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Review

Optimization of Pre-transplantation Conditions to Enhance the Efficacy of Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) are considered a potential tool for cell based regenerative therapy due to their immunomodulatory property, differentiation potentials, trophic activity as well as large donor pool. Poor engraftment and short term survival of transplanted MSCs are recognized as major limitations which were linked to early cellular ageing, loss of chemokine markers during *ex vivo* expansion, and hyper-immunogenicity to xeno-contaminated MSCs. These problems can be minimized by *ex vivo* expansion of MSCs in hypoxic culture condition using well defined or xeno-free media i.e., media supplemented with growth factors, human serum or platelet lysate. In addition to *ex vivo* expansion in hypoxic culture condition using well defined media, this review article describes the potentials of transient adaptation of expanded MSCs in autologous serum supplemented medium prior to transplantation for long term regenerative benefits. Such transient adaptation in autologous serum supplemented medium may help to increase chemokine receptor expression and tissue specific differentiation of *ex vivo* expanded MSCs, thus would provide long term regenerative benefits.

Key words: Mesenchymal stem cell, hyper-immunogenicity, chemokine receptors, xenogenic, autologous, allogeneic.

Introduction

The lineage committed progenitor cells or unipotent stem cells maintain cellular homeostasis [1]. Mesenchymal stem cells or mesenchymal stromal cells (MSCs) originated in bone-marrow, adipose tissue, dental pulp are involved in such homeostasis [2]. The number of MSCs increases in the peripheral blood during skeletal muscle injury [3] and osteoporosis [4]. Higher numbers of circulatory MSCs are also observed immediately after ischemic stroke and myocardial infarction [5, 6]. However, natural regenerative process alone is insufficient to repair a diseased or injured organ in case of myocardial infarction, stroke and spinal cord injuries because of the limited indigenous supply of the stem cells [7, 8]. Hence, adjunctive treatment such as stem cell based regenerative therapy has been given considerable attentions [7].

Due to pluripotency, embryonic stem cells (ESCs) are initially considered as the best source of stem cells for regenerative therapy [9]. Ethical issues over the use of ESCs compel researchers to search for suitable alternative [10]. In recent years, researchers developed a technology to generate induced pluripotent stem cells (iPSCs) that share characteristics of ESCs [11, 12]. Epigenetic memory, teratoma formation and immunogenicity related to the therapeutic potentials of iPSCs are yet to be resolved [13, 14]. Meanwhile, due to multi-differentiation potential, immunomodulatory effects, trophic functions, vasculogenesis potential of MSCs as well as its large donor pool make MSCs as the potential source for regenera-

tive therapy [2, 15, 16].

For each regenerative therapy, 50-400 million MSCs are required [17, 18]. The presence of very low number of MSCs within the tissues makes it impossible to isolate such a large number of MSCs from a single donor. Recently, derivation of MSCs from ESCs and iPSCs has been reported [19-23]. MSCs from these sources can also be used for cell based therapy and tissue engineering. Thus iPSCs may resolve patient-specific MSCs scarcity [20, 21, 23]. However, regardless of the sources, *ex vivo* expansion of MSCs prior transplantation is required to yield enough MSCs for cell based therapy [18, 24].

Several *in vitro*, *in vivo* and clinical studies reported encouraging regenerative potentials of MSCs [25-28]. However, low number of engrafted MSCs is considered as a major drawback for long term functional benefits [29, 30]. Different strategies were attempted to minimize such drawback such as intra-arterial delivery instead of intravenous delivery to avoid accumulation of MSCs in the lung [31, 32]; and modification of cell surface molecules through chemical, genetic and coating techniques to promote selective adherence to particular organs or tissues [33]. Several modifications in *ex vivo* or *in vitro* culture environment have also given due attention to overcome insufficient engraftment of MSCs such as culturing MSCs in hypoxic environment for partial [34] or entire [35] period of time; and culturing MSCs in medium that mimics the hypoxic condition [36]. Culture environment have an influential effect on cellular ageing and chemokine marker expression that may affect trafficking and engraftment of MSCs following transplantation [17, 18, 37]. In addition, there are safety concerns regarding hyper-immunogenicity to MSCs expanded in xenogenic serum [38] that might be a cause of acute rejection of transplanted MSCs.

To resolve the issue of poor engraftment of MSCs, this article elaborates the advantages and drawbacks of different approaches of *ex vivo* MSCs culture techniques. Finally a two phase *ex vivo* MSCs culture strategy is proposed as a possible way to produce clinical grade MSCs to enhance engraftment and regenerative outcomes. In phase 1, MSCs are initially isolated and expanded in human platelet lysate or pooled allogeneic AB-serum supplemented medium followed by the phase 2 where the expanded MSCs are cultured in autologus serum (patients' own) supplemented medium mainly to adapt the MSCs prior to transplantation (Figure 1).

Causes behind Poor Engraftment of MSCs Following Transplantation

For clinical trials, MSCs are mostly expanded in xenogenic serum supplemented media and incubated in ambient oxygen condition (Table 1). Use of MSCs (both autologous and allogeneic) for therapeutic purposes has been proven safe [41-55]. Clinical trials that used autologous MSCs to treat multiple system atrophy, renal transplant rejection, multiple sclerosis, ischemic cardiomyopathy, spinal cord injury and liver failure shown to have short term regenerative benefits or partial improvement [41, 42, 44, 46, 47, 50, 53, 55]. Clinical trials with allogeneic MSCs have also been shown significantly increased overall survival of graft-versus-host disease patients; improved forced expiration volume and global symptom score, and reduced infarct size in cardiovascular disease patients; improved Ankel Brachial Pressure Index in critical limb ischemia patient; and increased osteopoetic cell engraftment in patient with osteogenesis imperfecta [43-45, 48, 49, 54]. However, none of them have been reported the long term benefits from MSCs therapy.

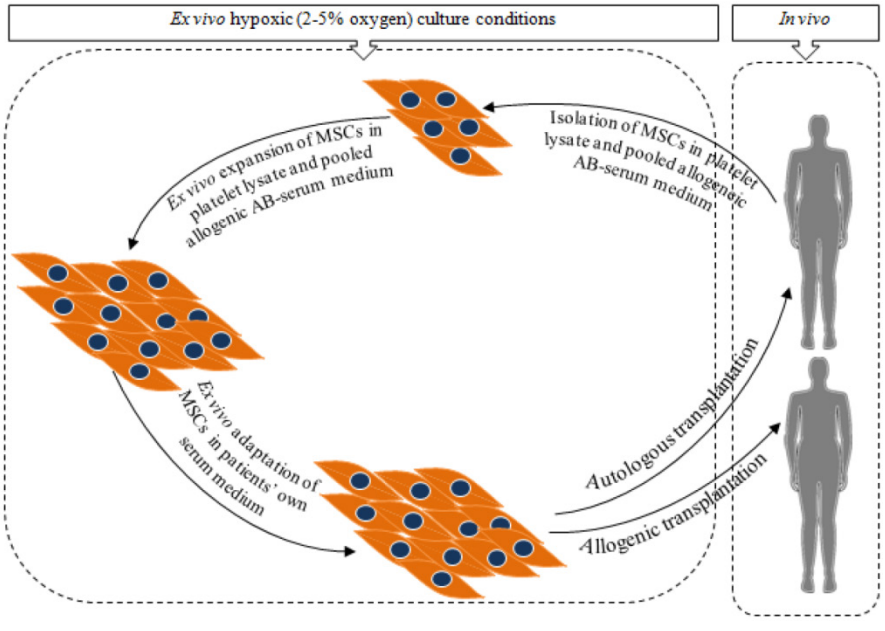

Figure 1: Steps to produce clinical grade MSCs for long term regenerative benefits. Isolation and expansion of MSCs in platelet lysate or pooled allogeneic AB-serum supplemented medium followed by adaptation in autologous serum (patients' own serum) supplemented medium. Hypoxic (2-5% oxygen) culture condition will be favourable for both the initial isolation and expansion later for adaptation [18, 36, 37, 39, 40].

Table 1: List of completed clinical trials using *ex vivo* expanded MSCs.

Clinical trial No.	Source of MSCS	Serum Supplement	Disease Treated	Dose No. of treatment	Route of Administration	Phase	Design	References
NCT00395200	Au BM	FBS	Multiple Sclerosis	1-2 $\times 10^6$ cells/ kg BW Single	Intravenous	I & II	Non-randomized, Safety/efficacy study, Single group assignment, Open label	[41, 42]
NCT00504803	Allo BM	Irradiated FBS	Graft-versus-host-disease	- Single	Intravenous	II	Non-randomized, Safety/efficacy study, Single group assignment, Open label	[43]
NCT01087996	Au BM Allo BM	-	Ischemic cardiomyopathy	20/100/200 $\times 10^6$ cells Single	Transendocardial	I & II	Randomized, Safety/efficacy study, Parallel assignment, Open label	[44]
NCT00114452	Allo BM	-	Myocardial infarction	0.5/1.6/5 $\times 10^6$ cells / kg BW Single	Intravenous	I	Randomized, Safety study, Parallel assignment, Double blind (Subject, Caregiver, Investigator, Outcomes assessor)	[45]
NCT00658073	Au BM	-	Renal transplant rejection	1-2$\times 10^6$ cells/ kg BW Twice	Intravenous	-	Randomized, Efficacy study, Parallel assignment, Open label	[46]
NCT00734396	Au BM	FBS	Renal transplant rejection	1$\times 10^6$ cells/ kg BW Twice	Intravenous	I & II	Non-randomized, Safety/efficacy study, Single group assignment, Open label	[47]
NCT00883870	Allo BM	-	Critical limb ischemia	2$\times 10^6$ cells/kg BW Single	Intramuscular (gastrocnemius muscle)	I & II	Randomized, Safety/efficacy study, Parallel assignment, Double blind (Subject, caregiver, investigator)	[48]
NCT00823316	Allo UCB	FBS	Graft rejection and graft-versus-host-disease	1 & 5 $\times 10^6$ cells/ kg BW Single	Intravenous	I & II	Randomized, Safety/efficacy study, Parallel assignment, Open label	[49]
NCT00911365	Au BM	FBS	Multiple system atrophy	40$\times 10^6$ cells Multiple	Intra arterial (1 time) Intravenous (3 times)	II	Randomized, Parallel assignment, Single blind (subject)	[50]
NCT01274975	Au AD	FBS	Spinal cord injury	400$\times 10^6$ cells Single	Intravenous	I	Randomized, Safety study, Single group assignment, Open label	[51]
NCT00683722	Allo BM	-	Coronary obstructive pulmonary disorder.	100$\times 10^6$ cells Multiple	Intravenous	II	Randomized, Safety/efficacy study, Parallel assignment, Double blind (subject, caregiver, investigator, outcomes assessor)	[52]
NCT00956891	Au BM	FBS	Liver failure	$\approx$100$\times 10^6$ cells Single	Hepatic artery	-	Case Control, retrospective	[53]
NCT00187018	Allo BM	FBS	Osteogenesis imperfecta	0.68-2.75$\times 10^3$ cells/kg BW Single	Intravenous	-	Non-Randomized, Safety/Efficacy Study, Single Group Assignment, Open Label	[54]
NCT00816803	Au BM	Serum free	Spinal cord injury	2$\times 10^6$ cells/ kg BW Multiple	Lumbar puncture	I & II	Safety/Efficacy Study, Parallel Assignment, Single Blind (Outcomes Assessor)	[55]

Au, Autologous; Allo, Allogeneic; BM, Bone marrow; UCB, Umbilical cord blood; AD, Adipose derived.

Prior to transplantation, MSCs are generally expanded in *ex vivo* culture conditions. Oxygen concentration of this culture environment is higher than MSCs' natural niche and the media contains xenoantigen [56, 57]. This culture conditions resulted in telomere shortening, early senescence, loss of chemokine receptors, and xeno-contamination in cultured MSCs [18, 37, 38]. Use of these *ex vivo* expanded MSCs may exhibit post-transplantation hyper-immunogenicity, improper trafficking and poor engraftment which in turn might result in failure of long term regenerative benefits.

Post-transplantation hyper-immunogenicity to MSCs cultured in xenogenic serum

MSCs are able to prevent expression of co-stimulatory molecules such as CD40, CD80, CD83 and CD86 and induce expression of inhibitory molecules such as B7-H1, B7-H4 and human leukocyte antigen G (HLA-G). At the same time, MSCs were reported to secrete soluble factors such as prostaglandin E2 (PGE2), transforming growth factor (TGF)-β, interleukin 10 (IL-10), nitric oxide (NO), hepatocyte growth factor (HGF) and indolamin-2,3-dioxygenase (IDO). These properties help MSCs to inhibit proliferation and function of cytotoxic T cells (T_C), natural killer (NK) cells and B cells, as well as prevent differentiation of monocytes into antigen-presenting dendritic cells (DCs). Notably, IDO plays an important role in activating immunosuppressive regulatory T cells (T_{regs}), facilitating differentiation of monocytes into M2 macrophages, and inhibit helper T cells (T_H) and T_C cells [58-60]. These immunomodulatory properties, makes MSCs a "universal donor" for stem cell based regenerative therapy [61].

In contrast, MSCs are described as immune evasive rather than immune privileged since differentiated MSCs or MSCs treated with interferon gamma (INF-γ) exhibit significantly higher expression of MHC class I and MHC class II. If mismatched, these MHC class I and MHC class II act as a source of hyper-immunogenicity thus the "universal donor" role of MSCs remains questionable [62, 63]. Besides, MSCs expanded in fetal bovine serum (FBS) supplemented media can be contaminated with bovine proteins that remains after multiple washings [64]. MSCs contaminated with N-glycolylneuraminic acid (Neu5Gc) xenoantigen [65, 66] originating from FBS potentially cause immunological reaction after transplantation

with anti-Neu5Gc antibodies present in human serum [67, 68]. Binding of anti-Neu5Gc antibody present in the human serum to xenoantigen Neu5Gc may cause post-transplantation lysis of the MSCs (Figure 2). Antibody dependent lysis of MSCs may take place in two ways: (i) complement-dependent cytotoxicity (CDC) and (ii) NK cell based antibody dependent cell-mediated cytotoxicity (ADCC).

MSCs cytotoxicity by complement activated membrane attack complex regardless of their source (autologous or allogeneic) has been reported in both *in vivo* and *in vitro* studies [66, 69]. However, CDC was less to autologous MSCs and this effect was greatly reduced when CD55 was highly expressed by MSCs [69]. In contrast, MSCs that show expression of complement regulatory proteins such as CD46, CD55, and CD59 are reported to be resistant to CDC [66]. The role of MSCs secreted factor H on inhibiting complement activation has also been reported [70]. For cell mediated cytotoxicity, higher phagocytic activity and ADCC was reported for the Neu5Gc-contaminated MSCs. In addition, reduced Neu5Gc contamination was reported to reduce cell mediated phagocytosis and lysis of the MSCs expanded in human serum supplemented medium [66]. Thus, CDC and ADCC to xeno-contaminated MSCs may lead to the acute rejection of transplanted cells [65, 66, 71]. Therefore, the effect of xenogenic serum on poor engraftment of transplanted MSCs regardless of autologous or allogeneic source should not be overlooked. Moreover, FBS supplemented media are potential source of viral or bacterial infections [72] and prions transmission [73].

Aging of MSCs during in vitro or ex vivo expansion

In standard culture conditions, MSCs reach senescence after a limited number of cell division [17]. Cellular ageing or replicative senescence affects proliferation and differentiation potentials of stem cells [74-77]. Senescence can be triggered by gradual loss of telomere repeat sequences, DNA damage and de-repression of the *INK4/ARF* locus [78]. Without any detectable telomere loss, oxidative stress-induced premature senescence may also take place in cultured cells [79, 80].

Among the different mechanisms of cellular aging, gradual loss of telomere sequence has been studied the most. Telomere is a guanine-rich DNA repeat sequence of the chromosomal end [81]. A reverse transcriptase named 'telomerase' plays key role in maintaining the telomeric repeats. Usually in rapidly proliferating germ cells and ES cells telomerase is highly expressed. After birth, telomerase level within cells including in MSCs gradually diminishes [81]. As a result, telomere repeat sequences in MSCs is gradually lost at a similar rate to non-stem cells [82]. Basic fibroblast growth factor (bFGF) was reported to maintain long telomeres without up-regulation of telomerase expression [83, 84]. However, the possible effect of bFGF on reduced differentiation potential and priming of MSCs should be taken into consideration when used in regenerative therapy [85].

Previous study has also shown that highly confluenced MSCs (100%) aged faster than the cells passaged at lower confluency (60-70%). During *in vitro* culture of MSCs, initial dense population showed prolonged population doubling time, higher expression of senescence associated β-galactosidase, and increased cell cycle arrest along with increased intracellular reactive oxygen species (ROS). However, difference in telomere length and alteration in p53 expression was not observed [80]. Contrary to this observation, the presence of ROS causes Wharton's jelly derived MSCs to be irregularly enlarged and flattened with granular cytoplasm and induce higher expression of other senescent markers namely p53, p21, p16 and lysosomal β-galactosidase [86]. Studies have also been reported that ambient culture environment cause higher ROS generation within cultured cells including MSCs compared to hypoxic culture environment (2-5%), and ROS is also responsible for faster telomere shortening and cellular senescence [17, 37].

These evidences suggest that aging of MSCs in culture is inevitable. It might not be possible to stop the aging process completely, yet it can be delayed and reduced by using proper growth factors and ma-

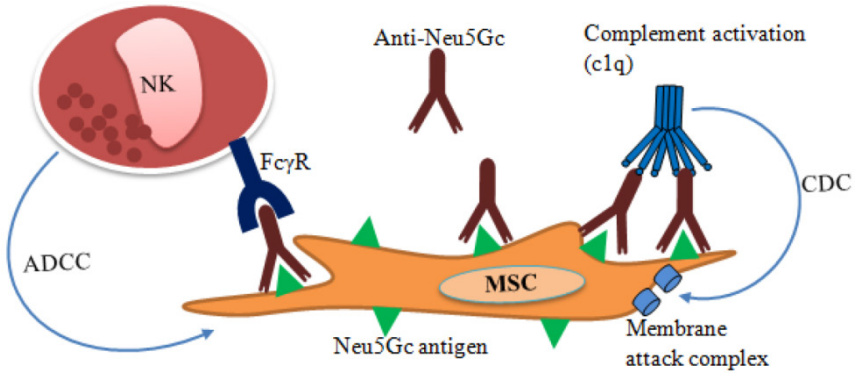

Figure 2: Immune response to transplanted xeno-contaminated MSCs. N-glycolylneuraminic acid (Neu5Gc) in FBS contaminates MSCs during *ex vivo* expansion. Anti-Neu5Gc antibody present in human serum may bind to the xeno-contaminated MSCs following transplantation. As a result, natural killer (NK) cells may bind to the antibody coated cells through Fc-gamma receptors (FcγR) and cause lysis by antibody dependent cell mediated cytotoxicity (ADCC). Anti-Neu5Gc antibody may also activate complement-dependent cytotoxicity (CDC) and cause lysis through membrane attack complex.

nipulating the culture practice and environment. As the success of stem cell based therapy depends on both the self-renewal and differentiation (towards the target cell populations) of the transplanted cells following engraftment [87], it is important to produce higher number of MSCs with longer telomere and regenerative potentials for successful regenerative therapy.

Ex vivo expansion in xenogenic serum may lead to improper trafficking and engraftment of the transplanted MSCs

Site specific trafficking and engraftment of transplanted MSCs are important in cell based regenerative therapy. These events are assisted by the affinity of chemokine receptors on MSCs (CXCR4, CXCR7, CX3CR1) to the chemokines (SDF-1, fractalkine) [34, 88-92]. Loss of these chemokine receptors during their *in vitro* or *ex vivo* expansion [93] is thought to affect the regenerative outcomes. Growth factors like platelet-derived growth factor (PDGF)-AB, PDGF-BB, insulin-like growth factor-1 (IGF-1), HGF, epidermal growth factor (EGF) and angiopoietin-1 (Ang-1) work as chemoattractants for MSCs [90, 94-97]. Inflammatory cytokine such as tumor necrosis factor alpha (TNF-α) also helps migration of MSCs towards the site of chemokines [90, 92]. All these paracrine signalling molecules are of primary importance for tissue specific migration and engraftment of MSCs. *In vivo* composition of these cytokines may vary depending on the type and stage of pathological conditions. Once isolated and expanded in *ex vivo* culture media, MSCs could embrace different cytokine composition, depending on the type of serum supplement. In other words, media supplementation with xenogenic serum, allogeneic human serum, platelet lysate or growth factors do not represent *in situ* cytokine composition of the serum of the patients undergoing stem cell based regenerative therapy. Therefore, paracrine signals to *ex vivo* expanded MSCs in those media supplement might cause improper trafficking consequently poor homing and engraftment.

Approaches to Enhance Engraftment and Regenerative Benefits of Cultured MSCs

In recent years, researchers have modified the culture media and environment (Figure 3) to improve engraftment efficiency of transplanted MSCs. Such modifications have shown partial improvement in the characteristics of MSCs. These modified *ex vivo* culture techniques have both advantages and limitations in producing clinical grade MSCs with higher engraftment potential.

Culture of MSCs in xeno-free media

From the very beginning of the development of synthetic cell culture medium by Harry Eagle in 1955, researchers were looking for suitable supplement to support cell viability and expansion. Animal serum especially FBS have been widely using to supplement media, as it provides almost all the necessary nutrients needed for the survival and proliferation of cells in culture condition [98, 99]. However, the uncertainty over the composition and concentration of cytokines and growth factors of FBS, possibility of disease transmission, and Neu5GC mediated hyper-immunogenicity [99, 100] are considered as drawbacks of FBS when used for isolation and expansion of stem cells for therapeutic purpose [64, 66, 101]. Hence, xeno-free media or well defined serum free media are being used as alternative [102-104]. Usually xeno-free media require different types of growth factors as supplement: recombinant human PDGF-BB, bFGF and TGF-β1 [105]. However, MSCs in both growth factors supplemented serum free media and FBS supplemented media showed similar growth kinetics and differentiation potential during *in vitro* expansion [105-107]. While, xeno-free media were found suitable for isolation and expansion of MSCs to maintain their multipotent differentiation capacity [102, 103], on the other hand there are also evidence that xeno-free medium does not support primary culture or isolation of MSCs. Indeed, after isolation of MSCs in any serum supplemented medium, MSCs can be further expanded and differentiated in xeno-free media [106, 107]. Moreover, xeno-free media does not offer solutions for early senescence, telomere shortening, and loss of chemokine receptors that are needed for site specific migration, engraftment and long term regeneration benefits.

Human serum and platelet lysate as alternative to growth factors and FBS

In the search for a solution to the problems related to severe immunogenicity to xeno-contamination caused by FBS, and limited isolation and expansion of MSCs in serum free media, researchers have proposed to use human serum, plasma and/or platelet lysate as possible replacement [56, 108-110]. The potential of autologous human serum in supporting the *in vitro* isolation and expansion of MSCs has gained considerable attention [56, 111-113]. Autologous human serum has been reported to have positive effect on the proliferation [112, 114] and differentiation potential of MSCs [56, 111, 114]. MSCs cultured in autologous human serum have shown more stable gene expression [56, 115] and higher motility [114] compared to MSCs cultured in FBS. Moreover, MSCs cultured in autologous serum sup-

posed to be more effective in immunomodulation as it significantly decreased the percentage of INF-γ producing activated T cells compared to MSC cultured in FBS [113]. Nonetheless, collection of blood from elderly, diseased and inflamed patients could be a limiting factor for serum preparation for the *ex vivo* expansion of MSCs prior to transplantation [111, 116, 117].

In addition to the autologous serum, allogeneic human serum and human cord blood serum has also been considered as suitable alternative to FBS [108, 118, 119]. However, it has been reported that allogeneic serum supplement during *in vitro* expansion of MSCs could cause over expression of genes that are responsible for growth arrest and cell death [56]. As opposed to that pooled allogeneic serum from adult AB-blood donors and pooled cord blood serum support isolation and expansion of MSCs while maintaining its differentiation potentials, motility and immunosuppressive property [114, 117, 120-124]. Lower level of hemagglutinin in pooled cord blood serum compared to adult serum, and lack of A and B hemagglutinin in pooled allogeneic AB- serum was attributed to be behind the success [120].

Among the different types of supplement from human source, platelet lysate was considered to be the best alternative to FBS because of its superiority in maintaining growth potential, genetic stability, immunomodulatory properties, and differentiation potential [110, 113, 125-130]. However, to produce clinical grade MSCs platelets free of infectious agents are of vital importance to prevent any possibility of disease transmission.

Transient adaptation of expanded MSCs in autologous serum supplemented media prior to transplantation

Despite the advantages of using the platelet lysate or allogeneic serum for *ex vivo* expansion, the microenvironment of the culture media with those supplement vary significantly compared to that of the patients' diseased organ. Hence, to make the *ex vivo* expanded MSCs accustomed with new microenvironment upon transplantation, incubation of the MSCs in well-defined or xeno-free media supplemented with freshly prepared autologous serum might be proven useful (Figure 4). Regeneration is a complex process and a large number of autocrine and paracrine signalling factors play a vital role in promoting this [131, 132]. Effect of cytokines, chemokines and growth factors on enhancing the chemotaxis and site specific migration of MSCs have been reported [90, 95, 133, 134]. Furthermore, enhanced site specific migration potential has been shown in MSCs pre-incubated with inflammatory cytokine TNF-α [90, 92]. In recent years researchers have acknowledged that the regenerative properties of microvesicles have been overlooked for years [135, 136]. Microvesicles are small (30-1000 nm) membranous vesicles released from the activated healthy cells or demaged cells during membrane blebbing [135, 137-139]. Rozmyslowicz *et al.* reported the transfer of CXCR4 receptor from the surface of platelets or megakaryocytes to the surface of CD4+/CXCR4-null cells through microvesicles [140]. Microvesicles are also able to transfer mRNA and miRNA from the cell of origin to the receiver cells [135, 141-143]. Induced epigenetic changes following internalization of microvesicles by receiver cells have been recognized as a universal phenomenon [135, 139, 144-146].

Several human and animal studies reported the increase of inflammatory cytokines, chemokines, growth factors and microvesicles in blood circulation following stroke and ischemic heart disease [5, 136, 147-151]. If the expanded MSCs are meant for transplantation in such pathological conditions where inflammatory cytokines, chemokines, growth factors and circulatory microvesicles are increased, positive response of the transplanted cells to the host microenvironment is highly important for successful regenerative therapy.

Notably, chemokines and inflammatory cytokines in the patients' freshly prepared autologous serum have the potential to enhance migratory potential of MSCs by inducing the expression of chemokine markers during incubation [5, 90, 92, 148]. Meanwhile, microvesicles present in the patients' autologous serum could enhance MSCs' migratory properties by delivering chemokine markers and as well as potentially cause epigenetic changes of MSCs by transferring host mRNA or miRNA [135, 137-139, 142-146]. Expression of chemokine markers on MSCs, transiently incubated in autologus serum, may facilitate tissue specific migration and engraftment. At the same time, the tissue specific modified cell population may produce microvesicles similar to that of injured tissues and organs [144] following engraftment. In turn, it might facilitate the migration and homing of circulatory MSCs and prevent apoptosis of cells in injured tissues or organs [136]. Since the number of circulatory MSCs and progenitor cells in circulation was found to be increased within 24 hours following stroke and myocardial infarction [5, 6, 8], incubation of MSCs for similar time period, i.e., 24 hours, would be considered sufficient for the transient *ex vivo* adaptation of the expanded MSCs.

Maintenance of hypoxic condition for genetic stability and stemness of MSCs

Tissues where the MSCs reside are hypoxic in

nature [57, 152-154]. *In vitro* hypoxic culture conditions (2-5% oxygen) help MSCs to grow faster while maintaining homogeneity, differentiation potential, increased chemokine receptors expression and retard the cellular ageing process as well [17, 18, 35, 37, 39]. Biosafety issue related to aneuploidy in expanded MSCs caused by oxidative stress [17] can be resolved by using hypoxic culture conditions [18]. Hypoxia inducible factor (HIF) especially HIF-1 plays an important role in maintaining the regenerative potential at hypoxic environment. Under hypoxic conditions, the lack of O_2 causes the prolyl-hydroxylation process to be suppressed resulting in stability of HIF-1α and this will facilitate translocation of its to nucleus. After nuclear translocation, it binds with HIF-1β to form the heterodimer. Then the HIF-1 heterodimer binds to a hypoxia-response element (HRE) in the target genes, associated with co-activators such as CBP/p300, and regulates the transcription of genes involved in metabolism, angiogenesis, cell migration and cell fate. Besides, through Notch signalling, HIF-1α regulate the expression of genes (e.g. HES and HEY) that maintain proliferation of cells [18]. To provide MSCs natural niche like oxygen concentration isolation, expansion and adaptation of MSCs should be done in hypoxic (2-5% oxygen) conditions. This culture environment will facilitate proliferation, site specific migration, and prevent early aging of MSCs. Moreover, hypoxic culture environment may increase biosafety by reducing aneuploidy [17, 18].

Serum Supplement	FBS	FBS	Xeno-Free	Allogeneic	Platelet Lysate	Autologous	References
Oxygen Tension	19-21%	2-5%	19-21%	19-21%	19-21%	19-21%	
Xeno-Contamination	√	√	×	×	×	×	[64-68]
Prion Contamination	√	√	×	×	×	×	[56, 73]
Supports 1° MSC Expansion	√	√	?	√	√	√	[102-107]
Proliferation	↓↑	↑	↓↑	↑	↑	↑	[17, 56, 110-130]
Senescence	↑	↓	NA	?	↓	NA	[17, 18, 56, 126]
Genetic Stability	↓	↑	NA	NA	↑	↑	[17, 39, 56, 125-127]
Motility	↓	NA	NA	↑	NA	↑	[114]
Suitability for Transplantation	?	?	√	√	√	√	[56, 66, 116, 119, 125]

Figure 3: Effect of culture media supplement on *in vitro* or *ex vivo* expansion of MSCs, and their suitability for clinical applications. FBS, allogeneic serum (pooled AB-serum), platelet lysate and autologous serum supplemented media support isolation and expansion of MSCs. Presence of xenoantigen in FBS make its use controversial. Although xeno-free media do not support isolation, they support further expansion of MSCs isolated in any serum supplemented media. MSCs expanded in xeno-free media and media supplemented with platelet lysate, pooled allogeneic AB-serum or autologous serum are considered appropriate for regenerative therapy as they are free from any xeno-contamination. Abbreviations are: MSC, Mesenchymal stem cells; FBS, fetal bovine serum. [↓= decrease; ↑= increase; ↓↑= regular/unchanged; ×=absent; √ = present; ?= controversial; NA= data not available]

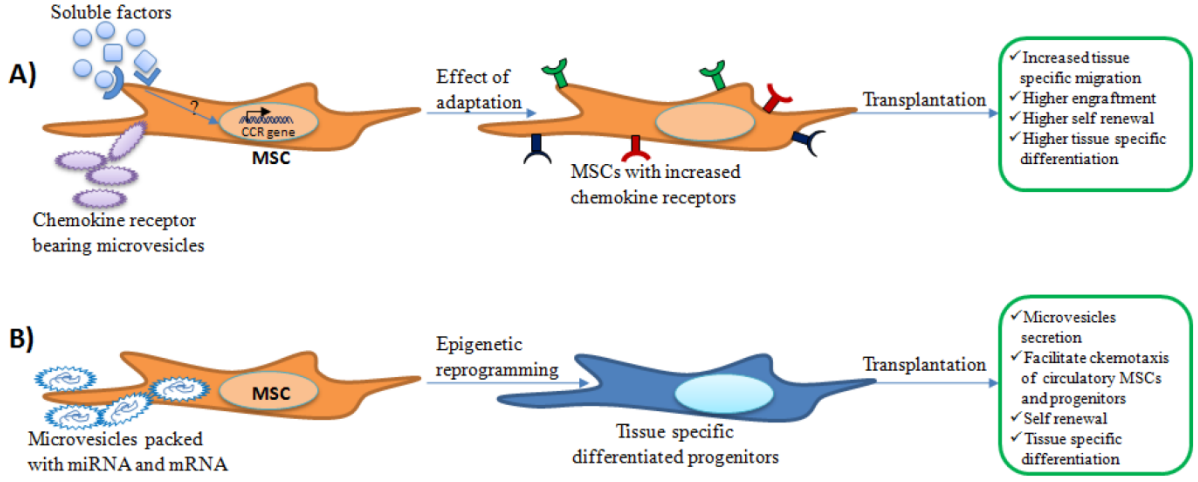

Figure 4: Possible effects of adaptation of expanded MSCs in autologous serum supplemented media on engraftment and regenerative efficiency. A) Cytokines and other soluble factors present in the freshly prepared autologous serum may increase chemokine receptor (CCR) expression on MSCs. Microvesicles present in the serum may deliver chemokine receptors that might enhance chemotactic properties of incubated MSCs. Expression of chemokine receptors may facilitate tissue specific migration and further regenerative benefits. B) In addition, mRNA or miRNA packed in microvesicle may be delivered to MSCs during incubation that could aid in tissue specific differentiation. Upon transplantation, these tissue specific differentiated cells may produce microvesicles similar to the cells within the injured tissues. This may help tissue specific migration of circulatory progenitors or MSCs and enhance regenerative outcomes.

Conclusion

MSCs have tremendous potential in regenerative medicine. It is the store house of several cytokines and paracrine signalling factors that facilitates the process of regeneration. For successful translation of the use of MSCs from bench side to bedside, *ex vivo* expansion of MSCs prior to transplantation requires appropriate supplement to minimize the impact of xenogenic serum. This article highlights comparative benefits of human platelet lysate and pooled human-AB serum as supplement for expansion of MSCs and subsequent transient *ex vivo* adaptation of the expanded MSCs in autologus serum supplement media prior to transplantation. Hypoxic culture environment must be maintained both for *ex vivo* expansion and adaptation. Collectively, *ex vivo* expansion using human platelet lysate and pooled human-AB serum and transient adaptation in autologus serum in hypoxic condition might prove useful in enhancing the regenerative potential of MSCs.

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Competing Interests

The authors have declared that no competing interest exists.

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