

Current Issues and Regulations in Tendon Regeneration and Musculoskeletal Repair with Mesenchymal Stem Cells

Short Title: Issues and Regulations with Stem Cells

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Abstract

Mesenchymal stem cells are multipotent stromal cells residing within the connective tissue of most organs. Their surface phenotype has been well described. Most commonly, mesenchymal stem cells demonstrate the ability to differentiate into mesenchymal tissues (bone, cartilage, fat, etc...), however, under the proper conditions these cells can differentiate into epithelial cells and neuroectoderm derived lineages. Their developmental plasticity also depends on the ability of mesenchymal stem cells to alter the tissue microenvironment by secreting soluble factors, as well as their capacity for differentiation in tissue repair. It is the cell-matrix interaction which defines the tissue characteristics. The molecular and functional heterogeneity of this cell population may confound interpretation of their differentiation potential, but it is this heterogeneity that is believed to provide for their therapeutic efficacy. Stem cell therapies are an attractive therapeutic approach for soft tissues as they offer a vehicle for repair and regeneration at the end of a needle. The early introduction of stem cell treatments into the therapeutic armamentarium involves both commercial and non-commercial multidisciplinary partnerships and has occurred in a climate of regulatory reform, so not all the relevant information resides in the public domain, but early clinical studies have shown promising results. Against this backdrop, novel techniques and early results of a small series of tendon and musculotendinous junction interventions are being published and other ongoing studies are yet to report their results. The issue of ensuring governance of these novel technologies falls upon both the scientific community and the established licensing authorities.

Keywords

Intervention; mesenchymal stem cells; registry; regenerative medicine; surgery; tendon

Mesenchymal stem cells (MSCs) are multipotent stromal cells residing within the connective tissue of most organs. Their surface phenotype has been well described. Most commonly, MSCs demonstrate the ability to differentiate into mesenchymal tissues (bone, cartilage, fat, etc...), however, under the proper conditions these cells can differentiate into epithelial cells and neuroectoderm derived lineages. Their developmental plasticity also depends on the ability of MSCs to alter the tissue microenvironment by secreting soluble factors, as well as their capacity for differentiation in tissue repair. It is the cell-matrix interaction which defines the tissue characteristics. The molecular and functional heterogeneity of this cell population may confound interpretation of their differentiation potential, but it is this heterogeneity that is believed to provide for the therapeutic efficacy of MSCs [1].

From embryological origins, the mesenchyme is characterized morphologically by a loose aggregate of reticular fibrils creating a matrix containing unspecialized cells [2]. As the animal grows, cells develop into different types of connective tissue; bone, tendon, cartilage, the lymphatic and circulatory systems [3,4] <http://en.wikipedia.org/wiki/Mesenchyme> - cite_note-isbn0-7817-8577-4-2. These cells thrive and survive within the collagen based matrix, which provides feedback to the cells based on architectural configuration, thought to influence certain activity in response to chemical stimuli [5]. As embryology progresses subsets of the mesenchyme will give rise to the bone and cartilaginous framework of the organism as well as the ligaments and tendons which make up the 'soft tissue skeleton'.

In adult life, lesions of this 'soft tissue skeleton' cause significant morbidity, commonly through attrition, secondary to over-use or overload injuries (trauma). This condition often affects the Achilles tendon, patella tendon and common extensor origin (Lateral Epicondylitis - Tennis Elbow). Based on *in vivo* and *in vitro* animal studies it is reasonable to presume that the process of regeneration and repair is mediated by MSCs [1] and can be enhanced therapeutically. It is the duty of surgeons and front line health care providers to harness and modulate this process to improve patient care in a responsible manner.

Basic Scientific Research

MSCs are a novel vector for treating such soft tissue disorders. They are traditionally understood as dividing asymmetrically – generating a daughter stem cell and a second progenitor cell. This approach to repair has more recently been questioned [6] with *in silico* modelling of stem cell niches in the gut [7] and haemopoietic systems supporting a different 'niche approach' [8-10]. Faced with the challenge of repair or regeneration to achieve equilibrium within the tissue, local cytokines encourage 'stemness' within the neighbouring cells [11]. The evidence is still uncertain as to the role of the bio physicochemical niche with respect to the differentiation to discrete cell lines, and thus, differentiation of pluripotent cells colonising such an environment. What is clear is that it is not just the cell alone, rather the cell and local environment interaction that effects change, influenced by factors such as matrix elasticity [12] and immunological privilege [13,14].

Though the question still remains as to how much the changes are due to the cells themselves or the positive provocative influence that they have on their neighbours, acting as a regenerative catalyst. In essence the outcome should be similar. For example, manipulation of the Smad8 signalling pathway [15] in MSCs can replicate a tenocyte gene expression profile *in vitro* and *in vivo* suggesting that genetic modification of MSCs may be an alternate route to achieving the desired output cell population. If the modification and transplantation process provides a suitable niche, then the cells should differentiate, to provide functional tissue. A compromised environment would therefore necessitate a more measured response. For example we know from animal experiments that expression in response to the stressed hypoxic environment encourages fibrosis [16] or even apoptosis, often associated on a gross scale with cyst formation and lacunae – a commonly observed degenerative change in tendons [17]. Clinically speaking this would not be a desired outcome and should be avoided, in the same way that miss-expression and differentiation down an inappropriate path could compromise the native tissue properties. Therefore, it would be essential to standardize any genetic modification of cells (although difficult) to limit MSCs sending inappropriate signals to the environment, as has been demonstrated in the laboratory [5].

Various animal studies have shown the ability of MSCs to be used successfully in early tendon regeneration [18,19] including the rabbit tendo-achilles model. These early studies were first published in the 1980's [20] with evidence of clinical potential and relevance demonstrated by 1994 [21]. In respect to a tissue like tendon, appropriate and controlled cell differentiation is not the only concern. If the repaired/regenerated tissue does not retain some resemblance of the native mechanical properties then this line of study will be in vain since this is the primary purpose of tendon. To this end, Awad et al. investigated the use of an MSC-collagen composite in a rabbit patellar tendon model and found a significant difference in mechanical properties compared to natural repair [22]. MSCs-collagen composites with an MSC density: 4×10^6 cells/ml implanted into long gap defects in the rabbit Achilles tendon displayed an improvement in biomechanical properties when assayed by biochemical and histological outcome measures. Furthermore, an tissue architecture and functionality of the tendon after injury [17,23] with maximum stress for the repair tissue around 35% of normal values by 12 weeks post-intervention was also observed.

There is also the possibility of improving the muscle component regeneration at the musculotendinous junction [24]. Most early musculoskeletal (MSK) efforts focused on regeneration of the cruciate ligaments [25,26] but with improvement in autologous hamstring grafting techniques, the focus is shifting to chronic degenerative conditions. Such tissue repair is judged by the quality of the mechanical resistance but does not reflect the longer term aspects of the ability for soft tissues to remodel. This is diminished compared with hard tissue but still exists.

Competing Treatment Modalities

Conventional therapy methods for such soft tissue (Tendon and ligament) conditions are based around modulation of the inflammatory process and thus encouragement of appropriate healing. Importantly, clinicians must act to reduce the risk of such adverse events through reduction in further damage due to the overloading of the healing tissues, as observed in lateral epicondylitis ('Tennis Elbow'), where there is a maladaptive healing response. Traditionally, the ladder of medical interventional hierarchy starts with expectant protective therapy; 'Rest, Ice, Compression, Elevate (RICE)'. This includes splinting and 'offloading' strategies with pharmaceutical support using non-steroidal medication. Controlled exertion may then be encouraged. Injections with anti-inflammatory agents such as topical steroid e.g. hydrocortisone [27] may also inadvertently fenestrate (needle) the affected tissue. This technique has itself been implicated in precipitating the healing process through encouraging neovascularisation.

Some success has been reported with similar techniques [28] such as the injection with autologous acellular supernatant of serum that has been reduced referred to as 'Platelet Rich Plasma' (PRP) in a technique originally pioneered in Australia. One might then consider cell therapy as the next stage of escalation before considering tenotomy. In this surgical procedure, a series of longitudinal surgical slits are made in the tendon to encourage neovascularisation. The procedure may include clearance of cystic degenerative tissue which acts as a physical barrier to regrowth. The progression to open excision of tissue and drilling of cortical bone at the osseotendinous interface provokes a significant healing response that may or may not be encouraged by local infiltration of cells from the bone marrow, though such a technique carries the morbidity of surgical complications. Surgeons have also reconstructed failing degenerative tendons using various different techniques successfully for at least 70 years. Such approaches range from simply providing the appropriate anatomical environment through the restructuring of host tissue to the insertion of prosthetic graft [29,30]. If good soft tissue perfusion is maintained, surgery ensures an optimal physiological environment also. It is within this window for therapy that clinicians can explore cell therapy rather than more aggressive approaches. This principle crosses disciplines, and has been addressed in earlier *in vivo* work that demonstrated correlation between transplantation of MSCs and repair of scarred myocardium after myocardial infarction in rats [31], suggesting the therapeutic potential for soft tissues. One potential concern is that cells can migrate but there is no reported evidence of stem cell migration to other tissues in the case of tendon regeneration. The incidence of alternate expression is however an issue, manifesting as heterotopic ossification with evidence of ectopic bone formation in 30% of cases. In this study, the maximum force and maximum stress for the cell-gel-suture repairs [32] were 20% of normal in the tissues in animal models, suggesting that the control of expression is not guaranteed. Improved cell viability in culture [33], eliminates ectopic bone in the repair site and improving repair biomechanics and histological appearance, and so this finding may simply be related to the cellular response to hypoxia which is observed clinically also.

Another ongoing trial [34] exploring the use of stem cell therapy to improve the muscle function of patients with partly denervated muscles of the arm would prevent the degeneration secondary to neurological injury, and thus indirectly influence muscles in this unfortunate group. The results of this ongoing non-randomised placebo controlled work are yet to be published and reflect the issues of experimental methodology seen in many pilot studies.

The aim is therefore to improve the benefit/risk ratio for patients by employing the MSC approach. Progress should be judged in this context. A similar clinical strategy employing injections with biologically active agents (biologics) proposes to accelerate the natural healing processes through the delivery of agents in the subjects' serum. Cells in effect act as 'vectors' or 'factories' either producing such agents to promote healing or 'redirect' a maladapted healing response. Studies appraising the impact of these novel modalities of treatment therefore need to define both the exact agents and components thereof, including the mode of delivery itself and also the local tissue's response, as has been seen in the case of the development of clinical cardiac trials.

Other areas of experimental musculoskeletal intervention [35] precede MSC tendon inoculation, with early successful results of stem cell mediated collagen regeneration reported [36]. Previous approaches to tendon therapy have therefore evolved from the autologous injection of blood [18], or PRP [28] which may be effective through 'fenestration' or the provocation of the inflammatory response or indeed by the mediation of stem cells when 'whole blood' is used. To date, only one cell-based pilot human trial has been carried out with regards to the treatment of lateral epicondylitis using skin-derived tenocyte-like cells [37]. The report of this prospective controlled cohort study of the clinical effects of MSC injection for the treatment of tennis elbow [18,38] suggests significant early improvement in outcomes using validated measures, in the short term.

This transition of biologically inspired therapy from simple autologous procedures to complex laboratory based culturing of MSC populations raises issues of how to investigate and monitor this novel treatment modality [39]. Stem cell characterisation is still an evolving science in its own right.

Early Clinical Studies and their Regulation

Presently there are early published results of the use of 'tenocyte-like' cells for the treatment of patellar tendinopathy [40] as well as for lateral epicondylitis [37] which have employed cells derived from skin through a proprietary pathway. These pilot studies suggest that ultrasound-guided injection of autologous skin-derived tendon-like cells can be safely used in the short term to treat patellar tendinopathy, with faster response of treatment and significantly greater improvement in pain

and function than with plasma alone. Such promising results fuel the enthusiasm for developing cell therapies. With a study population size of 60 for the treatment of patellar tendinopathy, the results are significant. Short term safety and efficacy have been demonstrated in a controlled study (Level III evidence), even if the cohort was not randomised. Cell therapies remain a controversial topic though, primarily, because of the ethical and long-term safety concerns.

The main ethical issue involves the use of embryonic stem cells, since in order to retrieve stem cells from an embryo, it has to be sacrificed. As a result, musculoskeletal stem cell work involves adult MSCs that are blood, bone marrow [41] and dermally derived [37], which overcomes this issue, though this distinction may not yet be fully appreciated beyond the biomedical community [42]. Stem cells are still regarded as controversial as they are unpredictable in terms of their differentiation and amplification, with pluripotent embryonic stem cells also representing a risk of teratoma formation in contrast to adult stem cells, which lack distinct teratogenic potential but remain multipotent [43].

Concurrent work in the cardiac domain of treating myocardial infarction, including treating 69 patients with acute myocardial infarction, has demonstrated significant left ventricular perfusion and Left Ventricular Ejection Fraction (LVEF) functional improvement, indicating potential myocardial regeneration using bone marrow derived mononuclear cells. This is considered to be due to the presence of MSCs though likely affecting the muscle units. The cardiac skeleton may also be influenced which has led to experimental cell/matrix augmentation patching techniques. Such an approach may ultimately be appropriate in treating isolated tendon pathology also.

With EU directives being centred on protecting patient welfare, this may restrict the application of MSCs in clinical trials, as they may pose a risk to the patient similar to the issues surrounding novel gene therapy [43,44]. It is therefore vital to demonstrate their safety as well as efficacy. As a result, there is pressure for them to be strictly regulated, as seen by the introduction of new UK Medicines and Healthcare Regulatory Agency (MHRA) classification and National Institute of health and Clinical Excellence (NICE) guidelines in 2009.

The EU Tissues and Cells Directive [45-47] was brought into UK law in 2007. As a result, the Human Tissue Authority (HTA) regulates stem cell lines grown outside the human body for human application [48].

Early delivery of these approaches needs to ensure sound scientific validation at each stage of the translational research path. The process involves translation of this understanding of the basic science. There must be compliance with the established regulations for handling of injectable biological agents. The same is true of the operational procedures derived from this strategic scientific approach.

The process of modification of the initial substrate, either cells or a biologically active agent must be a clearly defined by the 'end to end' regulated governing process, to ensure the appropriate compliance with MHRA regulations in the UK, European Medicines Agency (EMA) regulations across Europe and the Food and Drugs Administration (FDA) regulations in the USA. This requires Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), Good Clinical Practice (GCP) compliance *etc*, at the various stages of this Translational Research (TR) pipeline.

The quality assurance therefore depends on the Standard Operating Procedures (SOPs), which are usually protected Intellectual Property (IP) since the organisations have invested in developing and refining these processes. They describe the appropriate agents and timings for the filtering and expanding of the cell colonies to maintain differentiation into specific cell types or de-differentiation to maintain 'stemness'. This IP protection is to ensure a return on the investment initially made and reflects the regulatory interdigitation across jurisdictions, necessary for the secure development of the field. The evidence of induction of MSC differentiation for potential clinical applications [49], based on animal studies and human clinical trials, considers efficiency of MSC local injection in tendon repair [22,50]. Applying differing autologous MSC concentrations (1, 4 and 8×10^6 cells/ml) significantly improved tendon repair, but does not appear to be dose-dependent, but needs to be regulated to ensure consistent therapeutic approaches.

Any biological agent used should be monitored, including long-term surveillance. This has been promoted as a philosophy within the cartilage community represented by the International Cartilage Repair Society (ICRS) [18,51] and any autologous tendon grafting is monitored through the Human Tissue Authority [52] though the development of a cell based registry is still awaited. As with arthroplasty, this intervention registry should allow for non-repudiable anonymised data to reconcile with cancer registries in order to either detect early association or ultimately allay fears of inappropriate cell transformation which underlies any type of biological intervention.

Such MSCs have been used in a variety of musculoskeletal applications. Recently MSCs have been found to increase in bone formation around revision hip implants when implanted with allograft in sheep [53] suggesting a wide range of potential MSC applications. Other areas in which, MSC therapeutic potential is currently being explored includes cartilage repair, particularly for osteochondral defects, which at present, if cell based therapy is chosen, typically involves chondrocyte implantation which has its own disadvantages [54]. Meniscus repair in a rat model has also been carried out [55], but clinical tendon MSC research remains focused on treating common degenerative conditions of the patellar, Achilles and forearm 'common extensor' tendon problems.

MSCs are broadly grouped as residential or wandering. It is the latter which is to be discouraged, since inappropriate expression such as heterotopic ossification can be anticipated. This implies that the development of novel surgical approaches such as 'patching' might be safer than injection which is not contained. There is no evidence of such migration in the domain of tendon regeneration but the theoretical risk remains. The whole field of tissue engineering and regenerative medicine is a new science. It is therefore experimental in nature and as such, all results of experimentation both in animal analogues or human subjects should be logged, and appropriately followed up [56]. It is essential that the development of the field is not marred by inappropriate clinical exploitation of immature technologies as seen in the development of gene vectors [43,44] a decade ago. The ultimate clinical intention would be to achieve an alternative to the surgical solution with lower morbidity and assurance that no long-term sequelae are being initiated by this approach. The ideal solution would be to construct testing around randomised controlled trial [57] methodologies, as has been the case with other biological therapies such as PRP. The cost of adequate numbers [58] is prohibitive and so, as with conventional prosthetic insertion, clinicians must ensure the experimental cases are registered with long-term registries such as the implant and oncology registries to ensure that any adverse events can actually be identified passively, and flagged automatically to the attending physician rather than solely depending upon researchers actively seeking associations. The potential of MSC therapy is apparent. Long term robust proof of its effectiveness presently remains elusive.

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