Available online at www.sciencedirect.com

ScienceDirect

Procedia Engineering 110 (2015) 90 – 97

Procedia Engineering

www.elsevier.com/locate/procedia

4th International Conference on Tissue Engineering, ICTE2015

Mesenchymal Stem Cells and Biomaterials Systems – Perspectives for Skeletal Muscle Tissue Repair and Regeneration

A R Caseiro [a,b,c,e*] T Pereira [a,b,] P J Bártolo [d], J D Santos [e,] A L Luís [a,b], A C Maurício [a,b]

[a] *Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Porto, Portugal.*

[b] *Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA), Porto, Portugal.*

[c] *CDRsp- Centro para o Desenvolvimento Rápido e Sustentado de Produto, Instituto Politécnico de Leiria, Marinha Grande, Portugal.*

[d] *School of Mechanical, Aerospace and Civil Engineering & Manchester Institute of Biotechnology, University of Manchester, Manchester, UK*

[e] *CEMUC, Departamento de Engenharia Metalúrgica e Materiais, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal*

Abstract

Skeletal muscle is essential in voluntary movement and other major vital functions. Muscle injuries are important in clinical practice and, despite skeletal muscle's good regenerative ability, severe tissue loss impairs complete myofibre regeneration, limiting structural and functional recovery of the affected muscle, eventually leading to the development of non-contractile fibrous scar. The intrinsic healing mechanisms rely in great extent on the residing progenitor population but significant drawbacks to their practical application in regenerative strategies boosted the search for alternative cell sources, such as extra-fetal mesenchymal stem cells (MSCs). MSCs have demonstrated to positively influence the regeneration of different disease models. When severe volumetric muscle tissue loss occurs, the body is seldom capable of replacing the lost portions with fully functional tissue. A rational strategy to aid the healing of such situations is the application of biomaterial implants that provide a structural matrix for the ingrowth of regenerating muscle fibres. Both synthetic and natural biomaterials have been hypothesized for this purpose and some have reached as far clinical cases applications. Obvious improvements are observed in most cases, but reaction to some biomaterials and functional recovery are still a challenge. The addition of MSCs to the biomaterials seems to improve the systems' performance in the overall regenerative milieu. This strategies promote scaffold's vascularization and integration, as well as accelerated tissue ingrowth and reduces scar formation, resulting in improved recovery rates at both structural and functional levels.

Peer-review under responsibility of IDMEC-IST

Keywords: skeletal muscle; mesenchymal stem cells; Wharton's jelly; umbilical cord; dental pulp; biomaterials; secretome

* Corresponding author. *E-mail address:* rita.caseiro.santos@gmail.com

Peer-review under responsibility of IDMEC-IST
doi:10.1016/j.proeng.2015.07.014

Nomenclature

MSCs	Mesenchymal stem cells
ECM	Extra-cellular matrix
SC	Satellite cells
DPSCs	Dental pulp stem cells
VML	Volumetric muscle loss
BM-MSCs	Bone-marrow derived mesenchymal stem cells
AT-MSCs	Adipose tissue derived mesenchymal stem cells
UC-MSCs	Umbilical cord stroma/Wharton's jelly derived mesenchymal stem cells
CM	Conditioned medium

1. Introduction

Skeletal muscle is one of the major contributors to our body mass [1] and given its distribution and function is particularly prone to injury. Traumatic muscle affections are rather frequent in sports practice, as well as in more dramatic situations, such as accidents and war sceneries [2-5].

In order to develop optimized treatment strategies for such injuries, one must keep in mind the basic structure of skeletal muscle tissue, as well as its intrinsic response to events of insult.

Skeletal muscle results from a combination of muscle specific cells, nerves, blood vessels, and connective tissue support matrix. This tissue specific cells are multinucleated structures named myofibres that host a complex and highly organized contraction machinery within its sarcolemma. According to their contractile properties, myofibres can be classified into three types, and their relative content relates to each muscle specific function and training. Type 1 myofibres are slow contracting and fatigue-resistant, type 2A myofibres are fast contracting and have intermediate resistance to fatigue, and type 2B myofibres are fast contracting and have poor fatigue resistance. These contractile cells are supported by a complex extra-cellular matrix (ECM) [3]. The myofibres are further enclosed by connective tissue sheaths (the endomysium, perimysium and epimysium) associating them at three upscaling levels from involving a single myofibre to the whole muscle belly [6].

1.1. Skeletal muscle intrinsic regenerative mechanisms

The tissue response following skeletal muscle insult is classically divided into three inter-related and time-dependent phases, conveying the destruction, repair and remodeling of the affected tissue.

The first phase (destruction phase) consists on the rupture and necrosis and degeneration of the myofibres and associated neuro-vascular structures and ECM, and the formation of a haematoma (between the damaged/ruptured and retracted muscle cells), and the initiation of the inflammatory-cell response [3, 6]. The inflammatory phase peaks in approximately 3 days , and is characterized by the phagocytosis of the debris on site and the release of a cascade of inflammatory cytokines, mediated by a sequential influx of neutrophils and macrophages [3, 7]. The repair phase concludes the 'cleaning process' and initiates the regeneration of the myofibres, production of a connective-tissue scar by migrating fibroblasts and neuro-vascular re-growth [6]. In the final remodeling phase [8], the regenerated tissue matures and the formed fibrous connective tissue reorganizes and contracts [6]. Especially after severe tissue loss, the initial haemoderived matrix requires extensive remodeling [9], and an unbalanced process may derive in persistent fibrosis, impairing the muscle regeneration process and full functional recovery [10].

Satellite cells (SCs) are one of the main characters in the repays phase [11], since they form a population of muscle specific paired-box 7 (Pax7) positive myogenically committed, but yet undifferentiated, cells that reside between the basal lamina and the muscle fibre, holding functions of maintenance of tissue homeostasis and regeneration [12]. Upon injury, SCs are activated and undergo one of two faiths: differentiation into myogenic cells or 'stem-like' division, maintaining the pool of available cells for intervention in future events of injury [13]. Other populations with stem-cells' characteristics co-inhabit within the muscle tissue, such as mesangioblasts and PICs ($PW1^{+}/Pax7^{-}$ interstitial cells). More detailed discussions on the topics above are available elsewhere [14-18].

When unharmed, skeletal muscle regenerative cells remain in a quiescent state, devoid of active stimulatory or differentiating factors [19]. Upon insult however, the course of the regenerative response is strongly dependent on a delicate sequence of events which is mediated by and in turn mediates a cascade of modulatory and signaling biomolecules [18, 20-22]. These factors are released into the surrounding microenvironment, modulating resident (or delivered) populations [19], by triggering cell-type-specific programs [12], being essential for the timely activation of myogenic cells, re-vascularization and re-innervation of the lesion site and ECM deposition and remodeling.

These growth factors are mainly provided by the remaining tissue but also by the invading immune cells, participating in inflammatory response. Some of these molecules act as chemoattractant to additional inflammatory infiltration to the lesion site, such as MCP-1, IL-17, TNFα and TGFβ, amongst many others (a comprehensive table on the normalized nomenclature for some growth factors and cytokines is available as supplementary material on [23]) [24]. Other molecules such as TNFα, INFγ, IL-4 and IL-10 are essential at this stage of events [24, 25].

The evolution of the regenerative process relies on the compliance with specific patterns of interaction between these cytokines and growth factors play a crucial role in the outcome of the regenerative process [26]. Families of growth factors such as HGF, VEGFs, FGFs, TGFs, and IGFs are critical for this process, and detailed analysis on the specific functions and interactions of each can be found in [18, 20, 21], as well as in [27].

A separate mention to TGF-β1 is noteworthy [28], given its mainly detrimental role in the skeletal muscle regeneration process, contributing to exacerbated fibrosis and loss of contractile properties [10].

2. Extra-fetal tissue derived MSCs as alternatives to Muscle derived cells

When investigating cellular-based therapeutic strategies, the first thought generally goes to resident tissue populations. In the skeletal muscle, satellite cells and other 'alike' populations have been explored towards improving the regeneration process [29], but some obstacles to their effective application have arisen. They are present in limited numbers in the healthy skeletal muscle, which would require the harvest of large volumes of healthy tissue [9, 30]. To counterpart this one could advocate *in vitro* expansion prior to implantation, but this process is known to harm these cells regenerative potential, even in short term expansion [31]. Given these limitations, alternative sources of pro-myogenic agents gained relevance such as non-muscular MSCs.

MSCs are present in virtually all the body tissues, and are characterized by their clonogenic and proliferative capacities and multilineage differentiation abilities [32], into various mesodermal, ectodermal and endodermal cell types [33]. In order to gather some consensus the Mesenchymal and Tissue Stem Cell Committee, of the International Society for Cellular Therapy (ISCT) stated a series of recommendations regarding the acceptable criteria for the definition of 'Mesenchymal stem cell' populations. Briefly, MSCs population must: i) have plastic adherent ability; ii) be absent of definitive hematopoietic lineage markers (such as CD45, CD34, CD14, CD11b, CD79α, CD19 and HLA-DR), while expressing nonspecific markers CD105, CD90 and CD73; iii) have ability to differentiate into mesodermal lineage cells, osteocytes, chondrocytes and adipocytes [34].

In addition to the multi-lineage differentiation capacity, MSCs are considered immunologically privileged, mainly due to their lack of HLA-II expression [35], which widens the range of donor-recipient matching possibilities [36] as well as enabling the use of human MSCs in non-immunosupressed diseased models [37, 38] that more closely replicate clinical observations [39]. Besides, MSCs can also modulate immune responses, at both local [38] and systemic levels (benefiting cases of severe immunological disturbances)[39].

The bone marrow has been the preferential cell source for therapy development in general and skeletal muscle in particular, but its harvesting procedure is significantly harsh and the isolated cells' numbers and quality variable [40], which pushed the search for more easily accessible source tissues, as are the adipose tissue [30], the stromal tissue of the umbilical cord [36] and the dental pulp [41]. MSCs from these sources have been demonstrated capable of differentiating into myogenic lineages, and express transcription factor characteristic of skeletal muscle tissue embryogenesis [12]. Also, significant evidence on their benefits of their sole application to *in vivo* skeletal muscle regeneration in diverse models is available, as recently revised by the authors in [27].

3. MSCs and Biomaterials systems for VML repair and regeneration

As mentioned in section 1.1 skeletal muscle is equipped with powerful intrinsic response mechanism, but these can be exceeded by severe injuries consisting on the loss of significant volumes of muscle (volumetric muscle loss-VML)[42]. Some surgical treatment options are available targeting these situations, but outcomes are generally least satisfactory [43]. In the events of a VML, the support structures, blood vessels, nerves and regenerative cell populations turn absent and complete regeneration of functional tissue is hampered, even after long healing periods [2].

At this point, biomaterials appear as a possible path towards skeletal muscle tissue restoration, by providing a physical and biochemical support and guidance to the regenerating myofibres [2]. In some instances the biomaterial itself acts as such [44] or it can be functionalized with desired molecular cues [45].

The first category is dominated by ECM, which has been demonstrated to adequately fill a critical muscle defect, and to benefit structural recovery, although functional improvement was lacking [2]. Other report of the application of similar strategies revealed functional indexes recovery, but still insufficient to match undamaged muscles' response [46]. Using a larger pre-clinical canine model, these matrices provided slight functional recovery, and although no exogenous cells were associated to the implants, these were capable of stimulating endogenous progenitors migration into the regenerating area what possibly contributes to the outcomes [47]. In effective clinical applications, a ECM products successfully aided the treatment of a war-associated volumetric muscle injury case. The implant successfully restored the local body contour and promoted function improvement, as reported by the patient and confirmed by kinematic analysis. But still, improvements did not match the undamaged limb indexes [5]. A broader study including a larger group of patients also described functional recovery improvements, while only 2 out of the five patients were deemed irresponsive to therapy. Nevertheless, all patients reported significant improvements in quality of life [46], as did the one patient reported by Gentile et al [48].

Biomaterials other than ECM have also been described in skeletal muscle affections prospective treatment. Qazi et al quite thoroughly revises the biomaterials currently under study and significant achievements in the field in [49]. As an interesting example, a gelatin-poly(ethylene glycol)-tyramine (GPT) hydrogel successfully improved muscle regeneration following a laceration injury. This *in situ* forming gel was loaded with a pro-survival growth factor (basic Fibroblast Growth Factor – bFGF), sustaining controlled release for up to 3 weeks. *In vivo*, this significantly improved contractile force of healing muscles after 4 weeks, reduced local fibrosis and increased multinucleated regenerating myofibres and neurovascular structures on site, comparing to untreated controls [45].

Despite significant efforts, some key-points still remain challenging for optimal skeletal muscle regeneration, especially following event affecting support as associated structures, namely the re-vascularization and re-innervation of the regenerating tissue, as well as the attainment of proper myofibres alignment. All these factors are essential for optimal structural and functional recovery.

Considering the essential role of active cell populations in tissue regenerations, the association of cellular systems to these biomaterials was hypothesized to further boost their benefits and general performance. The first cell systems to be considered were muscle-derived populations, which led to observable benefits [50, 51]. However, given the challenges on the manipulation and application of such populations, other non-muscular cell sources came into consideration: such as BM-MSCs [52], AT-MSCs [45, 53], UC-MSCs [38, 54, 55], amongst others.

The most relevant observations when these cells are applied regard notorious enhancement of both structural and functional recovery, generally characterized by increased muscle tissue, blood vessels and nervous tissue ingrowth into the defect [2, 52], the reduction of fibrosis [45] and also the modulation of the body's reaction to the applied materials [38, 55]. It was also proposed that the cellular component could rather accelerate the whole regenerative process, rather than massively altering it [50].

4. MSCs Conditioned Medium – a new perspective for skeletal muscle regeneration

As earlier stated, MSCs therapeutic benefits to skeletal muscle tissue (and may other body tissues) regeneration relies on their paracrine effects on the surrounding populations. These assumptions are supported by compelling evidence that MSCs mostly remain in undifferentiated state at the lesion site or in its vicinity, or that are only identifiable for limited periods of time [56].

As such, it became of great interest to investigate what kind of paracrine factors were being secreted by those MSCs that so significantly affected regenerative outcomes, commonly designated as the MSCs' secretome, in which a wide range of growth factors, cytokines, chemokines and extracellular matrix components have already been identified [23, 57-61].

In the secretome of MSCs from the umbilical cord stroma, bone marrow, amniotic fluid, and adipose tissue, interesting levels of proliferative, chemotactic and immunomodulatory factors, such as TGF-βs, G-CSF, GM-CSF, MCP-1, IL-6 and IL-8 [23, 62] as well as other vasculogenic factors [63, 64]. However, exposure to inflammatory cytokines and other stimuli [65, 66], growth factors medium supplementation [66, 67] or unusual oxygen tension [63, 68] have been demonstrated to impact on MSCs activities. Hence the secretome composition of MSCs is not static and is dependent on either the cell type in consideration as well as on extrinsic microenvironmental factors [69], and its recognition may aid to understand variations observed in the regenerative response and to optimize therapies application. Comparative secretome analysis is also an interesting approach that will aid us to identify the most adequate MSCs source for each desired application. As an example, we have observed some clear differences between Wharton´s jelly and dental pulp MSCs secretion profiles (Figure 1), whilst the first seem to be richer is proliferative and immunoregulatory factors, such as TGF-βs, G- and GM-CSF, DPSCs catch up to comparable secreted levels of VEGF and RANTES (unpublished data).

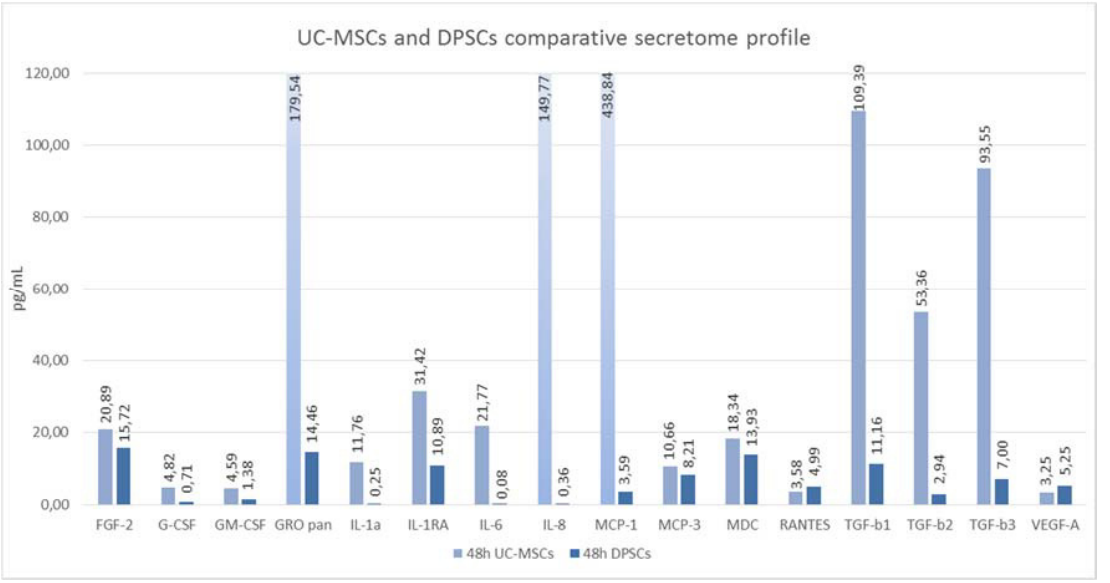

Fig. 1. Comparative secretome profile of UC-MSCs and DPSCs (passages 6-7) after 48 hours conditioning protocols in basal (no serum supplementation) culture medium. NOTE: GRO, IL-8 and MCP-1 levels in UC-MSCs cultures exceeded the defined maximum for the graphic representation (120,00 pg/mL), being represented by shortened bars accompanied by the real secreted value (179,54 pg/mL, 149,77 pg/mL and 438,84 pg/mL, respectively).

It is also essential to bear in mind that bioactive factors are not the only, or even the major, part of the array of molecules released by MSCs. A great variety of metabolic substrates is present in MSCs' conditioned medium (CM), most of which are involved in the synthetic activities of resident and delivered cell populations [23]. Amongst other metabolic fractions, aminoacids assume a preponderant role, providing basic molecules for further growth factors production by active cell populations, either native or delivered to the site.

As we have ourselves observed, the inclusion of Wharton´s jelly MSCs secretome in a biomaterial system eases the local inflammatory tissue response, as denoted by reduced ISO 10993-6 scores, which regard cell infiltrate and tissue reaction characteristics [38]. Recent work also demonstrated similar effects can be attributed to DPSCs' CM, which also contributed to the control of the short- to mid-term tissue response to a commercially available fibrin matrix, as depicted in Figure 2 from a preliminary study (unpublished data).

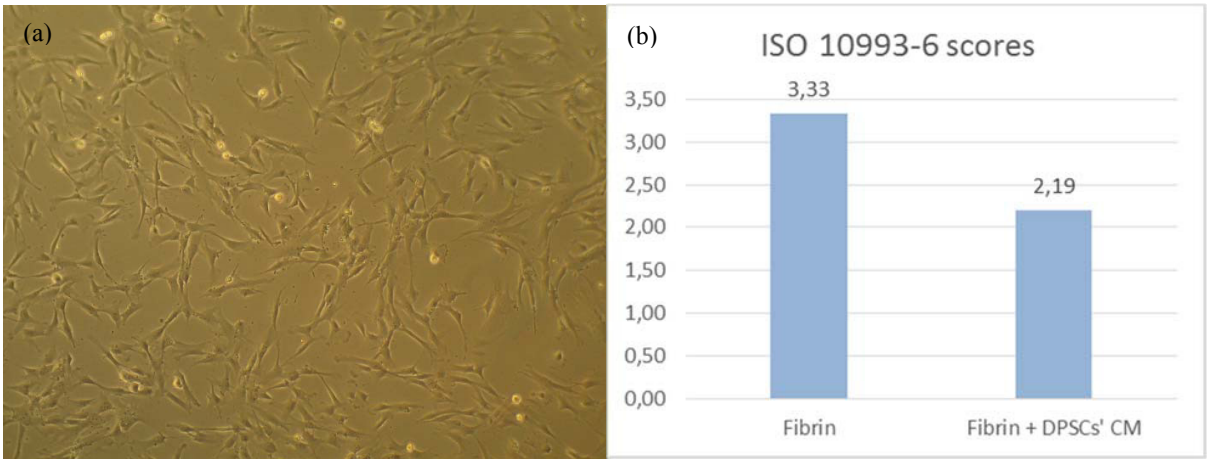

Fig. 2. (a) Passage 3 DPSCs in standard adherent culture conditions, 100x magnification; (b) ISO 10993-6 scoring of local tissue response, at 15 days post implantation into a tibialis anterior VML model, as described in [38] using Fibrin Matrix alone (Fibrin: 3,33 points) and in addition to DPSCs' CM following 48 hours conditioning protocol (Fibrin + DPSCs' CM: 2,19 points).

Remarkably, using both UC-MSCs and DPSCs, the CMs' effect was superior to that of the cells themselves. As we have also observed, besides the inflammatory response modulation, CM addition to the system also resulted in improved signs of skeletal muscle regeneration efforts, with increased regenerating myofibres ingrowth into the defect, as well as positively impacting on their alignment and orientation (unpublished data).

5. Final Remarks

In this brief overview on the state of the art regarding regenerative approaches to skeletal muscle cellular and biomaterial systems association it is evident that there is still a long road to cover until definitive therapies are available for ubiquitous application in clinical patients. Nevertheless, significant advances can be noted so far regarding biomaterials development, cell sources innovation, as well as other fast expanding concepts, such as the application of MSCs' CM to the systems. Detailed investigation on the specific mechanisms related to the observed benefits of the MSCs- biomaterials associations is ongoing, but holds promise to important breakthroughs in the field, by combining the advantages of each system component towards optimized recovery of severe skeletal muscle injuries.

Acknowledgements

This research was supported by System of Incentives for Research and Technological development of QREN in the scope of project nº 38853/2013 - DEXGELERATION : "Soluções avançadas de regeneração óssea com base em hidrogéis de dextrino - by the European Community FEDER fund through ON2 - O Novo Norte – North Portugal Regional Operational Program 2007-2013, by Project nº 34128 – BEPIM II: "Microdispositivos biomédicos com capacidade osteointegrativa por µPIM – BEPIM II, funded by AdI, and by the program COMPETE – Programa Operacional Factores de Competitividade, Project Pest-OE/AGR/UI0211/2011, by FCT project PTDC/CVT/103081/2008, and CDRsp's Strategic Project – UI-4044-2011-2012 (Pest-OE/EME/UI4044/2011) funding from FCT. AR Caseiro would also like to acknowledge the FCT for the PhD grant SFRH/BD/101174/2014.

References

1. Sicari, B.M., C.L. Dearth, and S.F. Badylak, *Tissue engineering and regenerative medicine approaches to enhance the functional response to skeletal muscle injury.* Anat Rec (Hoboken), 2014. **297**(1): p. 51-64.
2. Merritt, E.K., et al., *Functional Assessment of Skeletal Muscle Regeneration Utilizing Homologous Extracellular Matrix as Scaffolding.* Tissue Engineering Part A, 2010. **16**(4): p. 1395-1405.
3. Gates, C. and J. Huard, *Management of skeletal muscle injuries in military personnel.* Operative techniques in sports medicine, 2005. **13**(4): p. 247-256.
4. Järvinen, T.A.H., et al., *Muscle injuries: optimising recovery.* Best Practice & Research Clinical Rheumatology, 2007. **21**(2): p. 317-331.
5. Mase Jr, V.J., et al., *Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect.* Orthopedics, 2010. **33**(7): p. 511.
6. Järvinen, T.A.H., et al., *Muscle Injuries.* The American Journal of Sports Medicine, 2005. **33**(5): p. 745-764.
7. Tidball, J.G., *Inflammatory processes in muscle injury and repair.* American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 2005. **288**(2): p. R345-R353.
8. Serrano, A.L. and P. Munoz-Canoves, *Regulation and dysregulation of fibrosis in skeletal muscle.* Experimental Cell Research, 2010. **316**(18): p. 3050-8.
9. Page, R.L., et al., *Restoration of Skeletal Muscle Defects with Adult Human Cells Delivered on Fibrin Microthreads*. Tissue Engineering Part A, 2011. **17**(21-22): p. 2629-2640.
10. Sato, K., et al., *Improvement of muscle healing through enhancement of muscle regeneration and prevention of fibrosis.* Muscle Nerve, 2003. **28**(3): p. 365-72.
11. Mauro, A., *Satellite cell of skeletal muscle fibers.* The Journal of biophysical and biochemical cytology, 1961. **9**(2): p. 493-495.
12. Giordani, L. and P.L. Puri, *Epigenetic control of skeletal muscle regeneration: Integrating genetic determinants and environmental changes.* FEBS J, 2013. **280**(17): p. 4014-25.
13. Kuang, S., et al., *Asymmetric self-renewal and commitment of satellite stem cells in muscle.* Cell, 2007. **129**(5): p. 999-1010.
14. Mitchell, K.J., et al., *Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development.* Nature Cell Biology, 2010. **12**(3): p. 257-66.
15. Pannerec, A., et al., *Defining skeletal muscle resident progenitors and their cell fate potentials.* Development, 2013. **140**(14): p. 2879-91.
16. Pannerec, A., G. Marazzi, and D. Sassoon, *Stem cells in the hood: the skeletal muscle niche.* Trends Mol Med, 2012. **18**(10): p. 599-606.
17. Pannerec, A. and D. Sassoon, *PICs (PW1+ interstitial cells), a new muscle stem cell population.* Médecine/Science (Paris), 2010. **26**(10): p. 797-800.
18. Charge, S.B.P. and M.A. Rudnicki, *Cellular and molecular regulation of muscle regeneration.* Physiological Reviews, 2004. **84**(1): p. 209-238.
19. Santa María, L., C.V. Rojas, and J.J. Minguell, *Signals from damaged but not undamaged skeletal muscle induce myogenic differentiation of rat bone-marrow-derived mesenchymal stem cells.* Experimental Cell Research, 2004. **300**(2): p. 418-426.
20. Ten Broek, R.W., S. Grefte, and J.W. Von den Hoff, *Regulatory factors and cell populations involved in skeletal muscle regeneration.* Journal of cellular physiology, 2010. **224**(1): p. 7-16.
21. Husmann, I., et al., *Growth factors in skeletal muscle regeneration.* Cytokine & Growth Factor Reviews, 1996. **7**(3): p. 249-258.
22. Hayashi, S., et al., *Sequence of IGF-I, IGF-II, and HGF expression in regenerating skeletal muscle.* Histochemistry and Cell Biology, 2004. **122**(5): p. 427-434.
23. Pereira, T., et al., *MSCs Conditioned Media and Umbilical Cord Blood Plasma Metabolomics and Composition.* PLoS One, 2014. **9**(11): p. e113769.
24. Tidball, J.G., K. Dorshkind, and M. Wehling-Henricks, *Shared signaling systems in myeloid cell-mediated muscle regeneration.* Development, 2014. **141**(6): p. 1184-96.
25. Heredia, J.E., et al., *Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration.* Cell, 2013. **153**(2): p. 376-388.
26. DO, M.K.Q., et al., *Time-coordinated prevalence of extracellular HGF, FGF2 and TGF-β3 in crush-injured skeletal muscle.* Animal Science Journal, 2012. **83**(10): p. 712-717.
27. Caseiro, A.R., et al., *Chapter - Trends in Mesenchymal Stem Cells Applications for Skeletal Muscle Repair and Regeneration, in PROGRESS IN STEM CELL TRANSPLANTATION*. 2015: T Demirer
28. Li, Y., et al., *Transforming Growth Factor-β1 Induces the Differentiation of Myogenic Cells into Fibrotic Cells in Injured Skeletal Muscle: A Key Event in Muscle Fibrogenesis.* American Journal of Pathology, 2004. **164**(3): p. 1007-1019.
29. Koning, M., et al., *Current opportunities and challenges in skeletal muscle tissue engineering.* Journal of Tissue Engineering and Regenerative Medicine, 2009. **3**(6): p. 407-415.
30. Mizuno, H., et al., *Myogenic differentiation by human processed lipoaspirate cells.* Plastic and Reconstructive Surgery, 2002. **109**(1): p. 199-209.
31. Rossi, C.A., et al., *In vivo tissue engineering of functional skeletal muscle by freshly isolated satellite cells embedded in a photopolymerizable hydrogel.* The FASEB Journal, 2011. **25**(7): p. 2296-2304.
32. Chamberlain, G., et al., *Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing.* Stem Cells, 2007. **25**(11): p. 2739-49.
33. Muguruma, Y., et al., *In vivo and in vitro differentiation of myocytes from human bone marrow–derived multipotent progenitor cells.* Experimental Hematology, 2003. **31**(12): p. 1323-1330.
34. Dominici, M., et al., *Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.* Cytotherapy, 2006. **8**(4): p. 315-7.
35. Le Blanc, K. and O. Ringden, *Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2005. **11**(5): p. 321-34.
36. Kim, D.W., et al., *Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic*

Potential for Clinical Applications. International Journal of Molecular Sciences, 2013. **14**(6): p. 11692-712.
37. Conconi, M.T., et al., *CD105(+) cells from Wharton's jelly show in vitro and in vivo myogenic differentiative potential.* International Journal of Molecular Medicine, 2006. **18**(6): p. 1089-96.
38. Pereira, T., et al., *Effects of Human Mesenchymal Stem Cells Isolated from Wharton's Jelly of the Umbilical Cord and Conditioned Media on Skeletal Muscle Regeneration Using a Myectomy Model.* Stem Cells International, 2014. **2014**.
39. Caplan, A.I., *Why are MSCs therapeutic? New data: new insight.* The Journal of Pathology, 2009. **217**(2): p. 318-324.
40. Fossett, E. and W.S. Khan, *Optimising human mesenchymal stem cell numbers for clinical application: a literature review.* Stem Cells International, 2012. **2012**: p. 465259.
41. Kawashima, N., *Characterisation of dental pulp stem cells: a new horizon for tissue regeneration?* Archives of Oral Biology, 2012. **57**(11): p. 1439-58.
42. Grogan, B.F., J.R. Hsu, and S.T.R. Consortium, *Volumetric muscle loss.* Journal of the American Academy of Orthopaedic Surgeons, 2011. **19**(suppl 1): p. S35-S37.
43. Cittadella Vigodarzere, G. and S. Mantero, *Skeletal muscle tissue engineering: strategies for volumetric constructs.* Front Physiol, 2014. **5**: p. 362.
44. Wolf, M.T., et al., *Naturally derived and synthetic scaffolds for skeletal muscle reconstruction.* Advanced drug delivery reviews, 2014.
45. Hwang, J.H., et al., *Combination therapy of human adipose-derived stem cells and basic fibroblast growth factor hydrogel in muscle regeneration.* Biomaterials, 2013. **34**(25): p. 6037-6045.
46. Sicari, B.M., et al., *An Acellular Biologic Scaffold Promotes Skeletal Muscle Formation in Mice and Humans with Volumetric Muscle Loss.* Sci Transl Med, 2014. **6**(234): p. 234ra58.
47. Turner, N.J., et al., *Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction.* Tissue Engineering Part A, 2010. **16**(11): p. 3309-3317.
48. Gentile, N.E., et al., *Targeted rehabilitation after extracellular matrix scaffold transplantation for the treatment of volumetric muscle loss.* American Journal of Physical Medicine & Rehabilitation, 2014. **93**(11): p. S79-S87.
49. Qazi, T.H., et al., *Biomaterials based strategies for skeletal muscle tissue engineering: Existing technologies and future trends.* Biomaterials, 2015. **53**: p. 502-521.
50. Machingal, M.A., et al., *A tissue-engineered muscle repair construct for functional restoration of an irrecoverable muscle injury in a murine model.* Tissue engineering Part A, 2011. **17**(17-18): p. 2291-2303.
51. Corona, B.T., et al., *Further development of a tissue engineered muscle repair construct in vitro for enhanced functional recovery following implantation in vivo in a murine model of volumetric muscle loss injury.* Tissue Engineering Part A, 2012. **18**(11-12): p. 1213-1228.
52. Merritt, E.K., et al., *Repair of traumatic skeletal muscle injury with bone-marrow-derived mesenchymal stem cells seeded on extracellular matrix.* Tissue Eng Part A, 2010. **16**(9): p. 2871-81.
53. Peçanha, R., et al., *Adipose-derived stem-cell treatment of skeletal muscle injury.* The Journal of Bone and Joint Surgery. American volume, 2012. **94**(7): p. 609-17.
54. Pereira, T., et al. *Development of a skeletal muscle injury model in the rat and in vivo evaluation of the use of Human Mesenchymal Stem Cells (HMSCs) from the umbilical cord matrix in myectomy injury treatment*. in *17th annual Congress of the European College of Sport Science*. 2012. Bruges - Belgium.
55. Pereira, T., et al., *In vivo evaluation of muscular regeneration treated with Human Mesenchymal Stem Cells (HMSCs) from the umbilical cord matrix and different biomaterials in a rat anterior tibial myectomy model.* Journal of Tissue Engineering and Regenerative Medicine, 2012. **6**(suppl. 1): p. 396.
56. Phinney, D.G. and D.J. Prockop, *Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views.* Stem cells, 2007. **25**(11): p. 2896-2902.
57. Skalnikova, H., et al., *Mapping of the secretome of primary isolates of mammalian cells, stem cells and derived cell lines.* Proteomics, 2011. **11**(4): p. 691-708.
58. da Silva Meirelles, L., et al., *Mechanisms involved in the therapeutic properties of mesenchymal stem cells.* Cytokine & Growth Factor Reviews, 2009. **20**(5–6): p. 419-427.
59. J Salgado, A., et al., *Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine.* Curr Stem Cell Res Ther, 2010. **5**(2): p. 103-110.
60. Doorn, J., et al., *Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements.* Tissue Engineering Part B: Reviews, 2012. **18**(2): p. 101-115.
61. Baraniak, P.R. and T.C. McDevitt, *Stem cell paracrine actions and tissue regeneration.* Regen Med, 2010. **5**(1): p. 121-143.
62. Walter, M.N.M., et al., *Mesenchymal stem cell-conditioned medium accelerates skin wound healing: An in vitro study of fibroblast and keratinocyte scratch assays.* Experimental Cell Research, 2010. **316**(7): p. 1271-1281.
63. Chen, L., et al., *Paracrine Factors of Mesenchymal Stem Cells Recruit Macrophages and Endothelial Lineage Cells and Enhance Wound Healing.* PLoS One, 2008. **3**(4): p. e1886.
64. Mirabella, T., et al., *Amniotic liquid derived stem cells as reservoir of secreted angiogenic factors capable of stimulating neo-arteriogenesis in an ischemic model.* Biomaterials, 2011. **32**(15): p. 3689-99.
65. Lee, M.J., et al., *Proteomic Analysis of Tumor Necrosis Factor-alpha-Induced Secretome of Human Adipose Tissue-Derived Mesenchymal Stem Cells.* Journal of Proteome Research, 2010. **9**(4): p. 1754-1762.
66. Kilroy, G.E., et al., *Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors.* J Cell Physiol, 2007. **212**(3): p. 702-9.
67. McCoy, M.K., et al., *Autologous transplants of Adipose-Derived Adult Stromal (ADAS) cells afford dopaminergic neuroprotection in a model of Parkinson's disease.* Exp Neurol, 2008. **210**(1): p. 14-29.
68. Lopatina, T., et al., *Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo.* PLoS One, 2011. **6**(3): p. e17899.
69. Lavoie, J.R. and M. Rosu-Myles, *Uncovering the secretes of mesenchymal stem cells.* Biochimie, 2013. **95**(12): p. 2212-2221.