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Review

Key aspects of the mesenchymal stem cells (MSCs) in tissue engineering for *in vitro* skeletal muscle regeneration

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Tissue engineering, directly associated with Biotechnology and Biomedical Sciences, is an emerging field of research and development. The main issue of tissue engineering is to precisely and safely regenerate or reconstruct injured tissues of skeletal muscle, bone, teeth, neural, cardiac, cartilage etc. One of the primary requirements for tissue engineering development is a constant source of supplementary stem cells which have the ability to be differentiated into various tissue types such as condroblast, osteoblast or myoblast cells. In modern tissue engineering, mesenchymal stem cells (MSCs) take the most important part for *in vitro* growth or regeneration of the required tissues. Selective growth factors are also needed to optimize the growth process. In the preset review, an attempt has been made to focus on the crucial beneficial issues of mesenchymal stem cells for the skeletal muscle regeneration and repair. Though the detailed processes on how dystrophic muscles are replaced by fibrotic tissues inside living organs is still not very clearly understood, we have briefly discussed the overall ideas and future prospects of skeletal muscle regeneration (*in vitro*) using MSCs on 3D scaffold with optimum experimental conditions (use of various medias, growth factors etc.).

Key words: Mesenchymal stem cell, skeletal muscle, tissue engineering, tissue regeneration, growth factors, medical implant, biomaterials.

INTRODUCTION

Recently, considerable interest has been paid on the skeletal muscle regeneration by tissue engineering (Ground, 1999; Kagami et al., 2011; Grefte et al., 2007; Charge and Runiki, 2004; Jin et al., 2008). Tissue engineering research merges different branches of bioscience, engineering and medicine (Kagami et al., 2011; Mauro, 1961; Muir et al., 1965; Chen and Goldhamer, 2003; Shi and Garry, 2006). In case of a minor muscle injury, some growth factors based therapy seems to improve the muscle healing. The effects of growth factors on the activation, proliferation and differentiation of satellite cells have already been discussed elsewhere (Collins et al., 2005; Wagers and Conboy, 2005). Growth factors with stimulatory effects act *in vivo* to enhance the regeneration of the muscle tissue. However, in case of major muscle injuries, scaffold-based tissue engineering therapy (TET) is generally implemented to fill up the large defects. Discovery of different sources (like cord blood etc.) of multifunctional mesenchymal stem cells and rapid technological progress in biodegradable scaffold designing have encouraged tissue engineers and biotechnologists to apply their obtained results in therapeutics (Sadat et al., 2007; Schulze et al., 2005).

In the entire tissue regeneration process, a vital role is played by the Mesenchymal stem cell (MSC) which can differentiate into various tissue types such as bone, cartilage or skeletal muscle (Deans and Moseley, 2000; Harris et al., 2007; Hruba et al., 2008; Labarge and Blau,

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2002). Prockop (1997). Friedenstein and his research group (Friedenstein et al., 1966; 1974; 1976) first defined the bone marrow (BM) derived fibroblast-colony-forming-cells that adhered to cell culture surfaces. The fibroblast-colony forming cells were termed mesenchymal stem cells (MSC) or BM stromal cells (BMSC) (Pittenger et al., 1999). The MSCs might conventionally be defined as the adherent non-hematopoietic cells expressing positive markers such as CD90, CD105, CD73, and negative markers for CD14, CD34, and CD45 (Campioni et al., 2009; Jin et al., 2008). It has become a conventional and important method to derive MSCs from bone marrow. Subsequently, MSCs were also obtained from many other unconventional sources such as human placenta, adipose tissue (Zannettino et al., 2008), heart (Hoogduijin et al., 2007), Wharton's Jelly (Chao et al., 2008), dental pulp (Jo et al., 2007), peripheral blood (He et al., 2007), cord blood (Oh et al., 2008), menstrual blood (Patel et al., 2008; Hida et al., 2008) etc.

Recent researchers working with stem cells have realized the important functions of the mesenchymal stem cells (MSCs) in therapeutics and tissue regeneration. For example, much attention has recently been paid to overcome one of the major problems of tissue engineering associate with immune rejection (Martinez et al., 2011; Ichim et al., 2010) where MSCs take a vital role to overcome this problem along with the usual tissue regeneration process. In the present review, we have attempted to highlight some of the key functions of MSCs that trigger the *in vitro* tissue regeneration process without immune rejection. We have presented an overview of the progress and prospects of next generation skeletal muscle regeneration. For the sake of completeness, the importance of scaffold structure and growth factors in the tissue regeneration process has also been briefly discussed.

COMPARISON AMONG DIFFERENT SOURCES OF MESENCHYMAL STEM CELLS

The masenchymal stem cells for tissue engineering are available from different sources. Among the various available sources of MSCs, bone marrow (BM) has been widely accepted as the conventional source of MSCs. BM derived MSCs (abbreviated as BM-MSC) could be cultured rapidly and the process is also comparatively quicker. But other sources initially contain only mononuclear cell (MNCs), which have to be further differentiated into MSCs. Researchers have proposed that adult bone marrow-derived cells can gradually contribute to muscle cells (Jin et al., 2008). Even the transplanted bone marrow-derived cells have the potential to become satellite cells (mononucleated myogenic cells) that are found in muscle fibres and have the tendency to differentiate into specific type of cells (muscle cells) to repair muscle injury (Musaro et al., 2007). The main problems associated with bone marrow derived stem cells are their unavailability in large number, it needs suitable donors and the process of SC collection from bone marrow by surgery is rather painful. It has, however, been observed that adipose tissues (AT) have a higher capacity of muscle differentiation ability than that of the bone marrow derived MSCs (Hoogduijn et al., 2007). But it takes relatively much longer time to regenerate skeletal muscle from adipose tissue derived MSCs. To obtain MSCs from other sources like cord blood, adipose tissue or placenta, it takes more time even over a month (Semenov et al., 2009; Miao et al., 2006; Green et al., 2010; Kang et al., 2006). Compared to those obtained from other sources, above mentioned adipose tissue having more number of MNC has better quality in terms of ability to differentiate into MSCs. Some research reports also claimed highest concentration of MSCs found from adipose tissue (Green et al., 2010; Im et al., 2005; Puissant et al., 2005). However, the most easily available sources of stem cells are considered to be cord blood, placenta, and adipose tissues. No ethical problem is involved in the procurement of MSCs from such sources. Importantly, it is possible to obtain MSCs from cryo-preserved human cord blood which is an additional advantage. The obtained cells have also multi-differentiation capacity similarly to that of bone the marrow derived MSC (Lee et al., 2004a; Robinson et al., 2011).

Recently, umbilical cord blood (UCB) is being recognized as an alternative source of hematopoietic stem cells (HSC) and MSCs can be well used for transplantation and in regenerative medicine (Lee et al ., 2004b; Zhou et al., 2003). The SCs derived from the UCB (UCB-SCs) are younger and there is little or no problem of variation of number of UCB derived MSCs with age as encountered in the case of BM derived SCs (Gang et al., 2004). UCB-SCs have already been successfully used *in vitro* to differentiate into insulin and c-peptide-producing cells (Denner et al., 2007; Oh et al., 2008). Most interestingly, UCB-MSCs showed no adipogenic differentiation capacity, in contrast to BM- and adipose tissue (AT)-MSCs. Both UCB and AT are attractive alternative to BM in isolating MSCs. AT contains MSC at the highest frequency, while UCB seems to be expanded to higher numbers (Kern et al., 2066). There are also some other additional advantages of using UCB derived stem cells in tissue engineering as mentioned below: (i) The CB-SCs are available in large numbers. (ii) Lower risk of virus infection. Infectious agents such as cytomegalo virus are rare exceptions (Rubinstein et al., 1993). (iii) They are more potent in application in allograft (same patient) transplantation. The cord blood derived SC, compared to BM-SC, has a bigger telomerase length and demonstrates higher proliferation potential (Vaziri et al., 1994). (iv) The Human placenta derived MSCs can even be combined with HSCs from UCB to reduce the

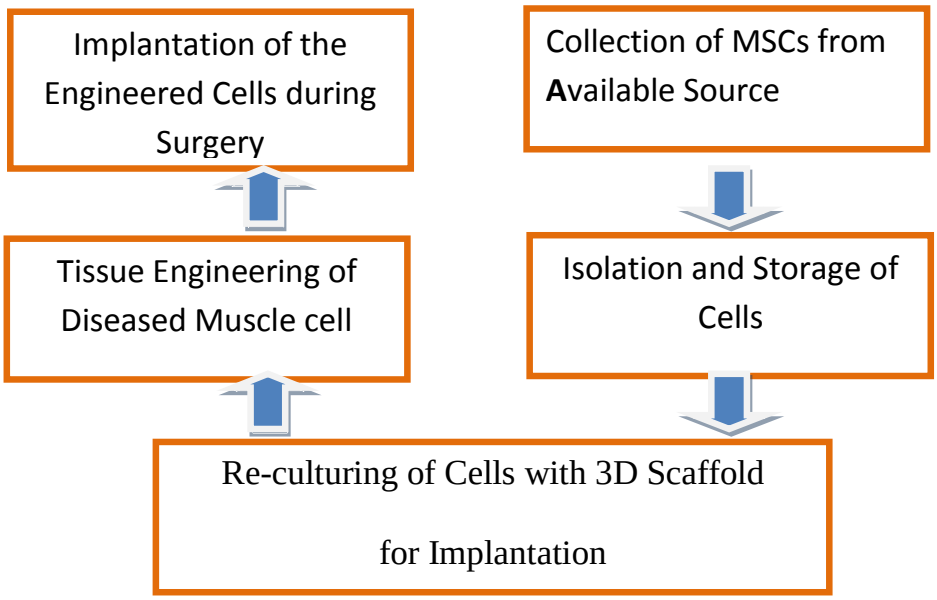

Figure 1. Stem cells collection from various sources, storage of stem cells, culturing on appropriate scaffold and its implantation for tissue regeneration in the body when required.

potential graft-versus-host disease (GVHD) in recipients (Magro et al., 2006). Usage of CB overcomes considerable problems encountered with other sources of CMs, such as allergenic, ethical, and tumorigenic issues. UCB-SC has been reported to repair myocardial hepatocytes (Yamada et al., 2007), muscles and neural tissues (Ikeda et al., 2004). Moreover, recently attempts are being made for the use of both autologous and allogeneic SCs as potential sources of safe and effective immunomodulation (Limbert et al., 2006). So when properly expanded in culture, UCB-MSCs are expected to be the most important practical units for skeletal, cardiac (Chen et al., 2004) or other muscle regeneration in the near future. However, to work with UCB-MSCs, the main drawbacks are the prolonged time to cell recovery, the early mortality associated with CB transplant and the overall lack of knowledge of working with UCB transplantation. Intensive research is going on to overcome these problems. It is to be noted that no significant differences concerning the morphology and immune phenotype of the MSCs derived from different sources were observed (Kern et al., 2006).

MESENCHYMAL STEM CELLS AND TISSUE ENGINEERING PROCESS WITH 3D SCAFFOLD

In the tissue regeneration process, as mentioned earlier, MSCs play the vital role due to the fact that it can differentiate into various tissue types such as bone, cartilage or skeletal muscle (Deans and Moseley, 2000). The first idea of the possibility of tissue engineering/tissue regeneration was put forwarded by the paediatric orthopaedic surgeon Dr. W T Green of Boston's Children Hospital. Dr. Green tried to implant chondrocytes on mouse to regenerate new cartilage (Green, 1977). Though this first experiment was only partially successful, but the outcome of this research paved the way for the use of stem cells in tissue regeneration. Dr Green's innovative idea about the use of stem cells for implantation and tissue regeneration later gave birth to the tissue engineering. Along with this, a new technique of biocompatible sophisticated scaffold designing was also subsequently developed for providing the growing tissues a desirable structure, which could be loaded with stem cells for implantation (Tan et al., 2003; Zein et al., 2002). In tissue engineering, the application of a three-dimensional (3D) scaffold is used for filling up the defect and to induce the formation of new muscles. In general, for skeletal muscle regeneration, the first step is to regenerate skeletal muscle cells *in vitro* on appropriate scaffold by using optimum culture conditions (growth factors etc.) and then to implanting it into the desired part of the body (Charge and Rudnicki, 2004). Such externally developed (i.e. bio-engineered) tissues are allowed to grow inside the body and reconstruct the affected muscle of the patient (Zammit et al., 2002; Lanza et al., 2007; Khademhosseini et al., 2009). A schematic diagram describing the tissue engineering process is shown in the self explanatory Figure 1.

In this context, it is to be noted that after any muscle injury, several series of well-coordinated optimum and compromising events take place inside the body, necessary for the proper development of the damaged

tissue (Zammit et al., 2002; Lazarus et al., 1996). The said optimum conditions triggering the cells to regenerate are initiated immediately after injury by the release of several growth factors and cytokines from various cells of the injured blood vessels as well as inflammatory cells. Interestingly, the entire tissue regeneration happens in a specific site and the tissue growth is optimally controlled (Jones et al, 1986). Though the actual mechanism of this phenomenon has not yet been conclusively understood, it is believed that the MSCs have immense potentiality to revolutionize the conventional therapeutic practices (Tedesco et al., 2010; Mauro, 1961).

SOME IMPORTANT ASPECTS OF MASENCHYMAL STEM CELLS IN TISSUE REGENERATION: IMMUNE RESPONSE, INFLAMATION AND HEALING

As mentioned earlier, one of the main problems of skeletal muscle tissue regeneration or myoblasts therapy is associated with chronic immune rejection (Fan et al., 1996; Qu et al., 1998). Various model experiments have been carried out clinically to reduce immune rejection, but these reconstructive strategies do not always yield satisfactory results (Ianni et al., 2008). Interestingly, it is found that MSCs can not only help prevent this problem, but also help to regenerate selective tissues without immune rejection. It was observed that MSCs prevent inflammation and simultaneously accelerates the healing process (Ripoll et al., 2011). This means that MSCs are capable of giving protection against autoimmune pathogenesis (Zhou et al., 2008). Furthermore, it has also been reported that human MSCs suppress *in vitro* allogeneic T cell responses. T cells play a central role in immunity and according to current research reports, T cells appear to mediate muscle damage through secretion of osteopontin (Vetrone et al., 2009). This indicates that T cells have the ability to directly promote fibrosis, as well as direct perforin-mediated cytotoxicity (Spencer et al., 1997).

It is to be noted that immediately after injury, there appears a phase of myofiber degeneration, which is initiated by the release of proteases at the place of damaged tissue (Hurme and Kalimo, 1992; Cantini and Carraro, 1995; Dipietro, 1995). Proteases automatically digest myofibers that result in tissue debris at the zone of injury. Along with this process, there is chemotaxis of neutrophils and macrophages related to this area. Due to macrophage activity, local debris is phagocytosed and proceeds to induce a local inflammatory response (Dipietro, 1995; Tidball et al., 1999; Robertson et al., 1993). So it appears that macrophages take part to induce inflammation. Some experimental studies also suggest that macrophages secrete several growth factors that enhance tissue regeneration process (Robertson et al, 1993; Summan et al., 2006).

It has been reported that MSCs posses various strong anti-inflammatory properties. For instances, MSCs suppress NK (natural killer) and T cytotoxic cell function (Selmani et al., 2008), reduce macrophage activities (Spaggiari et al., 2009; Yang et al., 2008) inducing generation of Treg cells (Casiraghi et al, 2008), inhibit Th1, Th17 cell generations (Batten et al., 2006), suppress Dendritic Cell (DC) maturation etc. Though the exact mechanism how MSCs regulate such immune suppression is not very clear, there are some important experimental results that demonstrate that different immune functions are suppressed by MSCs through the release of immune suppressive cytokines such as TGF-b, LIF etc., expression of T and NK inhibitory enzyme indolamine 2,3-deoxygenase, expressing contact-dependent inhibitory molecules such as PD-1L and via production of soluble HLA-G (Campioni et al., 2009; Bishopric et al., 2008; Nasef et al., 2008). These important immune modulatory properties are found to induce active immune response (Renner et al., 2009; Ryan et al., 2007; Opitz et al. 2009; Rizzo et al., 2008). As active immunity is cell mediated, it might be concluded that MSCs play very important role in this process. In bone marrow too, one of the main functions of MSC is to protect the hematopoietic precursor from inflammatory damage as MSCs have some control over immune system (Riordan et al., 2007). This also confirms anti-inflammatory effects of MSCs, which could reduce the chance of immune rejection and hence optimise the overall growth of tissue (Jones et al., 2007). Several other inhibitions of chronic inflammatory processes, such as models of autoimmune arthritis, diabetes, multiple sclerosis and lupus, have also been well documented which are optimised by the MSCs. It is also recognized that MSCs have the properties that allow transplantation across major histocompatibility complex (MHC) barriers (Blanck and Ringde, 2007). So, after allogeneic haematopoietic stem cell transplantation (HSCT), these beneficial immunomodulatory effects of MSCs could be utilized to prevent rejection of organ transplants and also to repair tissue damage caused by autoimmune-induced inflammatory diseases like Crohn's disease, ulcerous colitis, graft-versus-host disease (GVHD) of the gut etc. These are some of the advanced indications of enormous possibility of using multifunctional MSCs to regenerate selective or desired tissues free from immune rejection and also to protective against several common diseases. Currently intensive research work is going on in these directions.

SITE SPECIFICITY OF MESENCHYMAL STEM CELLS

Site specificity is another important factor of tissue growth. Regeneration of new tissue has to be highly précised and should also be at the required injured site

where replacement of old tissue with the new one is only needed. Here some crucial roles are performed by the Stromal cell-derived factor-1 (SDF-1). It has been observed that factor-1 (SDF-1) stimulates stem cell propagation in the desired sites. For example, SDF-1 has been demonstrated to be associated with the mobilization of masenchymal stem cells into the periphery and homing only to the site of injury (Penn, 2009). So it becomes possible to efficiently control the tissue growth as well as selection of the specific site. In addition, this property allows MSCs to differentiate into various specific tissues and to complement dystrophic deficiency. This also indicates the therapeutic aspects of MSCs for Duchenne muscular dystrophy (DMD), which is a lethal X-linked musculodegenerative condition and also a genetic defect whose manifestation is augmented by inflammatory mechanisms. MSCs produce paracrine factors that directly help to inhibit apoptosis, stimulate endogenous cell proliferation and activate tissue resident stem cells only at the site of injury (Tidball, 1995).

It has also been observed that under proper circumstances, MSCs can differentiate to specific tissues that had already been injured and urgently needed to be regenerated to support normal muscle function. Most importantly, the site specificity and efficiency are highly desirable to regenerate the damaged part without disturbing the rest where no further regeneration is required. Recently Tao et al. (2009) have precisely demonstrated that MSCs might differentiate selectively into tissue types that have only been injured. They have systemically administered the growth of MSCs to clone into immune deficient mice after subsequent carbon tetrachloride hepatic injury. Further to add, differentiation of MSCs only into albumin expressing hepatocyte-like cells was also observed in those mice. All these are strong evidences that MSCs possess some unique properties that are specific to the site of injury.

POTENTIAL RELATION OF MESENCHYMAL STEM CELLS WITH CHEMOKINE SIGNALLING

In the tissue regeneration process, chemokine signalling has a major role to support the entire immune response. MSCs continuously support the entire immune system even inside the body. This provides evidences that MSCs might also have the properties to influence cell signalling procedure especially for chemokines. Ichim and his group have shown that chemokines are directly related to major immune system (Ichim et al., 2010; Charge and Rudnicki, 2004). Based on cytokine production and arginine metabolism, two types of macrophages viz. M1 and M2 have been distinguished. The M1 macrophage is primarily antiangiogenic and shows some properties that can inhibit the tissue growth. For example, stimulation of M1 macrophage inhibits tumour growth in cancerous cells (Eriksson et al., 2009), whereas M2 macrophage shows more constructive role in the tissue regeneration process. Moreover, M2 macrophages are anti-inflammatory, support angiogenesis, and they are also associated with tissue repair via regeneration (Mantovani et al., 2004; Sica et al., 2008). Recent findings also show that regulatory interactions between cytotoxic M1 macrophages in dystrophic muscle and anti-inflammatory M2 macrophages are important in regulating the overall balance between the death of dystrophic muscle and regenerative processes. Manipulation of the balancing between the functions of M1 and M2 macrophages can, therefore, affect the severity of muscular dystrophy. This suggests that manipulation of macrophage phenotype *in vivo* may have potential therapeutic values for the treatment of various diseases (Tidball, 2002; Tdball and Villalta, 2010). In summary, MSCs, cytokine production, M1 and M2 macrophages must jointly play important part in the regenerative tissue engineering process. M1 might be used for the inhibitory function of tissue growth while M2 for the tissue regeneration process. This phenomenon was also described (Wang et al., 2007) as constructive in case of M1 and destructive in case of M2. So, attempts are being made to utilize the dual nature and activity of macrophages to control over tissue regeneration process by accelerating or controlling tissue growth according to our need.

One might find direct link between MSCs and several chemokine signals associated with MSC migration into specific injured tissues for the formation of new ones. The relevance of cytokines for the development of protective immune system has already been studied and well established. It is now quite clear that cytokines might regulate cell immunity during cell regeneration. However, at the present stage, it is not very clear whether cytokines can establish and maintain immunological memory in those stem cells. Further intensive research is indeed necessary for a deeper understanding of the relation between chemokine signalling and MSC migration.

STRUCTURE DESIGN OF NEO TISSUES

Stem cell research also shed light on the regulation of tissue structure determination. When muscle stem cells, present beneath the muscle basal lamina, are activated by massive proliferation and differentiation of myoblasts at the edge of injury, formation of new muscle fibres begins at that site to regenerate tissue. After that, fusion of myoblasts occurs themselves to the damaged tissue to regenerate new myofibers. But, our present knowledge to predict the structure of those muscles that accelerate regenerative medicine is not well developed. According to the recent experimental observations, understanding the control of cell-matrix interaction could revolutionize the idea of determining the tissue architecture to obtain the

desired shape. In this connection, extracellular matrix (ECM) has the potential to guide and support the differentiation of MSCs. The ECM, a part of animal tissue, is well known for providing the structural support to the animal cells (Lanfer et al., 2009). It is to be noted here that there might also have some link between MSCs and ECM and combined study of both could reveal the optimum control over the mechanism of tissue structure prediction. At the same time, morphogenesis of various tissue types could also be predictable for controlling the proper shape.

During skeletal muscle repair, muscle stem cells work on necrotic fibres that might be called basement membranes on which the tissue is to be grown to make sure that the newly formed tissue has the proper shape and position. Again, as there are strong relation between the basement membrane and extracellular membrane, there are also some new important aspects that help the regeneration process. The ECM proteins, for examples, fibronectin and tenascin-C are secreted to the wound surface, before the cell migration, to support the regeneration process (Tanaka et al., 1999; Tervo et al., 1991). Thereafter, the wound area is covered by an adjacent epithelial monolayer followed by cell proliferation covering the entire wound area.

3D SCAFFOLD FOR SKELETAL MUSCLE GENERATION

For the development of tissue engineering and regenerative medicine, the importance of biodegradable scaffold for controlling over the activities of stem cells is unimaginable (Lanza et al., 2007; Khademhosseini et al., 2009). In stem cell therapy, scaffold and its design contribute a lot to determine the desired shape and structure of the neo tissue (Sun and Lal, 2002). Recently computer aided scaffold designing has become more popular (Mulder de et al., 2009). Scientists working with Biomaterials are trying to control over pore geometry and architecture that would be most suitable for the cell growth (Moroni et al., 2006; Yan and Gu, 1996). Bioengineered scaffold made up of porous polyvinyl alcohol (PVA), silk, polycaprolactone (PCL), chitosan, polyhydroxylbutyrate, collagen, heparin, hybrinogen, elating etc. could be efficient choice of scaffold preparations (Mondrinos et al 2006; Miot et al., 2005; Benya and Shaffer, 1982; Yeong et al., 2004). Leong et al., 2003 used non-toxic polyvinyl alcohol (PVA) as it tends to dissolve quickly after implantation. There are several modern techniques used for scaffold preparation viz. Solvent casting (Mikos et al., 1994), Polymerization (Mooney et al, 1997; Bryant and Anseth, 2001), Melt quenching and moulding (Hsu et al., 1997), Phase separation (Thomson et al., 1995; Hua et al., 2000), Freeze drying (Lo et al., 1995) etc. Regeneration of neo muscle and degradation of scaffold should take place simultaneously. Ultimately the scaffold would disappear and that space would gradually be occupied by the neo muscles (Oh, Kang and Lee, 2006; Hutmacher, Goh and Teoh, 2001). This phenomenon might be compared with the phagocytosis of artificial (scaffold) basement membrane by neo muscles.

GROWTH FACTORS IN TISSUE OF SKELETAL MUSCLE

Skeletal or other muscle regeneration needs collective action of cells, scaffolds, signalling molecules and growth factors (Lee et al., 2011). Growth factors are soluble-secreted signalling polypeptides which instruct specific cellular responses in a biological environment. Under various circumstances, for instance, to regenerate affected tissues, cells secreted growth factors (GFs) protein perform various cellular actions viz. control over migration, differentiation or proliferation of a specific subset of cells and cell survival. Localised delivery of GFs is believed to be therapeutically effective for replication of cellular components directly involved in tissue regeneration and healing process (Chen et al., 2010; Vasita and Katti, 2006). Some essential GFs for tissue regeneration (Lee et al., 2011) are shown in Table 1.

Though all growth factors are important, some have more specific importance over the others. One important GF is Transforming growth factor (TGF)-beta1 which is very effective for fibroblast tissue regeneration. Immunohistochemical results predict TFG-beta as local stimulators for the tissue repairing process (Bourque et al., 1993). It has been shown that TFG-beta1 is one of the best fibrogenic mediators and it is over expressed in human dystrophic muscle (Leask and Abraham, 2004). With increased TFG-beta1, mRNA levels are directly associated with initial stage (Bernasconi et al., 1995) of tissue fibrosis which could be a positive indicator that the starting point of muscular tissue regeneration occurs through the TFG-beta1. It has also been shown that plasma TGF-beta1 level is elevated in patients with DMD and congenital muscular dystrophy (Ishitobi et al., 2000). TFG-beta also shows positive effect on reorganization of extracellular matrix and basement membrane surrounding the damaged myofibers. By stimulating the synthesis of collagens, fibronectin and novel matrix proteins, TFG-beta directly induces angiogenesis to regenerate new blood vessels (Husmann et al., 1996). For example, it has been examined that TFG-beta is expressed by regenerating skeletal muscle within a few days after trauma. So, TFG-beta is undoubtedly one of the major multifunctional growth factors that can motivate the entire skeletal muscle regeneration process. Moreover, TFG-beta also stimulates the production of Platelet Derived Growth Factor (PDGF) that is well known

Table 1. Some essential GFs for skeletal muscle regeneration.

Symbolic name	Name of GFs
Ang	Angiopoietin GF
bFGF	basic fibroblast GF
BMP	Bone morphogenetic GF
HGF	Hepatocyte GF
TGF-betas	Trans Growth Factor
IGF	Insulin- like Growth Factor
PDGF	Platelet-derived GF
TFG	Transforming GF
VEGF	Vascular endothelial GF
NGF	Nerve GF
CSF	Colony stimulating Growth or
FGF-R	Fibroblast Growth Factor Receptor
LIF	Leukaemia Inhibitory Factor
EGF	Epidermal Growth Facto

to cause cell migration to the injured tissues to accelerate regeneration (Canalis et al., 1989). PDGF also acts as a potent stimulator of cell division in fibroblast-like cells. So PDGF is likely to accelerate fracture repair in early stages.

Similarly, after tissue disruption, Fibroblast Growth Factor (FGF) released during inflammation, induces the satellite cells to further proliferate and hence accelerate the regeneration process (van den Boset et al., 1997). Like TFG-beta, it has also been found that FGFs are angiogenic in nature. So, they can also be involved in the growth process of new blood vessel from pre-existing vessels, which gives us another new aspect that TFG-beta not only regenerates new tissues but also helps in the formation of new blood vessels (Grounds, 1991; Baird and Ling, 1987). So it is quite evident that Transforming growth factor TGF-beta1 has some potentiality to directly influence to enhance the fibrotic process of human muscular dystrophy. Leukaemia inhibitory factor (LIF) has also been well examined and found to have some most important role in the regeneration of injured muscle (Husmann et al., 1996). LIF is also addressed as multifunctional cytokine that directly stimulates the growth of skeletal muscle after damage. Finally, growth factors have a great influence in proper growth and development of skeletal muscle regeneration.

SUMMARY AND CONCLUSION

We have discussed the unique differentiation potential of MSCs both *in vitro* and *in vivo* along with their ability to secrete various strophic factors and to modulate the immune system. All these make MSCs a promising nature gifted component for the development of next generation regenerative medicine. However, more research is needed to understand the full potentiality of MSCs in tissue engineering .The possibility of using MSCs from nonconventional sources provides an insight into the general processes involved in regeneration of the muscle which opens the perspectives of novel therapies. Though BM or adipose tissue derived MSCs are very important for the skeletal muscle regeneration, easily and abundantly available sources like cord blood, placenta etc. have great potentiality for their use in tissue engineering. During the last decade most of the researches on skeletal muscle regeneration have been done focusing on the characterization of MSCs and understanding their differentiation potential functions. *In vivo* tissue generation has mostly been traced in animal models. It has been established that the secretion of bioactive materials by MSCs in response to injury mitigates the inflammatory response leading to cure injury and promote repair. The basic mechanisms of tissue generation at the injury sites by the MSCs and their ability to repair are associated with the secretion of various chemo-tactic factors. However, the complex mechanisms and the pathways with which MSCs supports repair are yet to be understood. So, starting from wound healing to tissue regeneration at specific site of injury, giving proper structure (with the help of biocompatible bioengineered scaffolds) to the newly growing tissues to match the actual biological structure of body, and also to eliminate the chances of immune rejection, the success of tissue engineering research significantly depends on the proper use and functioning of the multifunctional MSCs. More successful clinical trials on human are to be made. There is immense scope of research and development in this field of tissue engineering. Finally, proper understanding and utilization

of the various novel aspects of MSCs will lead to enormous change of the conventional medicine to the next generation regenerative medicine for curing not only skeletal muscle but also many other acute diseases.

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