygen) in a skeletal muscle injury. Comparing to normoxia MSCs, HPC MSCs implantation shows improved vascular formation and enhanced muscle regeneration (Leroux L 2010). In a rat cerebral ischemia model, the implantation of HPC MSC (0.5% 0xygen) resulted in better angiogenesis, neurogenesis and recovery of locomotion activity (Wei L 2012). The HPC MSCs have an enhanced migration capacity. In a murine hind-limb ischemia model, HPC MSCs were able to achieve greater angiogenesis compared to unconditioned MSCs (Rosová I 2008). To test the effect of HPC MSC on IHD, Hu et al. preconditioned mice MSC with 0.5% Oxygen and transplanted into infarcted myocardium tissue. The preconditioned MSCs increased their expression of proangiogenic genes, including HIF-1a, angiooprotein-1 and VEGF. Not only it decreased cell apoptosis but also increased the angiogenesis and improved the function of the heart(Hu X 2008).

Some chemical agents have also been tested for additional benefits on MSC therapy. Diazoxide has been found to prevent appotosis of MSC by targeting the Fas protein (Suzuki Y 2010). Trimetazidine preconditioning protected against hydrogen peroxide (which induces oxidative stress), raised the expression of HIF-1a, survivin and other factors. Rats treated with Trimetazidine preconditioned BM-MSCs have a significantly better recovery of myocardium infarction(Wisel S 2009). Another reported agent is lipopolysaccharide, which enhances the engraftment of transplanted cells and promotes proangiogenetic secretion. This resulted in a superior therapeutic neovascularization of infarcted cardiac tissue (Yao Y 2009). Another agent, α-lipolic acid, addressed another important pathological process occurring in post-infarction microenvironment as it inhibits potential inflammation reaction resulting in MSC apoptosis (Byun CH 2005). In summary, the pharmacologic precondition shows promising therapeutic potential as adjunctive therapy to cardiac cell delivery.

Besides pharmacological agents, growth factors can also be used to precondition MSCs. gr Growth factor preconditioning can improve MSC therapy in two ways: firstly, by improving the ex

vivo expansions of MSC cultures; and secondly, by improving the post-implantation survival and function (Rodrigues M 2010). I will briefly discuss some key growth factors that have been studied as preconditioning agents prior to MSC transplantation.

SDF-1α: In a mice model of hindlimb ischemia, SDF-1α was shown to induce vasculogenesis and angiogenesis; In a rat model of myocardium infarction, SDF-1α brought increased cell viability and proliferation in the infarcted myocardium(Hiasa K 2004; Pasha Z 2008).

IGF-1: in a rat model of myocardial infarction, Guo et al. found the IGF-1 precondition upregulates the expression of CXCR-4 in vitro, which results in increased cell engraftment and survival, thus improving the efficacy of MSC (Guo J 2008).

TGF-a: TGF-a increases VEGF production by MSC in vitro, even in the presence of TNF-a or hydrogen peroxide, which indicates a strong proangiogenetic effect. Upon implantation, TGF-a preconditioned MSCs downregulated the expression of inflammatory and apoptotic factors like IL-1β, TNF-a, which resulted in pro-survival effect (Herrmann JL 2010).

PDGF-BB: This is strong stimulator of proliferation and migration for MSC after wound-scratching in vitro. The blockage of its receptor leads to hindered bone repair in vivo in a rat model (Chung R 2009). For cardiac repair, PDGF-BB precondition reduces the loss of cells on site after implantation, which indicates a potential improved therapeutic potential(Krausgrill B 2009).

TSG-6: It is has been shown to reduce the inflammatory response and the infarction size after systemic coadministration with human MSC. There is great potential that TSG-6 also serves as an excellent preconditioning agent for MSC therapy (Lee RH 2009).

4.3 Use of Nanoparticles

There is a clear need to find clinically applicable delivery strategies that are both safe and

efficient. Moreover, in order to pave the way for the future, "naked SC" delivery, while efficacious in and of itself to a certain extent, is not sufficient. Many strategies have been explored to improve targeting of stem cells. For example, cardiac tissue engineering offers scientifically appealing solutions to enhance engraftment: from acellular collagen matrices to improve cell recruitment, soluble 3-D scaffolds with cells embedded within them to decellularized organs that can be reperfused and repopulated with cells as artificial organs.(Zimmermann, Melnychenko et al. 2006; Suuronen, Zhang et al. 2009; Taylor 2009) Nevertheless, most of the current proposed solutions (for example, cardiac patches and bioartificial constructs) still require surgical intervention, which likely limits the type and number of eligible patients.

Nanotechnologies have considerable potential for biomedical application. Currently, critical issues to be resolved are their stability and biocompatibility in circulatory system, and surface functionalizations that conjugate the targeting spacers or therapeutic agents.(Xu, Hou et al. 2007; Fang, Bhattarai et al. 2009) Core/shell structures have been proposed in an effort to address the stability and biocompatibility problems.(Gupta and Gupta 2005; Zhang 2007; Stamopoulos, Manios et al. 2008) Among all the potential candidates, silica-based shells are more superior, due to their low cost, relatively simple synthesis, low toxicity and their potential to create nanoporous shells offering higher surface areas needed it employed as drug carriers or for magnetic concentration. Attaching functional groups onto silica shells remains a critical issue that can allow nanoparticles to function as linkers for a large variety of biomolecules and drugs. This is usually done by either of two main strategies, post-functionalization or co-condensation. For examples, Fernandes-Pacheco et al. reported a simple arc-discharge method for producing silica-coated magnetic NPs, which were post-functionalized with primary amine or carboxyl groups and covalently coupled to antibodies.(Fernandez-Pacheco 2006) Schoenfisch et al. recently synthesized secondary amine-functionalized silica NPs by co-condensation with tetraethoxy- or tetramethoxysilane, which can be used as carriers for the storage and release of

nitric oxide (NO).(Shin, Metzger et al. 2007; Hetrick, Shin et al. 2008; Hetrick, Shin et al. 2009) However, it still remains a challenge to find a simple method to integrate the superparamagnetic cores into a nanoporous silica shell, which is simultaneously functionalized, thus offering both magnetically targeting possibilities as well as the surface functionalization for coupling with targeting key proteins such as antibodies.

Nevertheless, nanoscale inert biocompatible materials can be functionalized to either impart or modulate precise biological functions. Surface modifications and coating of nanomaterials could modulate their toxicity, immunogenicity and pharmacokinetic properties as well as impart efficient targeting. Several key nanoscale biomaterials have been reported to show promise for diagnostic or therapeutic use (quantum dots, biopolymers and magnetofluorescent particles). In recent years, magnetic nanoparticles (NPs) have been mainly investigated for their potential applications in fields such as cell delivery, magnetic separation magnetic resonance imaging and targeted drug delivery. Thus, nanotechnologies will likely help extend the therapeutic reach of established or novel, promising cardiovascular therapies such as cardiac cell therapy.

VII RESEARCH PROJECT

Optimization studies to improve MSC-based Cardiac Cell Therapy：

In order to overcome the limitations related to MSC delivery into an ischemic environment (such as cell death and poor cell tracking), my research will focus on in vitro studies to optimize MSC therapy for cardiac cell therapy.

Research Objectives:

1. To investigate the effect of cytokine preconditioning to improve MSC cell survival, under conditions mimicking the cardiac ischemic environment.
2. Investigate the biocompatibility a novel nanoparticle functionalized to specifically couple with MSC.

Research Hypotheses:

1. MSC preconditioning with key cytokines will improve cell survival.
2. Functionalized nanoparticles coupled to MSC will form a biocompatible unit.

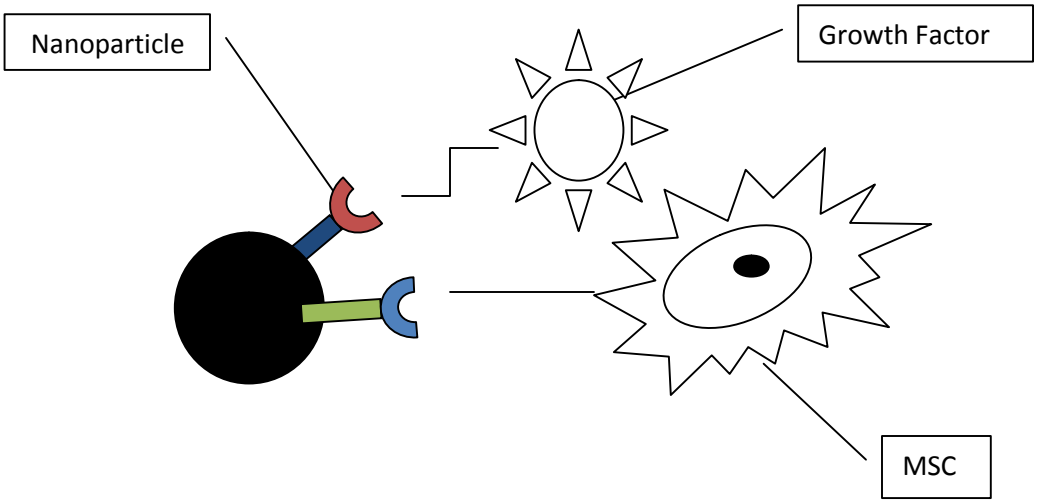


Figure: 6 Nanoparticle-Antibody-Mesenchymal Stem Cell complex: A schematic presentation of the proposed NP-Antibody-MSC-Growth factor complex. Both the antibody and the growth factor are attached to the NP core. Antibody is used to attach the SPIO nanoparticle to the MSC, and the growth factor offers stimulation of the MSC.

VIII MATERIAL AND METHODS

Chapter 1 Isolation and Characterization of MSC

The Ly laboratory works on numerous small and large animal studies involving MSC, therefore my studies will involve BM-derived MSC from both rat and swine origin.

1.1 Isolation of Bone marrow derived mesenchymal stem cell from rat

Male Lewis rats at the weight of 100g were humanely sacrificed according to the Montreal Heart Institute Animal Ethic Review Board. Following sacrifice, 70% ethanol was applied to the hindlimbs, and then the tibia was surgically removed to a 100mm culture dish containing preheated PBS. Muscles attached to the bones were carefully removed by scissors and rubbed with gauze. To assure no muscle cell contamination, cleaned bones were dipped in 70% ethanol for 10 seconds and then washed with PBS and dried with sterile gauze immediately. The two ends of the bone were swiftly excised by scissors; the bone cavity was flushed with 5ml culture medium until the bone became white, using number 23 needle with a syringe. The cell suspension was passed through number 23 needle with a syringe for 3 times to obtain a unified cell suspension. Then the cell suspension was transfered equally to two T150 Flasks supplied with 20ml culture medium each and cultured in 37°C and 5% CO2. After 72h, the medium was changed for the first time. Cells were passed when the confluence reached 80%, and the medium was changed every 3-4days.

Culture medium: For each 500ml: 50ml FBS (Gibeco), 450ml DMEM, 5ml Streptomycin/Penicilin, 3ml HEPES.

1.2 Isolation of Bone-marrow-derived mesenchymal stem cells from pig

Landrace-Yorkshire male swine (30-35kg) were used for large animal studies. Following sedation and general anesthesia (with Isoflurane 1.5%), bone marrow aspiration was performed

at the level of the iliac crest. Bone marrow was isolated by a syringe containing heparin and kept in room temperature. In less than 1 hour, isolated bone marrow was processed. Briefly, 9ml of Ficoll Paque Plus (GE healthcare) was added to the bottom of a 50ml tube. Then 16ml of BM solution was gently transferred on top of the Ficoll without mixing(a ratio of bone marrow to Ficoll was maintained at 2:2.4). The tube was centrifuged at 1880rpm for 40 min without brake and with slow acceleration. The white layer between the two phases was collected with a sterile transferring pipette to a new 50ml tube. Wash three times with 50ml PBS (2000rpm, 5min). Resuspend the cells in 5ml culture medium and seed the cells in a T25 flask and culture in 37°C and 5% CO2. Change the medium every 3-4 days and pass the cells when the confluence is around 80%.

1.3 Flow cytometry

Even though there is no simple set of putative surface marker that characterize MSCs, certain markers are repeatedly used from article to articles. For the purpose of my research work, CD44 and CD90 were chosen as positive markers; CD45 and HLA-DR were used as negative markers (All antibodies are conjugated with FITC, Biolegend, CA). Passage 5-6 cells were expanded and harvested, and for each marker $70x10^4$ cells were prepared. Cells were washed twice with PBS, and then blocked for 10min with serum from which the antibody is derived. Cells were spun down and washed once with PBS. The antibody was added as recommended by the manufacturer. The staining solution used to dilute the antibody was 0.025% BSA in PBS. Incubation was carried out in 4 degree for 30 min after which cells were washed twice with PBS and suspended for immediate analysis by flow cytometry (Beckman Coulter Inc).

1.4 MSC Differentiation Assays

1.4.1 Adipogenic differentiation and oil red-O staining

Passage 6 MSCs were seed in 24-well dish at the density of $4x10^4$ cells/well. When the cells came to confluence (70-80%), the medium was changed to Adipogenic Differentiation Medium (StemPro®, Invitrogen, CA), 500ul/well. Medium was changed every 3 or 4 days. After eleven days, lipid accumulation or vacuoles in cells was checked by Oil-red-O staining. Thus, medium was removed, the wells were washed once with 1ml/well PBS; and then in each well, 200ul oil-red-o staining solution was added (0.5g oil-red-O in 100ml isopropanol, 0.5%). After incubation for 30min, the oil-red-O staining solution was removed and wells were washed with distilled water, 1ml/ well, until the background was clear. Photos were taken by a light microscope equipped with digital camera.

1.4.2 Osteogenic differentiation and Alizarin red-O staining

Passage 6 rat MSCs were seeded into 24-well dish at the density of $5x10^4$cells/well. When the cells come to confluence (70-80%), the medium was changed to differentiation medium (10^{-7}M dexamethasone, 10mM β-glycerol phosphate and 50uM ascorbic acid in culture medium). Then the medium was changed every 3 to 4 days. Two-eight days later, cells were processed for Alizarin red-O staining for detection of calcium accumulation. Briefly, cells were fixed in cold ethanol 70% for 30min, 500ul/well. Alizarin-red-O working solution was made by adding alizarin red-O into distilled water, 1%, PH was adjusted to 4.10-4.30 and filtered through 0.22um filter to remove any insoluble particles. After washing the wells once with PBS, 1ml/well, 200ul Alizarin red-O working solution was added to each well. Five minutes later, the staining solution was removed; then wells were washed with PBS, 1ml/well until the background was clear.

1.4.3 Chondrogenic differentiation and Alcian Blue staining

Passage 6 rat MSCs were seeded into 24-well dish at the density of $15x10^4$cells/well. Two-four hours later, medium was changed to differentiation medium (StemPro®, Invitrogen, CA). Medium was changed every 3 or 4 days. Two-eight days later, cells can be processed for

chondrogenic differentiation by Alcian Blue staining. Briefly, 0.1g Alcian Blue was dissolved in 100ml 0.1N HCl and filtered through 0.22um filter to make working solution. Differentiated cells were washed first with distilled water, 1m/well, twice and then fixed with cold methanol for 20min. Then the cells were washed once again with distilled water. Then 200ul Alcian Blue working solution was added to each well and incubated for 45 min. Then the cells were washed once with 0.1N HCl, and then the wells were washed with distilled water until the background was clear.

1.5 MSC Proliferation Assays

MSCs were seeded in seven 24-well plates at the density of $1X10^4$/well. Each day, one plate was taken to run PMD/MTS assay to determine the number of living cells in each well.

PMS/MTS solution: To detect the change of cell active cell numbers, Celltiter 96well nonradioactive proliferation kit was employed. Before usage PMS (1ml) and MTS (20ml) were mixed according to manual provided. Then the mixed solution was aliquot into 2ml/vial and stored in -20°C. When needed, PMS/MTS was thawed before application. When used, PMS/MTS was mixed with culture medium at the ration of 1:5;

PMD/MTS assay: Briefly, the culture medium in each well was replaced with 200ul PMS/MTS culture medium. Then cells were incubated in the incubator for 1h. After incubation, the absorbance at 490nm of each well was measured by an ELISA reader.

Chapter 2 Apoptotic Assays

2. 1 Preconditioning agents

As discussed in the previous section, preconditioning represents a major option to improve cell survival in the ischemic microenvironment. In all studies, $50x10^4$ cells of passage 6-8 rat MSC were seeded to each well of six-well dish, overnight before starting the assays. My in vitro studies will explore the following agents: TSG-6, SDF-1α and PDGF-BB

2.1.1 TSG-6, SDF-1α and PDGF-BB preconditioning

Every vial of TSG-6(R&D system, CA) was dissolved in 500ul PBS to a concentration of 10ug/ml. precondition medium spans 5 concentrations: 0.5ug/ml, 1.0ug/ml, 1.5ug/ml, and 2.5ug/ml. Five precondition time-spans were also employed: 2hours and 24hours. Briefly，passage 8 rat MSCs were seeded at the density of 8,000 cells/well in 96 –well dishes at 100ul culture medium per well and allowed to equilibrate overnight. Then medium were changed to precondition medium and incubated for different time spans. According to reported data, two concentrations for SDF-1α, two concentrations for PDGF-BB and one concentration for TSG-6 were examined. SDF-1 α: 0.05ug/ml and 0.025ug/ml; PDGF-BB: 10ng/ml and 50ng /ml and TSG-6 2.5ug/ml. Incubation time-spans were 2 hours and 24hours. Briefly，passage 8 rat MSCs were seeded at the density of 8,000 cells/well in 96 –well dishes at 100ul culture medium per well and allowed to equilibrate overnight. Then medium were changed to precondition medium and incubated for different time spans.

2. 2 Oxidative Stress Assay

Oxidative medium: 30% stabilized H_2O_2: 100ul was added into 900ul PBS to make initial solution. For 10mM H_2O_2 medium: 102ul initial medium was added into 10ml culture medium.

2mM H_2O_2 culture medium was made by adding together two portions of 10mM H_2O_2 medium and 8 portions of culture medium. The rest concentration was mixed accordingly.

Passage 6-8 rat MSCs were preconditioned according to precondition protocol described in section 2.1.1. Then the culture medium was changed to oxidative medium at the concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0mM in different wells, with an incubation time of 2 hours. Afterwards, the oxidative medium was removed and wells were washed once with 100ul/well culture medium. Then the culture medium was replaced by PMS/MTS culture medium, 100ul/well. After 1hour of incubation, absorbance at 490n was examined for each well by an ELISA reader.

2.3 Serum Deprivation

Passage 6-8 rat MSCs were preconditioned according to precondition protocol. Then serum-deprivation wells were kept in DMEM only; control wells were kept in normal medium. 24h later, PMS/MTS test was performed according to previous protocol to examine the cell viability.

2.4 Hypoxia

Passage 6-8 rat MSCs were preconditioned according to precondition protocol as described in 2.1.1. Then medium was changed, and the cells were transferred into a hypoxia chamber where the oxygen concentration was set at 1.8% by influx of 1% oxygen in nitrogen, then the hypoxia chamber was incubated for 24h. PMS/MTS assay was performed according to previous protocol to examine the cell viability.

2.5 Combined Hypoxia and Serum Deprivation

The protocol of 2.3 and 2.4 were combined to perform Combined Hypoxia and serum Deprivation Assay.

Chapter 3 Wound Healing Assay

3.1 Preconditoning

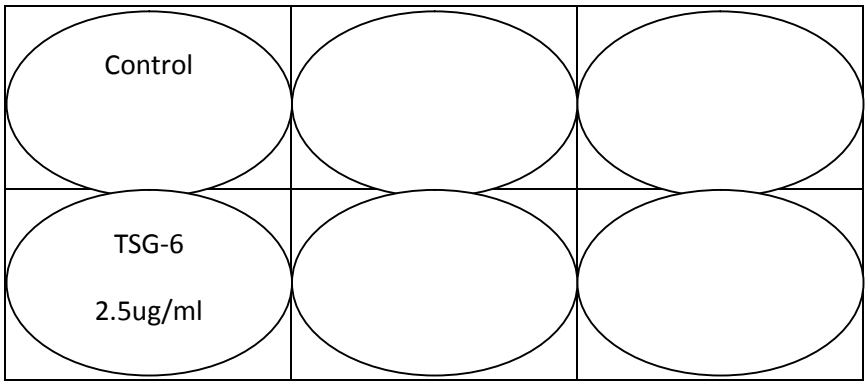
$50x10^4$ cells/well of Passage 6-8 rat MSC were seeded to six-well dish, cells were allowed to equilibrate overnight. Then medium was changed to precondition medium containing SDF-1 α: 0.05ug/ml; PDGF-BB: 50ng/ml and TSG-6: 2.5ug/ml respectively. Cells were then incubated for another 2 or 24 hours before wounding healing assay. For each condition 5 replicates were done (**figure 7: A**).

3.2 Wound Healing

Wound healing, or gap closure in a confluent cell monolayer, incorporates the two cellular phenomena of migration and proliferation. At the end of the precondition, a 20ul pipette tip was used to create the wounds. One vertical wound was created in the middle and three horizontal crossing wounds were created as markers. Photos were taken close to the crossings. Three photos for each well were taken at time-points of 0h, 6h, and 24hours after scratching. The width of the wounds was measured for the healing ability of the cells at fixed points of each well (**figure 7: B**).

A

Plate 1:



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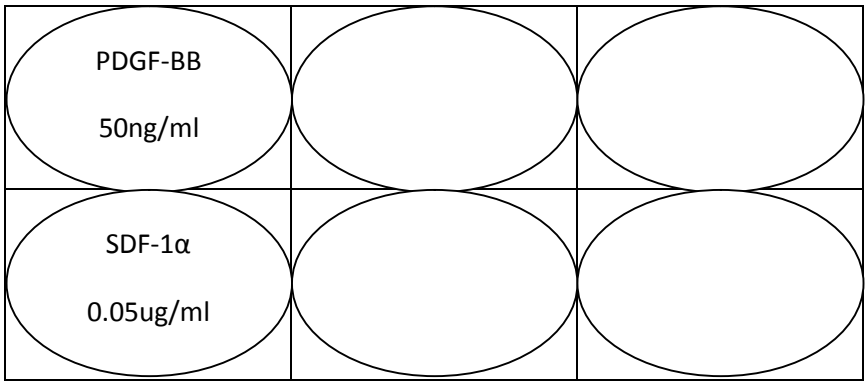

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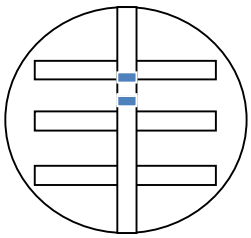


Figure: 7 Wound Healing Assay Arrangement: **A**: Plate plan of precondition and would healing Assay. For each condition three wells were prepared and from each well three data points per time were obtained. **B:** Wounds created in one well of six-well dishes for the wound healing assay. Three crossings were created and photos were taken at the blue points at three time points: 0, 6, and 24 hours.

Chapter 4 Ferromagnetic Nanoparticles

4.1 Synthesis and characterization of Nanoparticles (NPs)

The Veres Research Group, part of the National Research Council of Canada at the Institute of Industrial Materials in Boucherville, closely collaborates with the Ly lab. Nanoparticles were designed and manufactured at their site and transported to our laboratory for cell coupling and biocompatibility studies.

The following paragraphs provide a summarized version of the complex process of nanofabrication of the ferromagnetic, silicone-shell nanoparticles. For NP synthesis, a 2-step procedure will be employed starting by the hydrolyzation of tetraethyl orthosilicate (TEOS) and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAP3) molecules. The synthetic route that will be employed for the FeO-based nanoprobe (NP1) is briefly outlined. The first step consists of the synthesis of Fe_3O_4/silica nanoparticles by hydrolyzing TEOS in a water-in-oil microemulsion that contains the Fe_3O_4/OA (oleic acids) NPs as seeds. In this process the Fe_3O_4/OA NPs will be first dispersed in cyclohexane, at a concentration of 1 mg/mL, and then 0.5 ml of the Fe_3O_4-containing cyclohexane dispersion will be rapidly injected into a mixture of Triton X-100, anhydrous 1-hexanol and cyclohexane under a strong vortex for one hour. Subsequently, 0.5 ml of ammonia solution (28-30% ammonia solution: water=1:4) will be added to the above solution and shaken for another hour. Finally, 25 µl of TEOS will be added and the mixture will be allowed to react for a full 24 hours. The As-fabricated products are then separated by centrifugation at 9000 rpm, washed with ethanol, and the centrifugation/wash procedure is repeated three times. Subsequently, the resultant NPs will dried under vacuum, or directly dispersed in de-ionized water for characterization.

In the second NP synthesis step, 25 *µ*l of AEAP3 are injected into the reaction mixture for another 24 hours in order to form the outer nano-nanoporous shell. The resultant product denoted as Fe_3O_4/silica (amino) NPs will be washed with anhydrous ethanol three times, and

finally dispersed in de-ionized water for use. The resulting Fe_3O_4(15nm)/silica(10 nm)/silica(amino) (10nm) NPs, with a total particle size ranging of about 35 nm will contain 350-400 primary amine groups/particle. The latter will be used to couple on surface of the MSC by targeting the surface marker, CD44. The latter was shown to be specific for BM-MSC during in vivo tracking NIR studies performed by Ly et al.(Ly, Hoshino et al. 2009) The covalent coupling of the antibody and antigen to the NPs can be carried out via glutaraldehyde as the amine reactive homobifunctional crosslinker (Bangs Laboratories). As a control experiment, FITC-labeled antibody will be employed for covalently coupling with aims of straightforward characterizations by fluorescence spectra. The intensity changes of fluorescence spectra will be used to monitor the conjugated contents. After synthesis as described above, the "naked" antibody will be conjugated to CD44 antigen, followed by a reconfirmation of FITC-labeled antibody. Finally, once successful coupling is confirmed, phantom models using low melting point agarose (1% at 37^{o}C) for MRI in vitro studies will be performed.

4.2 Preparation of the NPs

Stocking solution of NP was at 200μg/ml. To make NP culture medium, stocking solution was first taken according to designed volume. Then under a chemical hood, the stocking solution was put into a magnetic field for 10min before careful aspiration of the supernatant without touching the NPs. Then replenish the vial with culture medium to make NP culture medium.

4.3 Proliferation of MSC with NPs

In 7 24-well plates, seed in each plate 14 wells of pig MSCs, 1X10^{4}/well, three groups, control, 5ug/ml NP and 10ug/ml NP. At time point of day 1, 2, 3, 4, 5, 6, 7, take one plate and run PMD/MTS assay to assess the cell number in each well. Briefly, the medium is replaced with PMS/MTS in medium (1:5). In each well 200ul PMS/MTS in medium is added. Then the dish is incubated in the incubator for 1h. After incubation the absorbance at 490nm is measured using an ELISA reader.

4.4 Incubation and Visualization of NPs on MSCs

Histological Visualization

In a six-well plate, seed $5x10^4$ pig MSCs to each well, let the cells attach to the bottom of the well for 16 hours. Then change the medium with medium containing 5ug/ml or 10ug/ml NPs. Incubate in the incubator for 1h, then wash twice with PBS 1X. After that, fix the cells with methanol for 15 min, and then wash the cells with distilled water, then incubate first with 2% potassium ferrocyanide in 6% hydrochloride acid for 30min, and then with Hematoxyline QS for 1 min. Then wash the cells once with robinet water, and then wash with distilled water until the background is clear. Take photo of the wells under microscope under 20X.

MRI Visualization

In a six- well plate, seed $5x10^4$ pig MSCs to each well, let the cells attach to the bottom of the well for 16 hours. Then change the medium with medium with medium containing 5ug/ml or 10ug/ml NPs. Incubate in the incubator for 1h, then wash the cells twice with PBS 1X. Then put the plate into the MRI scanner for MRI imaging.

4.5 Adhesion Assay of pig MSC combined with NPs

Prepare the Nano Particles (NPs) medium according to the protocol above. And then cell suspension was prepared by harvesting cells from a T75 flask. Three groups of cells were prepared, control, 5ug/ml NPs, 10ug/ml NPs. For each group, a sub control without cells is also prepared. Seed the cells into a 12-well dish at the density of $2x10^4$cells/well. Incubate at 37°C for 24 hours. Then remove the medium and wash twice with preheated PBS, then trypsize the cells in each well. After the cells are fully detached, add in each well 0.8ml culture medium and then incubate for another 4 hours. Remove the medium and wash 3 times with preheated PBS. In each well, add in 1ml culture medium and 200ul PMS/MTS and then incubate in the incubator for 1 hour. Measure the absorbance at 490nm in an ELISA reader after.

4.6 Test of toxicity of NPs on MSC

In six-well plates, seed $5x10^4$ pig MSCs to each well. Let the cells attach to the bottom of the well for 16 hours. Then change the medium with medium containing 5ug/ml or 10ug/ml NPs. And then incubate for 1, 4 and 24 hours respectively. Then trypsize the cells and count the number of viable cells with trypan blue. For each condition and time point, do triplicate.

4.7 Migration Assay of MSC with NPs

Add the NP-containing medium (5ug/ml and 10ug/ml) into T75 flasks with MSC when a confluence level of 80% was ascertained. Incubate in the incubator for 1 hour, then trypsinize the cells and count the number of viable cells using Trypan Blue Dye exclusion test. At the start of the assays, 10^5 cells were added to the superior chamber of migration dish in a volume of 0.5ml, whereas 0.5ml predetermined medium was added to the interior well. For each condition, experiments were performed in triplicate.

4.8 Differentiation Assay of MSC with NPs

In six-well plates, seed 5x104 pig MSCs to each well. Let the cells attach to the bottom of the well for 16 hours. Four groups of pig MSCs were prepared: control positive, control negative, 5ug/ml NP and 10ug/ml NP. Then change the medium with medium containing 5ug/ml and 10ug/ml NPs. Incubate in the incubator for 1h, then wash the cells twice with PBS 1X. Then the medium was changed to differentiation medium (10-7M dexamethasone, 10mM β-glycerol phosphate and 50uM ascorbic acid in culture medium). Then the medium was changed every 3 or 4 days. Two-eight days later, cells were processed for Alizarin red-O staining for the detection of calcium accumulation. Briefly, cells were fixed in cold ethanol 70% for 30min, 500ul/well. After washing the wells once with PBS, 1ml/well, 200ul Alizarin red-O working solution was added to each well. Five minutes later, the staining solution was removed; then wells were washed with PBS, 1ml/well until the background was clear. Alizarin-red-O working

solution was made by adding alizarin red-O into distilled water, 1%, PH was adjusted to 4.10-4.30 and filtered through 0.22um filter to remove any insoluble particles.

4.9 Statistic Methods

All the data acquired in the experiments was subjected to statistic analysis. Analysis of variance was performed for every experiment. Different strategies were chosen according to the number of factors and the number of responses. One-way Anova was used for experiments with one factor. Main-effect ANOVA was used for experiments with two factors. Statistica from stasoft was used to analyze all the data. All graphs were presented as mean+SEM of its group. Statistical significance was set at p value below 0.05.

IX RESULTS

Chapter 1 Isolation and Characterization of MSCs

1.1 Isolation and culture of BM-derived MSCs

After 72 hour of incubation of the fresh isolated Bone Marrows, cell colonies were observed on the bottom of the flask. Pass of the adherent cells resulted in clean culture of spindle-like cell monolayer. The cells grow in DMEM supplied with FBS 10%, adhered to plastic surface and exhibited typical Mesenchymal stem cell morphology (**figure 8:A**).

1.2 Surface marker expression of the BM-derived MSCs

Isolated cells were examined by flow cytometry for the expression of several major Mesenchymal Stem cells surface markers. The cells express CD44, CD90 and are negative for RT1D and CD45, which are markers for the Hematopoietic cells (**figure 8:B**).

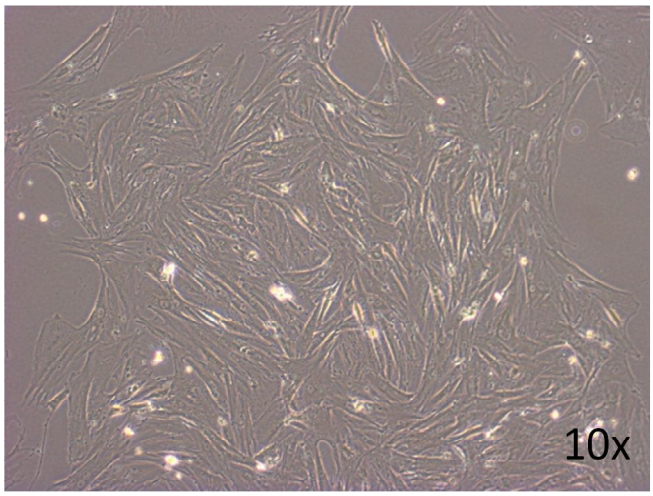
1.3 Differentiation capacity of BM-derived MSCs

The multipotency of mesenchymal stem cells is one of the major reasons that make mesenchymal stem cells promising candidate for stem cell therapy. Our mesenchymal stem cells were tested as standard procedure for osteogenic, adipogenic and chondrogenic differentiation capacities. The cells were positive in all three aspects of stem cell multipotency (**Figure 9**).

1.4 Proliferation Assay

The rMSC is very proliferative as reported. The cell number started to increase right after being plated, and then reached a plateau from day 4 to day 5, and then experienced another proliferation peak at days 5-6. The proliferation started lowering down from day 6. (**figure 10**).

A:



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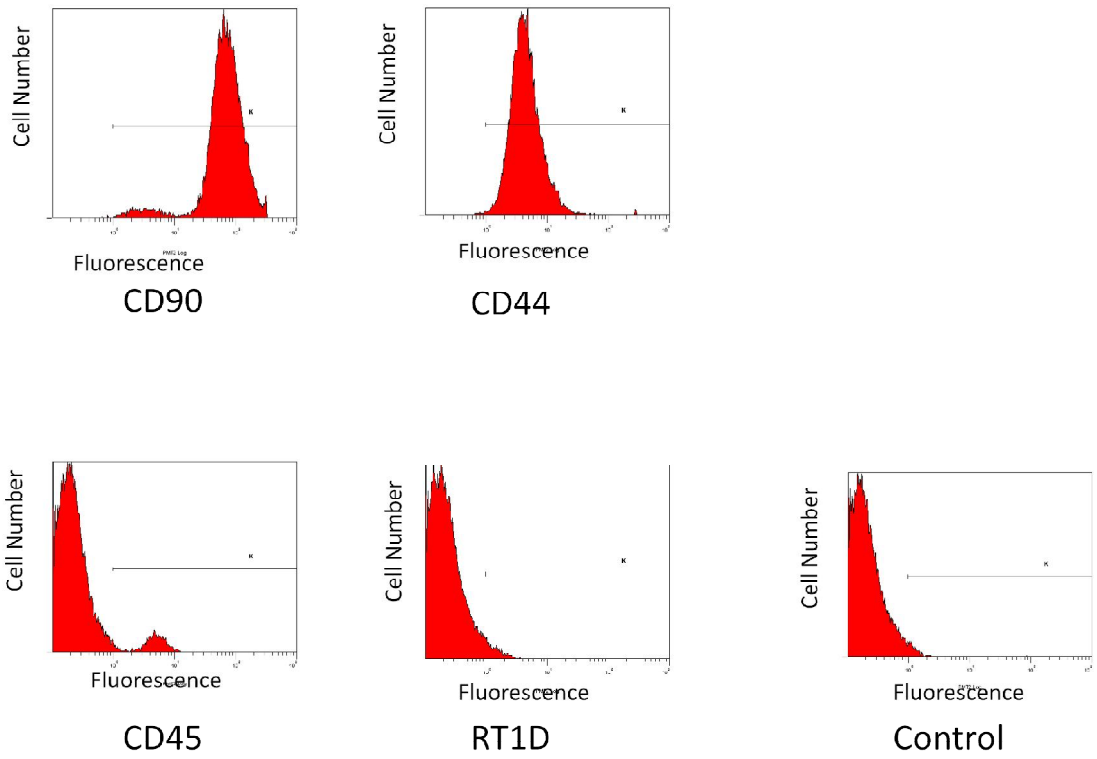


Figure: 8 Characterization of Mesenchymal Stem Cell: A: Freshly isolated Bone Marrow MSC colonies. B: Established Bone Marrow MSC line was examined by flow cytometry for recommended markers. Cells are positive for mesenchymal markers CD44 and CD90 and negative for RTID (rat counterpart of HLA-DR) and CD 45. P2 rat mesenchymal stem cells were used.

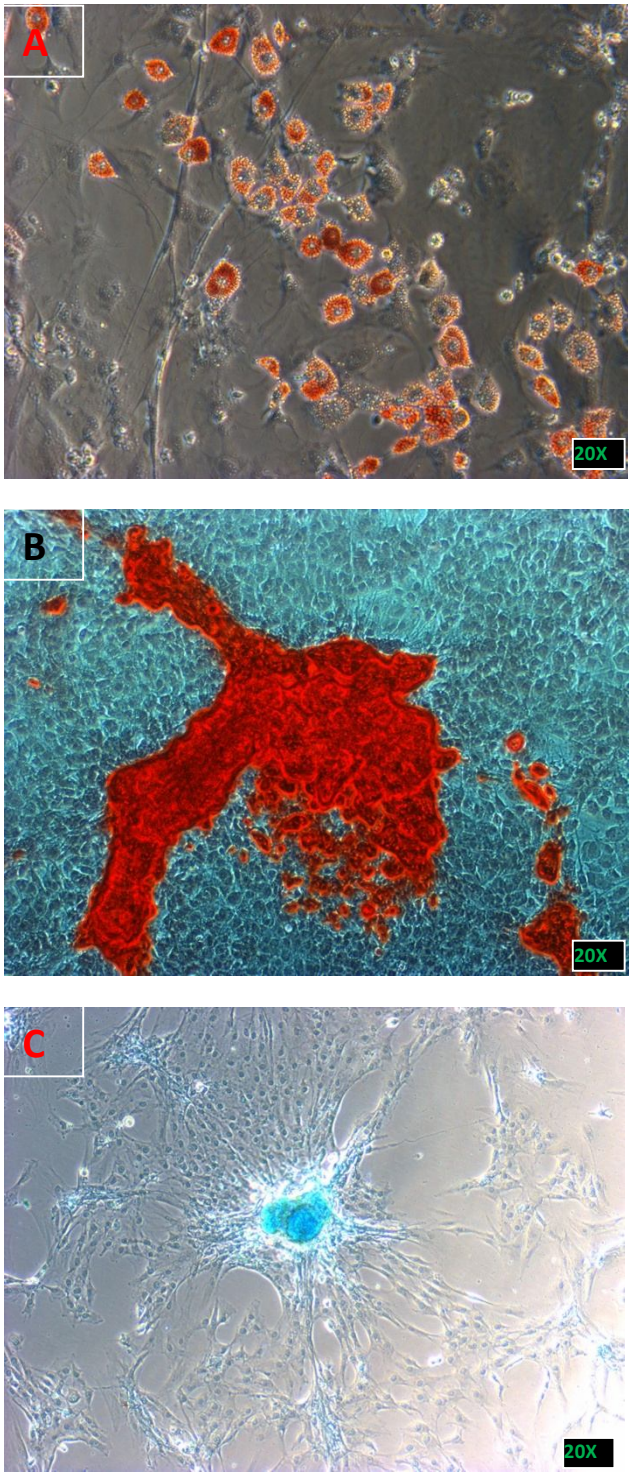


Figure: 9 Differentiation of Mesenchymal Stem Cell: A: p6 Bone-marrow derived rat mesenchymal stem cells were induced in adipogenic differentiation medium for eleven days. Lipids accumulation was revealed by the staining of oil-red-O. lipid vacuoles are revealed by oil-red-O staining as red spots.

B: p6 Bone-marrow derived rat mesenchymal stem cells were induced in osteogenic differentiation medium for twenty-eight days. Calcium accumulation was revealed by the staining of Alizarin-red-O as red spots. Cells are positive for osteogenic morphology (3D cell structures) and calcium (red staining).

C: p4 Bone-marrow derived rat mesenchymal stem cells were induced in chondrogenic differentiation medium for 28days. Chondrogenic differentiation was revealed by the staining of Alcian Blue as blue stains. Cells are positive for chondrogenic morphology (3D cellular structures) also.

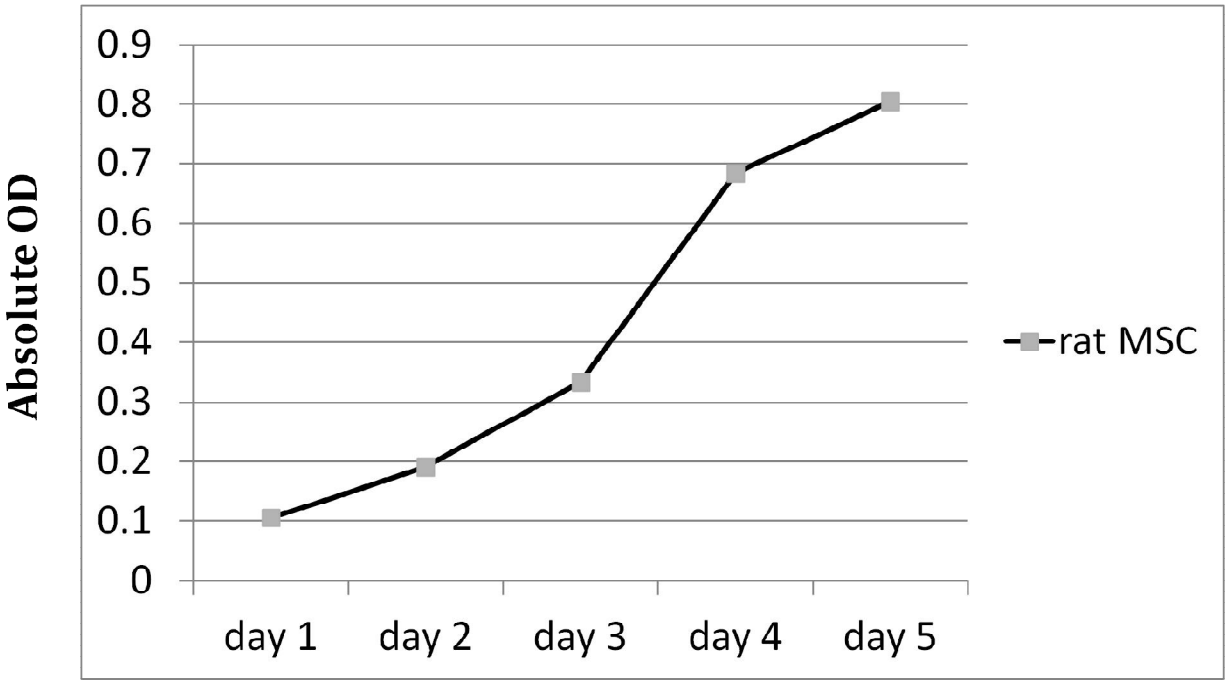


Figure: 10 Proliferation of Mesenchymal Stem Cells: The proliferation pattern of rat MSC over a 7-days time span. No dormant phase is seen in the curve and a twelvefold increase of OD value indicating a high proliferation profile of rat MSC. Each data point is an average of 4 wells.

Chapter 2 Cell Necrosis Assay Results

2.1 TSG-6 preconditioning

Since there is no available data for rat MSC preconditioning with TSG-6, both time range and dose ranging were carried out to locate the optimal time and dose for TSG-6. However, the data suggest that TSG-6 has no additional effect on the viability of rMSC under the combination of Hypoxia + Serum Deprivation or Oxidative stress, for both 2 hours precondition and 24 hours preconditioning. Similarly, in doses ranging studies from 0.05ug/ml to 2.5ug/ml, TSG-6 did not influence on the response of rat MSC under oxidative stress. TSG-6 did not decrease the viability of rat MSC, which indicates that TSG-6 is a safe growth factor to be used in MSC therapy **(Figure11).** In accordance to oxidative stress results, TSG-6 is not improving the viability of rat MSC undergoing Hypoxia+Serum Deprivation since no significant differences were found among the 5 different concentrations and 2 different precondition timings. This suggests that the positive results from TSG-6 therapy in the mouse myocardium infarction model are from the effects exerted on other cells by the TSG-6. The MSCs are not a target of TSG-6 **(figure 12).**

2. 2 SDF-1α and PDGF-BB Preconditioning

Oxidative stress

Preconditioned for 2h: SDF-1α and PDGF-BB were not found to be protective against oxidative stress. On the contrary the presence of SDF-1α and PDGF-BB corresponded with lower viability of the rMSCs. And the viability is lower when the concentration of SDF-1α or PDGF-BB is higher, with lowest viability in PDGF-BB groups **(figure 13:A**). However, when the H_2O_2 concentration is higher than 1.0mM, the viability is similar with or without cytokines.

Preconditioned for 24 hours: The protective effect of SDF-1α and PDGF-BB on rMSCs undergoing oxidative stress changed dramatically according to preconditioning time. While SDF-

1α and PDGF-BB were detrimental to the rMSCs when would for 2 hours, they were strongly protective when preconditioning time increased to 24 hours. Both SDF-1α and PDGF-BB are protective against oxidative stress with PDGF-BB being more protective than SDF-1α. Interestingly, the protection did not increase with increasing concentration; on the contrary, lower concentrations provided better results. The protective effect was more prominent at concentrations of 0.025ug/ml than at 0.05ug/ml for SDF-1α whereas 10ng/ml was better than 50ng/ml for PDGF-BB (**figure 13: B**).

Protection with SDF-1α and PDGF-BB is affected by the severity of the oxidative stress: With low concentration of H_2O_2 (< 0.5mM), damages caused by the oxidative stress were completely abolished, with viability higher than 100%. Higher concentration of H_2O_2 (> 1.5Mm) nullified the protective effect of cytokines.

These results therefore suggest that both SDF-1 α and PDGF-BB preconditioning protects MSCs against cell death caused by oxidative stress conditions.

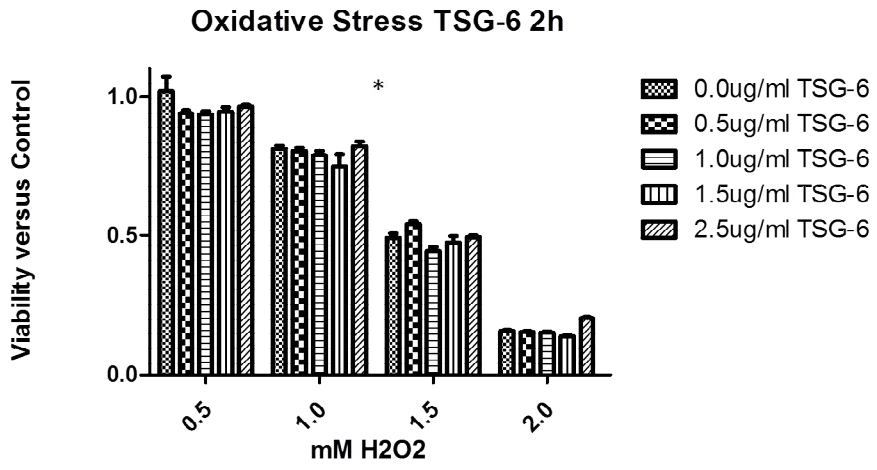
Combined Hypoxia and Serum Deprivation

Under hypoxic conditions alone, the cell viability ranged from 80% to 90% regardless the preconditioning agent. Under serum deprivation conditions, the viability dropped to almost 60%. When serum deprivation was combined to hypoxia, the viability did not change, which suggests that serum deprivation is the predominant stressor. Preconditioning with SDF-1α or PDGF-BB is conducive to the survival of rat MSC under combined hypoxia and serum deprivation conditions. PDGF-BB offered a better protection than SDF-1α, and its protective effects improved with progressive concentration. At 50ng/ml of PDGF-BB, the rMSCs' viability was restored to an estate similar to what would be obtained with hypoxic stress alone. This suggests that PDGF-BB nullifies the deleterious effect of serum deprivation has on viability (**figure 14: A**).

Preconditioning timing also altered findings. As the precondition time increases, the total culture time from seeding to the end of the assay also increased. Hypoxia appeared to provide additional protection against cell death. Even when combined with serum deprivation, hypoxia increased the viability of rat when we compared viability from serum deprivation only relative to serum deprivation +hypoxia. Serum Deprivation remained the dominant cause of the decreased cell viability. SDF-1α did not improve the survivability under hypoxia. Regardless of SDF-1α concentrations, viability remained unchanged both in hypoxia and hypoxia+serum deprivation. Conversely, PDGF-BB still exerted protective and proliferative effect on the rat MSC. PDGF-BB stimulated the proliferation of rMSCs. PDGF-BB+hypoxia showed the highest proliferation, with 40% above the control. MSC proliferation was less under serum deprivation conditions and at lower PDGF-BB concentrations. However, all serum deprivation groups had higher proliferation than control groups. In the hypoxia groups, the proliferation did not increase much with the increase of the concentration, as the concentration of PDGF-BB rose from 10ng/ml to 50ng/ml, the proliferation only increased by 3%. However in the hypoxia+ Serum Deprivation groups, the proliferation increased almost by 21% when the concentration increased to 50ng/ml.

Therefore, these above data suggest that, following 24 hours of preconditioning, PDGF-BB represents an excellent protective growth factor for MSC under hypoxia and serum deprivation conditions **(figure 14**).

A:



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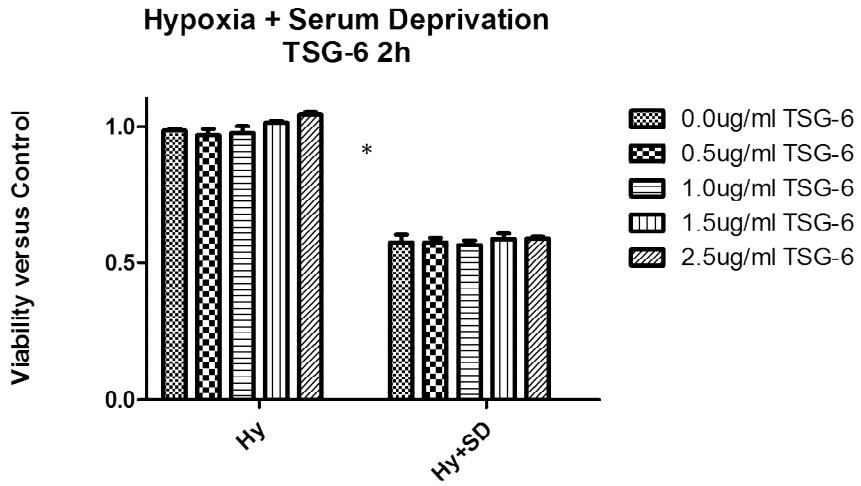
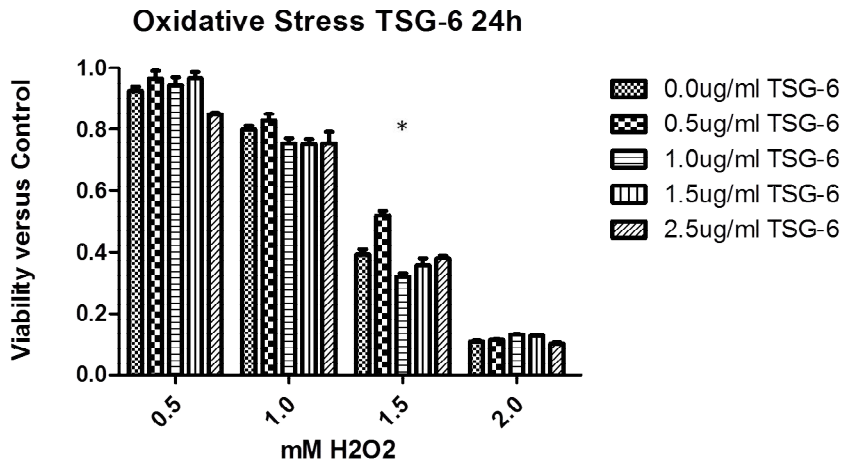


Figure: 11 Apoptotic Assay of Tumor Necosis Factor Stimulated Gene-6 Protein precondition 2h: Oxidative stress and Hypoxia+ Serum Deprivation of rat MSC after 2 hours precondition with TSG-6. **A**: TSG-6 showed no protection of MSC against oxidative stress throughout the tested concentrations. **B:** TSG-6 had no effect on the viability of MSC under hypoxia+serum deprivation throughout the tested concentrations. Each column is an average of 5 wells. *: comparisons are found non-significant.

A:



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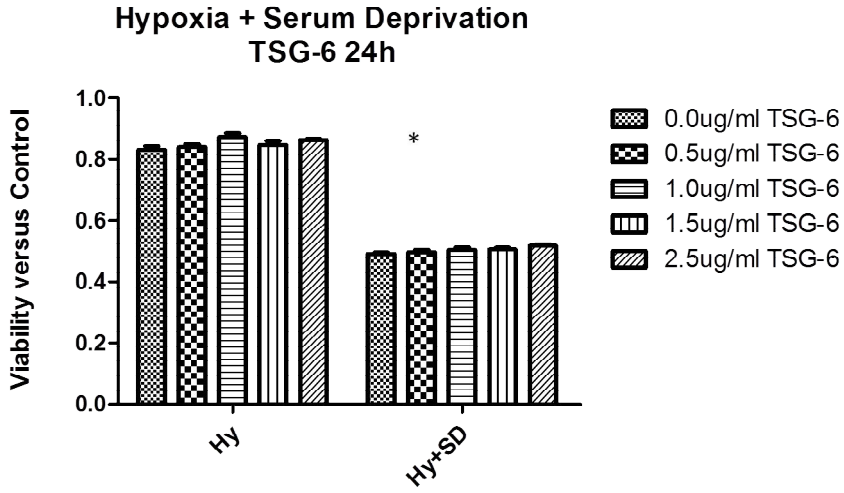


Figure: 12 Apoptotic Assay of Tumor Necosis Factor Stimulated Gene-6 Protein precondition 24h: Oxidative stress and Hypoxia+ Serum Deprivation of rat MSC after 24 hours precondition with TSG-6. **A**: TSG-6 showed no protection of MSC against oxidative stress throughout the tested concentrations. **B:** TSG-6 had no effect on the viability of MSC under hypoxia+serum deprivation throughout the tested concentrations. Each column is an average of 5. *: comparisons are found non-significant.

A:

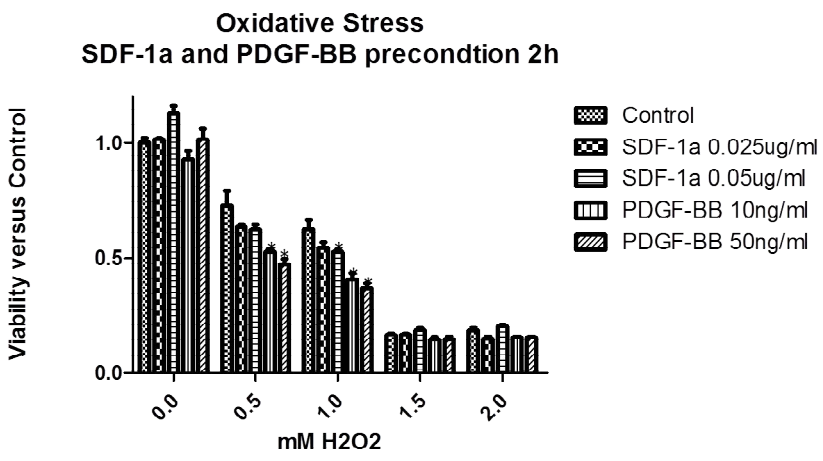
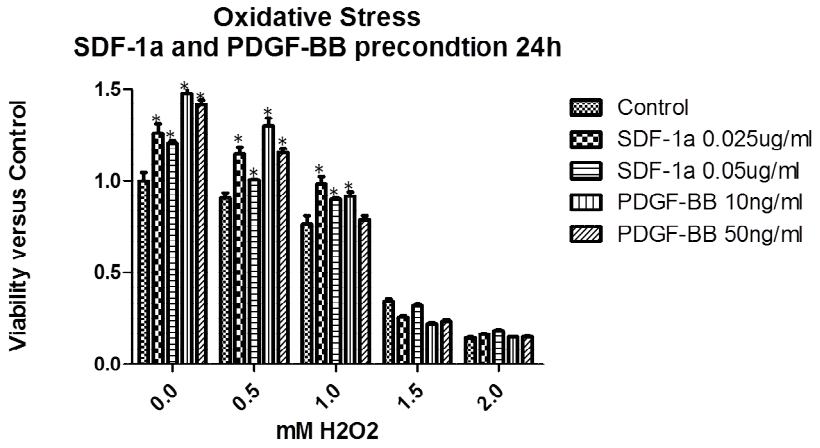
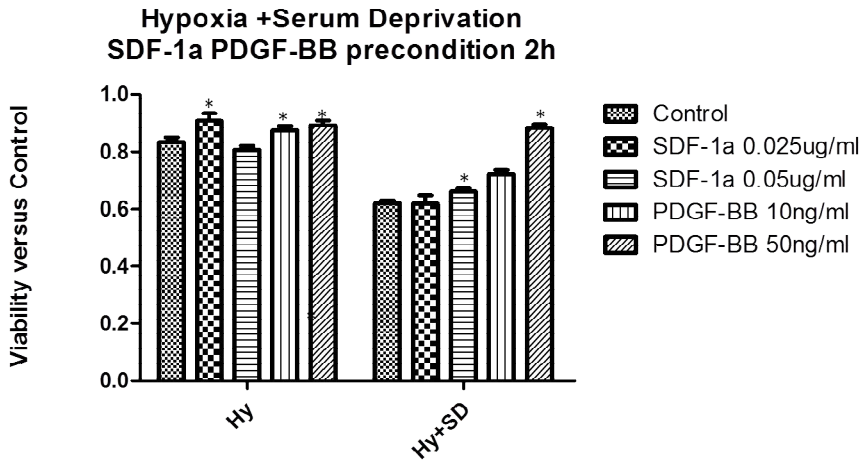


Figure: 13 Oxidative Assay of Stromal Derived Factor-1α and Platelet Derived Growth Factor-BB precondition: A: Oxidative stress assay on rat MSC preconditioned with SDF-1a and PDGF-BB. No protected was observed when the preconditioning time was 2 hours；
B: 24 hours precondition leaded to protection against oxidative stress by SDF-1a and PDGF-BB with H202 concentration under 1.5mm. Each column is an average of 5. *: P<0.05, comparing to their controls, t-test.

B:



A:



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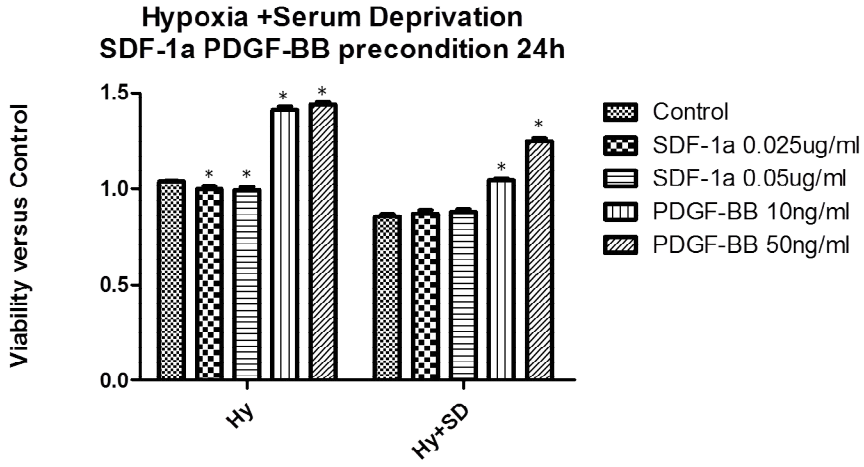


Figure: 14 Hypoxia and Serum Deprivation Assay of Stromal Derived Factor-1α and Platelet Derived Growth Factor-BB precondition: **A:** Hypoxia alone did not cause serious drop of cell viability at the test time of incubation regardless precondition or not. After two hours precondition, PDGF-BB showed protective effect against serum-deprivation, and the protection increases with dose; **B:** After 24 hours of preconditioning: PDGF-BB promoted the proliferation of MSC and completely nullified the effect of serum-deprivation on. n=5 for each column. *: $P<0.05$, comparing to their controls, t-test.

Chapter 3 Wound Healing Assay Results

3.1 Wound Healing Results

MSC demonstrated strong wound healing properties even without preconditioning. The wound healing process is characterized with the closure of the wound by cells migrating in from the two sides over time. When preconditioning growth factors are added, the MSC reacted accordingly (**figure 15**).

At 24 hours after the scratching: PDGF-BB preconditioned MSCs achieved full scratch closure regardless of the preconditioning time i.e.100% closure of the wound. Very dense cell monolayers were documented only in the PDGF-BB preconditioned cells (**figure 15**). The other groups are left with small gaps that are still visible. PDGF-BB precondition stimulates the proliferation and migration of MSCs, and its effect is time-dependent.

MSCs showed consistent wound healing properties among the study groups: at 6 hours after wound scratching, 29% of the gap was closed for the 2 hour-preconditioning group versus 32% for the 24 hour-precondition group; At 24 hours after scratching, the two groups had closed the gap between cell margins for up to 80% and 90%, respectively.

At 6 hours after the scratching: In the 2 hours preconditioning group, the best healing is seen in the SDF-1a preconditioned cells at 32%, but no statistically significant differences was observed among all the study groups (control: 29%, PDGF-BB: 28% and TSG-6: 27%) (**figure 16**). In the 24 hours preconditioning group, PDGF-BB and TSG-6 showed significant improvement on healing capacity compared to the control (PDGF-BB:45% versus 32% and TSG-6: 41% versus 32%, respectively) (**figure 16**). This indicates that, PDGF-BB and TSG-6 are stronger stimulator of migration for MSC, however longer incubation times are needed.

Therefore, this data shows a degree of similarity with the above assays is that PDGF-BB is a strong pro-proliferation and pro-survival factor for MSC.

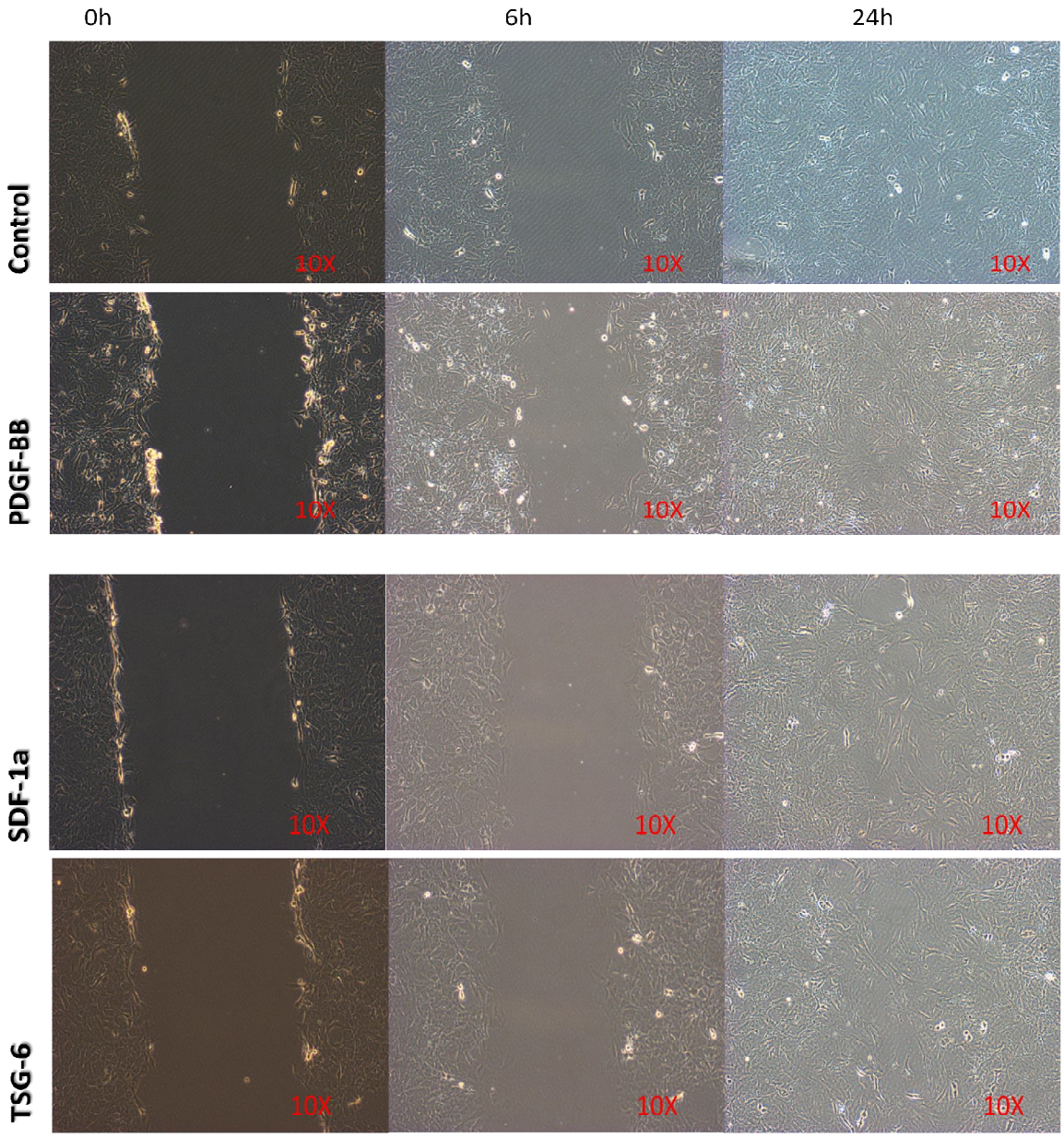


Figure: 15 Photos of Wound Healing Assay: PDGF-BB preconditioned MSC heals the best both after 2 hours preconditioning and 24 hour precondition. The density of the cells is the same as original unwounded areas. For the other groups, unfilled gaps can still be observed after 24 hours.

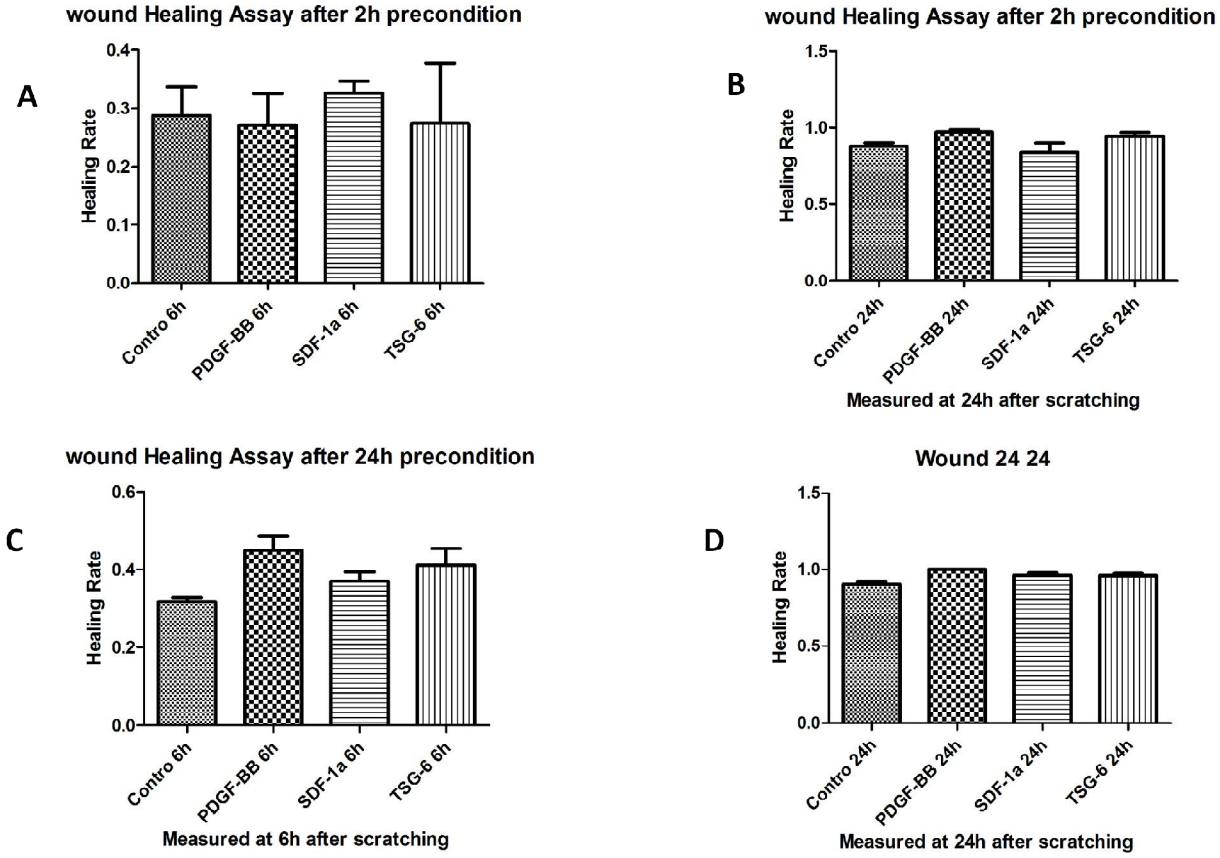


Figure: 16 Wound Healing Assay: **A** and **B** were preconditioned for 2 hours; **C** and **D were** preconditioned for 24 hours. **A:** At 6 hours after wound healing assay, the healing rate was similar among the different conditions. **B:**At 24 hours after wound healing assay, PDGF-BB preconditioned MSC healed the best, followed by TSG-6 preconditioned MSC; SDF-1a was similar to control. **C:** At 6 hours after wound healing assay, PDGF-BB preconditioned MSC healed the best, 13% better than the control (45% versus 32%), followed by TSG-6 preconditioned MSC (41%); SDF-1a was similar to control (37%). **D:** At 24 hours after wound healing assay, PDGF-BB preconditioned MSC healed the best, 100%, followed by TSG-6 and SDF-1a preconditioned MSCs were similar, both better than control. For each column triplicate was performed, for each replicate n=3, comparisons are found non-significant. The healing rate is defined as 1-Wound-width/(initial Wound-width).

Chapter 4 Nanoparticles and MSC Results

4.1 Visualization of Attachment of NP to MSC

Histological Visualization of MSC-NP coupling

The NPs provided by the Veres Research group were all functionalized with an anti-CD44 antibody firmly anchored on the NP complex. Thus, MSC coupling was achieved via CD 44, which was highly expressed in MSCs. After incubation for 1 hour in culture medium, cells are mixed with NPs. The multi-step staining process (with multiple washings) did not affect the attachment of the NPs. Under light microscopy examination, NPs conjugated with CD44 antibody are able to attach firmly to MSC cells and appears as aggregated dots that are visible under optical microscope. As expected, higher concentration of NPs resulted in denser appearance of the dots on the MSC (**figure 17: A**).

MRI Visualization of MSC-NP coupling

Ultimately, a future direction of the project is to use these cells in preclinical, swine model of cardiac injury with cell tracking by serial cardiac MRI. Thus, a key first step, consistent with my project was to document MRI images show that NPs retained by MSC in the cultured dishes were detectable by this imaging modality (**figure 17: B**). The control dish is transparent without any NPs. Both 5ug/ml and 10ug/ml NPs treatment result in detectable MRI signals, which stronger signals at 10ug/ml. Thus the conjugation with these novel functionalized, ferromagnetic NPs visualized MSC under MRI.

4.2 Cell Death

The NP-CD44(+) complex was incubated with MSC over 24 hours. Cells were tested with trypan blue for necrosis. NPs did not affect cell viability at any given point. In fact, NPs treated wells

are containing slightly more cells than the control. Thus, there does not appear to be any cellular toxicity associated with NPs (**figure 18**).

4.3 Cell Adhesion

Following standard plating protocols, compared to wild type-MSCs, NP-CD44(+) conjugated to MSC did not lose their ability to adhere to plastic surface (**figure 19**).

4.4 Cell Proliferation

The proliferation curve of pig MSC is relatively smooth over the 7 days span, indicating a stable proliferation profile. The conjugation with NPs changes the proliferation profile of MSC, i.e. slows down the proliferation of pig MSC to a downward curve other than the upward slope observed in the control. The cell number drops initially and then stabilizes. A greater concentration increase in NPs did not induce further decrease in proliferation. The two concentrations of NPs presented a similar proliferation profile. The NPs used in this experiment down-modulated the proliferation of MSCs, but did not completely stop it (**figure 20**).

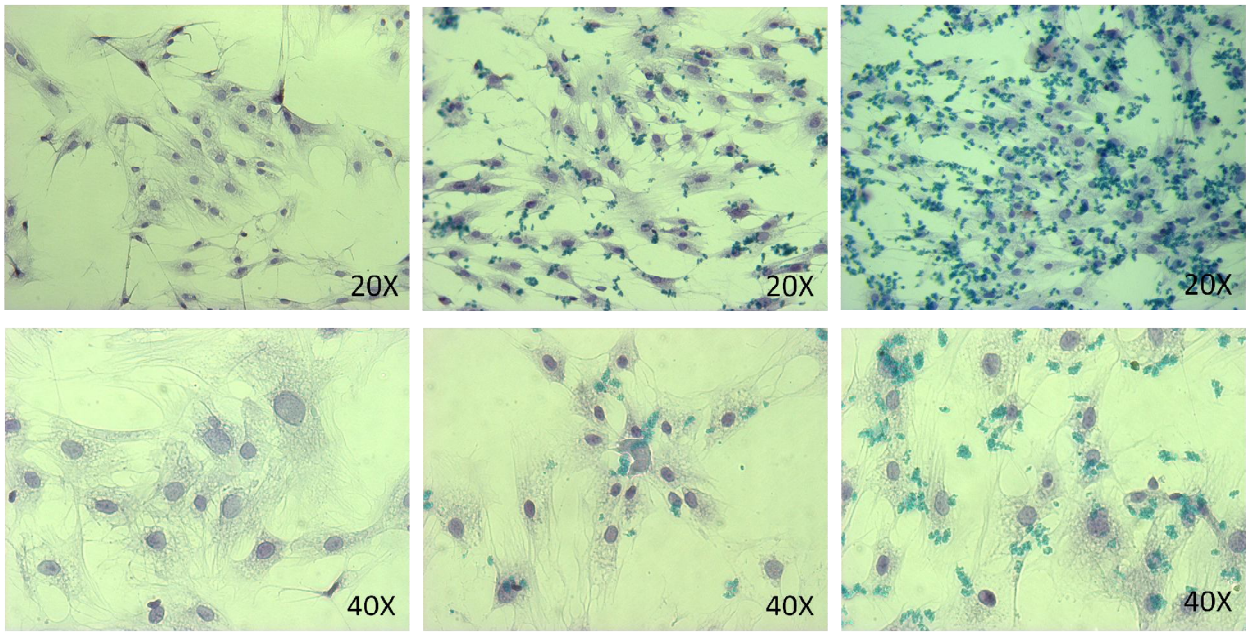
4.5 Wound Healing

The gap closure ability of the NP+MSC complex was significantly altered after the conjugation with NPs, while the control cells healed over 70% of the wound after 32 hours. This alteration did not depend on the NPs concentration and was completed with both of the tested concentrations: 5ug/ml and 10ug/ml (**figure 21**).

4.6 Cell Differentiation

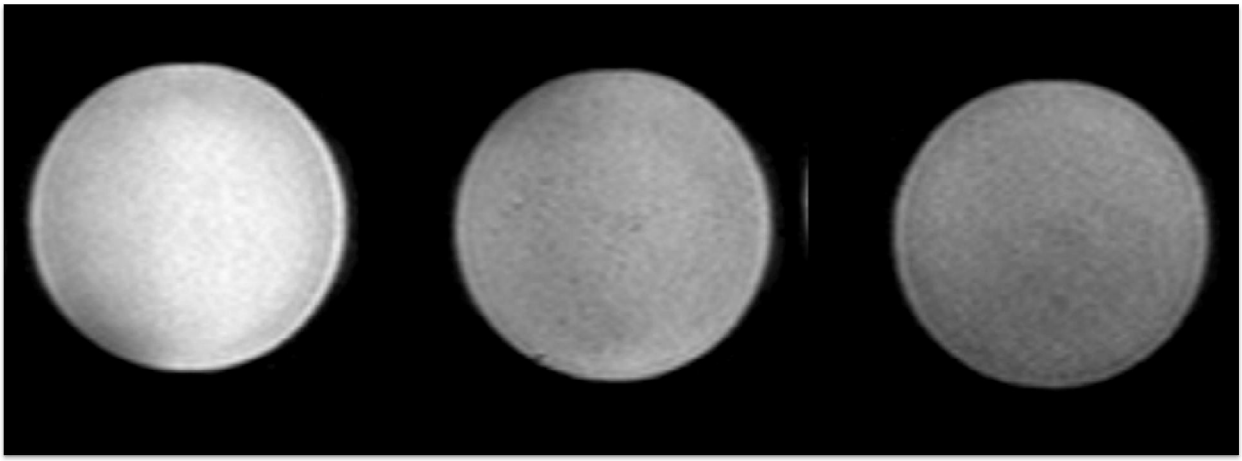
Osteogenic differentiation was performed with pig MSC to assess NPs' impact on MSCs. The NP-conjugated MSC accumulated calcium after the osteogenic induction. NPs did not interference with this process. MSC completed osteogenic differentiation after being incubated with 5ug/ml NPs. The accumulation of calcium was revealed by alizarin red-O staining (**figure 22**).

A:



Control NP: 5ug/ml NP: 10ug/ml

B:



Control NP: 5ug/ml NP: 10ug/ml

Figure: 17 Visualization of Nanoparticle: A: Hematoxylin staining of MSCs and NPs. NPs appeared in aggregated dots and attached to the cell body of MSCs. Only a small portion of the cells surface was in contact with the NPs. In 10ug/ml group more attached NPs are found. **B:** MSCs conjugated with NPs were detectable by MRI. The intensity of MRI signal of MSCs was correlated with attached NP numbers; 10ug/ml pretreatment resulted in stronger signal.

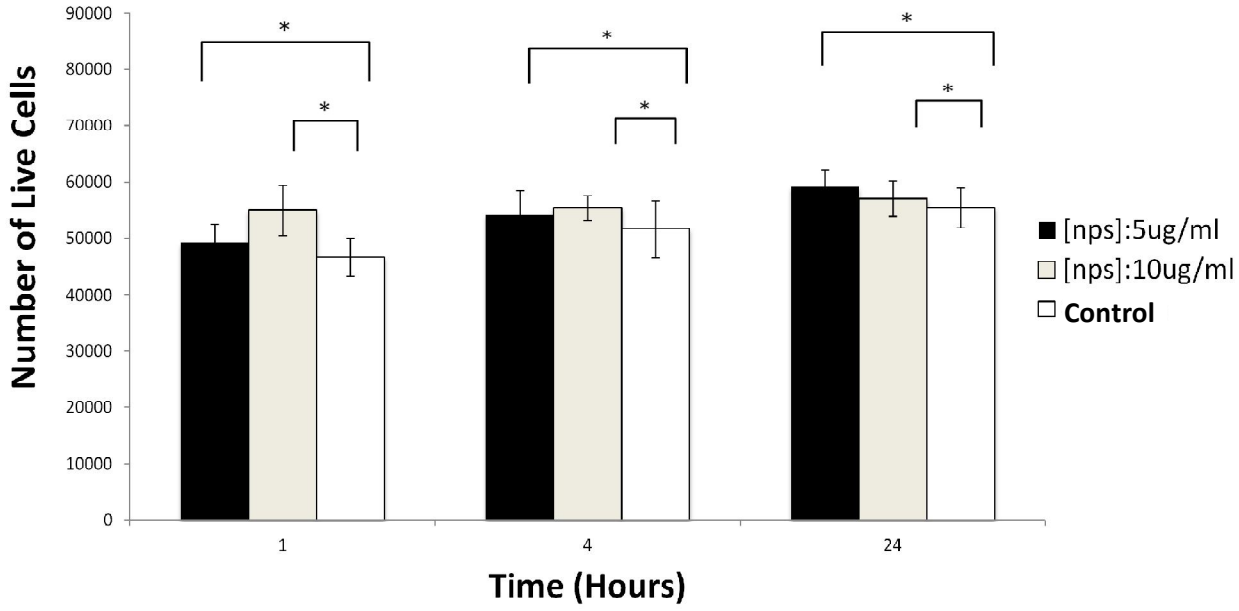


Figure: 18 Adhesion Assay of Nanoparticle-Mesenchymal Stem Cell: NPs did not affect MSC viability. The numbers of viable cells were similar in NP groups and the control group. There is no change at different incubation times: 1, 4 and 24 hours. Each column represents an average of 3 wells, *p>0.05, not significant.

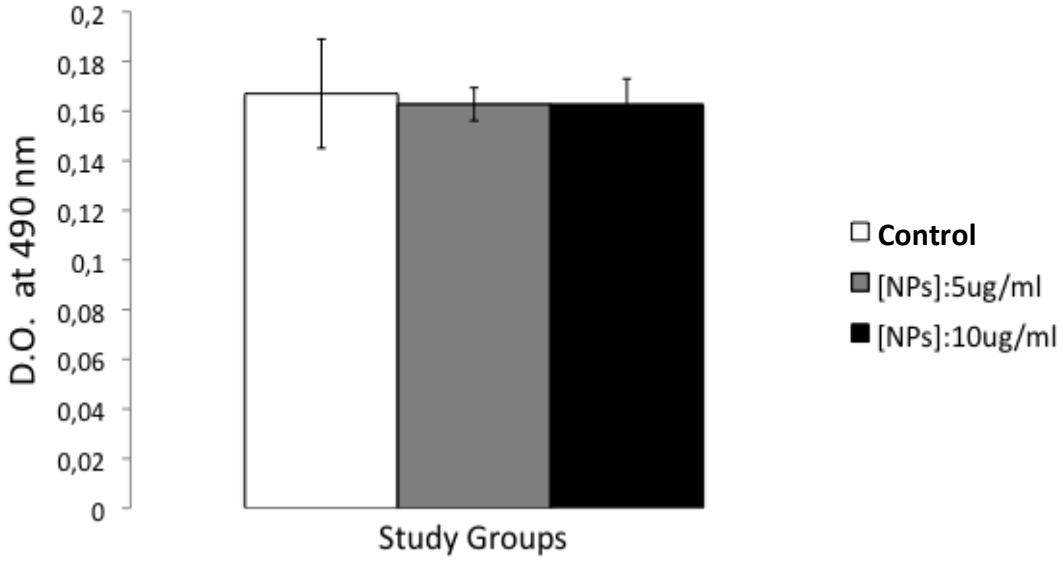


Figure: 19 Cellular Toxicity of Nanoparticle on Mesenchymal Stem Cells: NP conjugated pig MSC adhered as fast as the control pig MSC. Each column represents an average of 3.

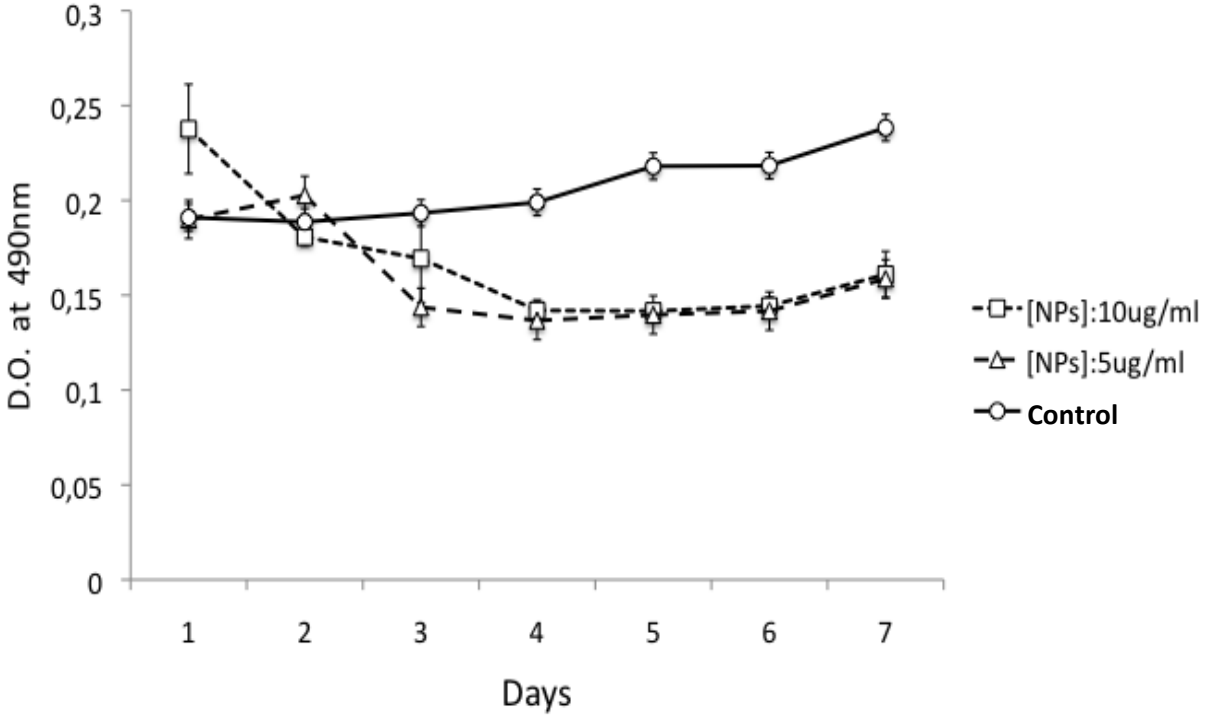


Figure: 20 Proliferation Assay of Nanoparticle-Mesenchymal Stem Cells: Instead of proliferation, the numbers NP conjugated MSCs dropped first and then stayed the same for the rest of the assay. 5ug/ml and 10ug/ml are the same in the later days of the assay (t>4 days). Control MSC displayed a normal increasing curve over time. Each data point is an average of 3 wells.

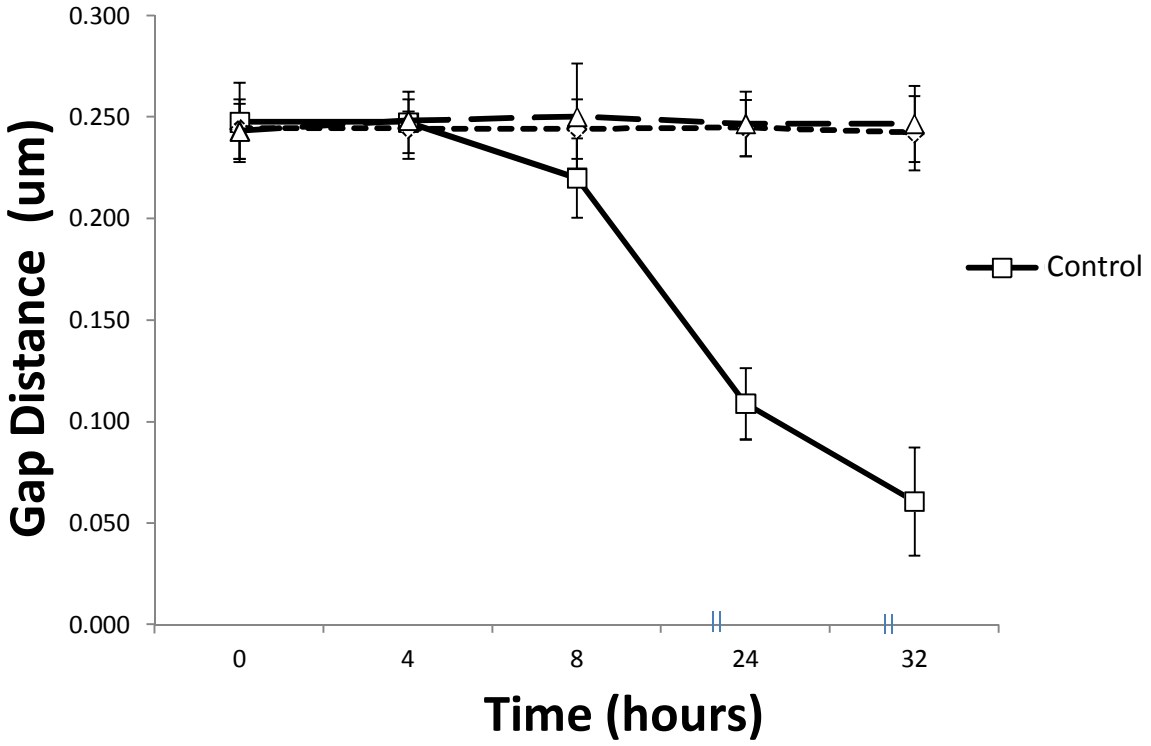


Figure: 21 Wound Healing Assay of Nanoparticle-Mesenchymal Stem Cells: Wound healing Assay of pig MSC conjugated with NP. The control MSCs closed up the wound significantly over time. However the NP conjugated MSC (both 5ug/ml and 10ug/ml group) did not migrate at all, and no change of wound width over 32 hours. Each data point is an average of 3 replicates.

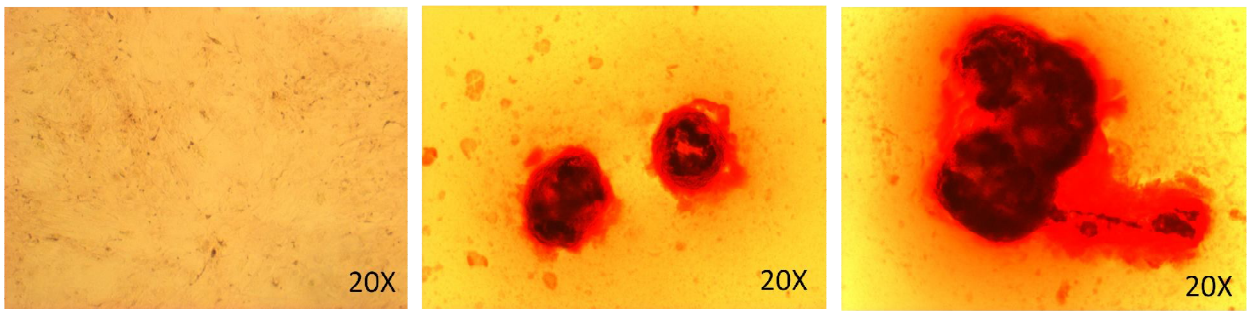


Figure: 22 Differentiation Assay of Nanoparticle-Mesenchymal Stem Cells: The pig MSC differentiated into osteocytes after osteogenic induction regardless of the presence of NPs. The calcium accumulation was revealed by Alizarin Red-O staining.

X Discussion & Conclusion

Isolation and ex vivo expansion of MSCs

BM-MSCs have shown tremendous therapeutic potential for cell and tissue replacement therapies due to their multipotency, homing properties and paracrine-mediated effects.(Saito, Kuang et al. 2002; Barbash, Chouraqui et al. 2003; Rombouts and Ploemacher 2003; Zimmet and Hare 2005; Chamberlain, Fox et al. 2007; Fox, Chamberlain et al. 2007; Abdallah and Kassem 2008; Penn and Mangi 2008) For my research projects, the isolation of BM MSC was successful from both rat and pig, species with success rates concordant to what have been reported for the isolation of MSC (Kern S 2006). MSC expressed typical surface markers, can differentiate and expand easily in vitro. BM remains an efficient source of MSCs. While we only explored BM-derived MSCs, alternatives source could have been pursued for the procurement of MSC, such as the adipose tissue. MSCs are presented in much higher abundance in adipose tissues but are phenotypically and functionally comparable (De Bari C 2001). No surface marker is known to specifically identify MSCs. But some works have shed some light. A new marker CD271 has been identified as a potential specific marker for MSC (Bühring HJ 2007).

Preconditioning

PDGF-BB has long been known to promote neovascularization by recruiting smooth muscle cells and endothelial cells to form new vascular network. Battegay et al found that PDGF-BB specifically stimulates cord/tube forming endothelial cells to construct new vessels and promote angiogenesis. The expression of PDGF-BB by angiogenic and non-angiogenetic endothelial cells are dramatically different (E J Battegay 1994). PDGF-BB has been shown to induce metastasis in tumors. Nissens et al found that in lymphatic system, the presence of PDGF-BB is important for lymph angiognesis and tumors metastases (Renhai Cao 2004). In the work by Ågren et al, PDGF-BB was found to stimulates the proliferation of fibroblasts isolated from

chronic venous leg ulcers, indicating a strong reparative ability (Magnus S Ågren 1999). Moreover, PDGF-BB also stimulates MSC. First PDGF-BB stimulates the proliferation(Pierre Cassiedie 1996) , and the migration of MSCs(Jörg Fiedler 2002). In previous studies, PDGF-BB has been shown to promote the MSC-related recovery of injured myocardium(Chung R 2009; Krausgrill B 2009). The results in our study correlate with the published literature, proving that PDGF-BB is a potent stimulator of MSC. Beyond its beneficial effects on MSC, PDGF-BB alone protects the heart after an ischemic injury. Hsie et al. found that by delivering PDGF-BB in (a nanomesh of fibers) into ischemically injured hearts, PDGF-BB stimulated endothelial cells to protect the heart(Patrick C.H. Hsieh 2006). In our study, PDGF-BB has been identified as an effective growth factor that offers protection against oxidative stress, serum deprivation and hypoxia. PDGF-BB has been reported to be able to enhance the engraftment of MSC in vivo (Krausgrill B 2009). This could be attributed to the anti-apoptotic effect of PDGF-BB on MSC against the oxidative and nutrition-low post-infarction environment. In addition to survival benefits, PDGF-BB also stimulates the cell migration and proliferation of MSC. The results from the time-dependent experiments of PDGF-BB provide insight to guide future studies. Longer PDGF-BB preconditioning time should be favoured to prevent early cell death for future translational studies.

It has been well documented that SDF-1α is a strong chemotactic factor for stem cells, improving their homing to the sites of injury. Otsuru et al found that SDF-1α expressed by vascular endothelial cells and de novo osteoblasts successfully attracted circulating BM-derived osteoblast progenitor cells to sites of bone formation (Satoru Otsuru 2008). Using SDF-1α, Schantz et al achieved polarized tissue formation in MSC-based tissue engineering. Using a customized micro-delivery system, SDF-1α guided cell migration in 3-dimensional polycaprolactone scaffold (Jan-Thorsten Schantz 2007). Preconditioning MSC with SDF-1α has been shown to be anti-apoptotic, to favor survival, and engraftment of MSC in the infarcted

myocardium (Pasha Z 2008). Yin et al. showed that SDF-1α preconditioning reduces hypoxia and serum deprivation induced apoptosis in MSCs through PI3K/Akt and ERK1/2 pathways (Qi Yin 2011). Thus, choosing SDF-1α was a reasonable choice for searching an appropriate preconditioning agent. However, in my studies, SDF-1α modestly improved MSC survival under stressful conditions and modestly improved their migration capacity. Our results are not consistent with favorable effects reported by other groups in the other animal models. The reason could be SDF-1a may be many a recruiting factor and the body provides a series of growth factors that synergized with SDF-1a. The reason could be that SDF-1α depends on a serial of growth factors to exert their beneficial effects. Since no such factors are present in in-vitro experiments, no beneficial effects could be seen. Likewise, the SDF-1α recruitment depends on a gradient of concentration, which is absent in the wound healing assay. This could be changed in the in vivo experiments, where the concentration gradient would be inevitable.

TSG-6 improved cardiac function after systematic co-administration with MSC; however preconditioning MSCs with TSG-6 did not offer any advantage to MSCs. Possible reasons could be: 1) the target cells of TSG-6 are not MSCs, but other cells, like cardiomyocytes; 2) TSG-6 may increase the paracrine effect of MSC which can be determined in further studies. No other studies have looked at the therapeutic potential of TSG-6 following ischemic cardiac injury.

Interestingly, hypoxia is not a threat to MSC, but somewhat of a rescuing factor. In my studies, MSCs exposed to both hypoxia and serum deprivation led to enhanced viability comparing to MSCs exposed to serum deprivation alone. This indicates that hypoxia alone is not a threatening factor to MSC. The native environment of the BM-derived MSCs likely provides an answer to this observation. In the bone marrow, blood flow is low and oxygen concentration is around 2%. Our observations are consistent with the reported beneficial effect of hypoxic preconditioning of MSCs. As discussed previously, a key effect of hypoxia on MSCs is to enhance the paracrine secretion of factors, such as SDF-1a, VEGF, HIF-1a, and AKT, (Chacko

SM 2010) and angioprotein-1(Hu X 2008). However, a stand-alone strategy of HPC might not provide as much benefits as the enhanced proliferation and migration that we observed with PDGF-BB. At present, most preconditioning strategies are using either a single-factor or a combination of similar. Adding the beneficiary effect of different strategies may synergize the effects of MSCs to CCT. When combined with PDGF-BB preconditioning, the MSCs displayed significant proliferation and full recuperation from the serum deprivation-induced stress. The combination of PDGF-BB with hypoxic preconditioning could be an interesting venue of investigation.

NPs and MSC

The conjugation of ferromagnetic NP with functionalized CD44 antibodies was successfully coupled to MSCs. MSC labeling allowed the visualizing using a clinically available 1.5 Tesla MRI. The NP-C44 (+) showed low toxicity to the MSCs and did not interfere with their adhesion ability. Cell proliferation was initially slowed but stabilized, thus was never impaired following coupling. However, a complete loss of in vitro migration was documented.

CD 44 is a highly expressed surface maker in MSCs, thus using it to anchor NPs was a logical decision. Nevertheless, docking of NP-Ab complex may have influenced with CD44 function. It has been documented that CD44 is involved in MSC migration and proliferation(Zohar R 200; Bühring HJ 2007). Thus, my experiments have shown that the coupling of NPs to CD44 could have partially altered cell physiology (mainly migration) but not viability. Conversely, of the loss in migratory capacity may not reflect a dysfunction of the cell. An additional explanation is that attachment of the bulky NP complexes could have impeded MSCs from migrating through the migration chamber pores. There is also a possibility that coupling MSCs with NPs may alter their therapeutic potential and immunological status, which are to be confirmed. Changing the antibody may restore the migration of conjugated MSC. This could be explored in future studies

either by synthesizing smaller scale NPs or functionalizing current NP's with a different antibody to other key MSC markers such as CD 90, CD105. From a practical standpoint, the NP-MSC complexes will be delivered directly into the injured heart (intracoronary or transmyocardial route) and not injected via intravenous route. The ability for them to migrate to the site of injury is of lesser importance for them to reach their target to secrete their beneficial paracrine factors.

Future Directions

The scope of the research projects is to explore two optimization strategies ie. nanotechnology and preconditioning. One could envision to cross-link PDGF-BB molecules to NPs on which functionalized antibodies (for example CD44 or CD90 or CD105) would provide specific coupling with MSC. Furthermore, the ferromagnetic components of the nano-shell can contribute to MSC labeling for cell tracking (figure 14). Such a strategy could be quite efficient as MSC will not be labeled and preconditioned simultaneously. The growth factor attached to the NP could not only stimulate the MSC after the implantation with high precision and potency, it could also contribute to stimulate the repair and the recovery of myocardial tissue. This latter aspect would need further studies to assess the effect of PDGF-BB on the infarcted myocardium. Finally, delivery of cell and growth factors can be further improved by NPs. The ferromagnetic components would thus offer the possibility for site-specific targeted delivery of cells and growth factors either by site-specific antibodies (antibodies to cardiac tissue epitopes) or by the guidance and retention via manipulation by external magnetic fields.

CONCLUSIONS

In summary, the clinical importance of IHD is ever growing especially with an aging population and a greater number of patients surviving to myocardial infarction. Among the potential stem-progenitor cells to heal the infarcted myocardium, MSCs is a promising population that offers many advantages, most notably their paracrine effects and their immunomodulatory properties.

Nevertheless, there are many challenges that need to be overcome before MSC-based cardiac cell therapy becomes a reality. Thus, strategies to optimize their efficiency need to be investigated.

In my research work, I have found that preconditioning can improve MSC viability against cellular stressors commonly found in the ischemic environment (hypoxia, serum deprivation and oxidative stress). Most notably, PDGF-BB appears to be the most promising cytokine to protect (pro-survival), increase the proliferation and favor the migration of MSCs. In addition, I documented that novel ferromagnetic NPs functionalized to a common MSC surface epitope, CD44, was detectable by MRI. Thus, my research work opens the doors for more focused research on multifunctional nano-based methods for cell imaging, preconditioning and targeting to improve MSC-based cardiac cell therapy.

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