From tendon to nerve: a MSC for all seasons

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Abstract

The potential of mesenchymal stem cells (MSCs) to regenerate damaged tissue is well documented as this specialized progenitor cell type exhibits demonstrated superior cellular properties, which allow straightforwardly overcoming medical as well as ethical limitations. By now, MSCs have been successfully introduced in a manifold of experimental approaches within the newly defined realm of Regenerative Medicine. Advanced methods for in vitro cell expansion, defined induction of distinct differentiation processes, 3D-culture on specific scaffold material and tissue engineering approaches have been designed and many clinical trials, not only have been launched, but could recently be completed.

To date most of the MSC-based therapeutic approaches have been executed to address bone, cartilage or heart regeneration; further prominent studies showed efficacy of ex vivo expanded and infused MSCs to countervail graft-versus-host-disease. Yet more fields of application emerge, in which MSCs unfold beneficial effects, and presently therapies that effectively ameliorate non-healing conditions after tendon or spinal cord injury are forged ahead by scientific research to enter the clinical stage.

Keywords: MSCs, differentiation potential, tendon injury, spinal cord injury, regeneration, tissue engineering, scaffolds

Introduction:

Bone marrow primarily comprises hematopoietic cells embedded in supporting stroma. The latter also houses specialized precursors. First evidence for the presence of non-hematopoietic progenitors in bone marrow was put forward more than 140 years ago by the German pathologist Julius Friedrich Cohnheim (1839-1884); he was the first to notice the occurrence of “mesenchymal precursor cells” there (Cohnheim, 1867). This observation was affirmed not until 1970, when Alexander Friedenstein specified these particular cells in culture as firmly adhering to the surface of culture dishes and vastly forming colonies (Friedenstein, Chailakhjan, & Lalykina, 1970). Besides self-renewal potential, this cell type also exhibited multipotential differentiation capacity, and thus two decades later, Arnold Caplan coined the term of the “mesenchymal stem cell” (Caplan, 1991). As of now the biological attribution “mesenchymal stem cell” is still debated for at least in the embryo myogenic and the skeletal tissue do not share a common primordial precursor (Paolo Bianco, 2011)

In the early days, the heterogeneity of the non-hematopoietic cell isolates made it difficult to precisely specify single, distinct entities in the bone marrow and other stromal tissues representing specific mesenchymal precursors. Thus besides MSCs, other names were introduced by various research teams, e.g. “multipotent adult progenitors cells” (MAPCs), “marrow-isolated adult multilineage inducible cells (MIAMIs), or “very small embryonic-like stem cells” (VSELs) (Asahara et al., 1999; D'Ippolito et al., 2004; Y. Jiang, 2002; Kronenberg & Schipani, 2009; Reyes et al., 2001). In 2006, a group of peers in the field acting under the auspices of the ‘International Society for Cellular Therapy’ refined the minimal criteria for multipotent stromal cells: firstly MSCs should exhibit strong attachment to the surfaces of culture dishes (plastic adherence), secondly MSCs have to bear a set of, albeit not unique, surface markers such as CD90, CD73, CD105, CD146, CD44 while at the same time lacking the expression of CD34 and CD45, CD31, CD11b, CD14 and CD19, CD79α as well as HLA-

Class II, and thirdly, MSCs need to show tri-lineage differentiation potential into osteoblasts, pre-adipocytes and chondrogenic cells (Dominici et al., 2006).

In addition to the enlisted surface markers, MSCs also express transcription factors such as Oct4, Nanog and stage-specific embryonic antigen-4 (SSEA-4), which are actually prominently present in embryonic stem cells (Rastegar et al., 2010). The potential of differentiating into many different tissue-determining cell types, to name only a few bone, cartilage, muscle, tendon, heart, liver and blood vessels, distinguish MSCs not only as an important source for ubiquitously present mesenchymal precursor cells, but more than that, a powerful asset for tissue engineering strategies and clinical therapies.

MSCs can be easily obtained from various tissues such as bone, bone marrow, adipose tissue, periosteum, synovial membrane or fluid, skeletal muscle, dermis, placenta, liver, spleen or thymus (F. H. Chen, Rousche, & Tuan, 2006; Liu, Zhuge, & Velazquez, 2009; Rastegar et al., 2010). Traditionally MSCs are isolated by means of gradient centrifugation of bone marrow aspirates in order to separate erythrocytes from mononuclear cells. The latter are then being seeded onto plastic dishes and subsequently cultivated in the presence of media containing fetal calf, or bovine serum under controlled cell culture conditions. After 24 hours, the non-adherent cell fraction is stripped off from those firmly adhering, and by doing so eventually one MSC may be isolated starting from 10,000 bone marrow cells (Fehrer et al., 2007). In normal culture, clonogenically growing human MSCs are capable of accumulating up to 50 population doublings before becoming irreversibly growth arrested and replicatively senescent (Laschober et al., 2011; Lepperdinger, 2011). The individual cell line's "Hayflick Limit" differs donor-wise, yet also greatly depends on the fashion of aspirate preparation (Fröhlich et al., 2008). Compared to hematopoietic stem cells (HSCs), which are capable of repopulating niches in the bone marrow to efficiently sustain the production of blood cell precursors, a comparable proof for MSC stemness has only recently been adduced by Paul Frenette and colleagues, by and large enabled through the observation that MSCs express the neural-specific

intermediary filament nestin. Hence this research group together with cooperating laboratories could prove the MSCs potency to self-renew in serial transplantation in vivo by employing cells derived from transgenic mice that express GFP under the nestin promoter (Mendez-Ferrer et al., 2010).

MSCs in regenerative medicine and clinical applications

By virtue of their inherent multipotentiality as well as their ubiquitous appearance in many tissues, yet also due to the ease of expansion in culture, MSCs have by now been widely adopted in tissue engineering, and more than that, also tested in advanced clinical therapies (Rosenbaum, Grande, & Dines, 2008). As Regenerative Medicine paraphrases innovative clinical applications, in particular aiming to establish methods for integral restitution of traumatized or surgically removed organ parts and tissues, this highly interdisciplinary field is gathering many different research areas and topics such as cell biology, gene therapy, tissue engineering, scaffold testing and biomolecular signaling, altogether firstly tested in appropriate animal models. One major goal is to develop efficient tools for the treatment of immedicable diseases, or to design powerful methods to counteract lingering tissue degenerations, which come forth and accumulate with advancing age.

In this very context, it has been emphasized that bone-marrow derived MSCs, which have only recently been introduced in the clinics very successfully for curing graft-versus-host-disease (Le Blanc et al., 2008; Tolar, Villeneuve, & Keating, 2011) will be the prime cell source for cell-based clinical therapies. Further on along this general line, it could be shown in baboons that the survival of skin grafts is greatly supported by concomitant treatment with MSCs derived from bone marrow (Bartholomew et al., 2002). Although immune modulatory processes guided by MSCs are well documented, and the phenomenal anergic properties of MSCs have been highly appreciated by the stem cell community (Trento & Dazzi, 2010; Uccelli, Moretta, & Pistoia, 2008), the underlying molecular mechanism still await detailed elucidation to be fully understood (Tolar et al., 2011).

Certainly, various critical points regarding donor as well as host specificity, such as age, sex and systemic health status need to be carefully considered, and proper standards have to be defined and broadly accepted to warrant future success in presently hardly curable pathologies (DiGirolamo et al., 1999; Rastegar et al., 2010).

Tendon

Tendons connect muscle to bone and exhibit properties such as durable strength due to the compact collagenous structure of extraordinary elasticity. The repair of damaged tendon tissue is an intricate process, requiring a lot of time to regain biomechanical levels, which are sure enough necessary for proper function and mobility. The tendon structure is dominated by collagen organized into fibrils, fibers, fiber bundles, and fascicles in concert with other extracellular matrix proteins. The structure of tendons, being actually organized into single fibrils to be individually harmed, warrants only minor damage taking place before the entire tendon ruptures. Tendon injuries happen with increasing frequency these days as people are more physically active, and also turn older. Despite good condition and fitness, elderly person may individually suffer from tendon abrasions and tendon weakness. Most frequently affected tendons are the supraspinatus tendon of the rotator cuff, the Achilles tendon, flexor tendons of the hand as well as the anterior cruciate and medial collateral ligaments of the knee (Aslan, Kimelman-bleich, Pelled, & Gazit, 2008). Therapeutic options to repair injured tendon tissue exist by implanting autografts, allografts and synthetic prostheses, but unfortunately none of these therapeutic regimens provide symptom-free long-term solutions. Possible side effects of surgical treatment are nerve damage, donor site morbidity, muscle atrophy, stiffness, scar formation and decreased mobility (Longo, Lamberti, Maffulli, & Denaro, 2010). One of the most prevalent side effects in tendon healing is scar formation, which not only hinders function but also bears the increased risk of further tissue damage through exuberant inflammation. Hence tendon may not only be harmed by acute trauma but substantially weakened when suffering from

119 chronic inflammatory insults during enduring tendonitis, tendinosis, bursitis, or epicondylitis
120 eventually resulting in tendon rupture.

121 Besides the use of growth factors, cytokines or gene therapy, the field of tissue engineering based on
122 MSCs is vastly emerging (Longo, Lamberti, Maffulli, & Denaro, 2010). Despite well-established
123 surgical procedures and subsequent therapeutic regimens, MSCs are today considered an interesting
124 option for tendon regeneration, and therefore MSC-based applications are presently being studied in
125 humans, rabbits, rats and horses (Violini, 2009).

126 Interestingly, there is no commonly accepted standardized recipe to convert naïve MSCs into
127 tenogenic precursors. Moreover, no reliable protocols have been established for differentiation of
128 MSCs into tenocytes. Members of the bone morphogenetic protein (BMP) and the transforming
129 growth factor (TGF) superfamily as well as fibroblast-like growth factors (FGF) have been tested (for a
130 recently published comprehensive review see Longo et al., 2010). Exceptionally, rat bone marrow
131 MSCs can be stimulated in vitro by BMP-12 to differentiate along the tenogenic lineage (Lee et al.,
132 2011). This finding is strengthened by the observation that BMP-12 pretreated MSCs improved
133 tendon healing in a calcaneal tendon defect. More than a decade ago, experimental attempts have
134 been commenced by loading scaffold material with MSCs prior to transplantation. The material
135 composed of a pretensioned polyglyconate suture was first cellularized with synovium-derived MSCs
136 and thereafter grafted into an iatrogenically introduced, 1-cm Achilles tendon gap in a rabbit. This
137 resulted in regeneration with well-organized collagen fibers and enlarged cross-sectional areas within
138 the defect (Young et al., 1998). Since then, both direct delivery of MSCs, or engraftment of MSCs in
139 combination with biomatrices have been thoroughly investigated in more than 20 published studies
140 (see Table 1). One particular study yielded important results: when loading increasing numbers of
141 bone marrow-derived MSCs (1, 4 and 8 million of rabbit MSCs/ml) onto collagen gels, those defects
142 that contained MSC-collagen scaffolds showed indeed much higher maximum stresses and moduli
143 compared to natural healing defects, but the increasing cell numbers showed no distinct differences
144 with regard to organization of the regenerated tissue or matrices (Awad et al., 2003). This

observation raised the question how MSCs contribute to tendon healing and regeneration, in particular whether the delivered MSCs are actually dominantly influencing wound healing and regeneration through secreted factors. In this context it is also important to note that MSCs show beneficial long-term effects in the course of tendinitis. In an equine flexor digitorum superficialis tendon model of collagenase-induced bilateral tendinitis lesions, bone marrow-derived MSC treatment induced no aberrant tendon-specific marker expression such as collagen I or III, insulin-like growth factor, cartilage oligomeric matrix protein, matrix metalloproteinases and aggrecanase-1. Yet those tendons treated with MSCs showed indeed improved histological scores (Schnabel et al., 2009), again raising the up till now insufficiently answered question what are the molecular mechanisms that govern this effect. Due to these results, regeneration of ruptured or damaged tendon tissue has received a lot of interest in veterinary medicine, with the greatest resonance in equine orthopedics. To treat tendon injuries in horses, marrow cells are now often explanted from the sternum, and re-implanted into the damaged tendon tissue (Richardson, Dudhia, Clegg, & Smith, 2007).

Addressing age-related tendon pathology, effectiveness of MSCs has been investigated in regeneration of the enthesis, the site where tendon attaches to the bone. Often this part degenerates with increasing age leading to mobility failures. In comparison to delivery of chondrocytes, MSCs treatment strongly enhanced regeneration of the enthesis actually resulting in morphological and biomechanical properties similar to those of a normal enthesis morphology and function (Nourissat et al., 2010).

Bone and cartilage

In many aspects of orthopedics, bone and cartilage regeneration after trauma and tumor surgery, as well as in the geriatric patient are of increasing relevance since molecular mechanisms which had been elucidated by means of experimental osteologic and biogerontologic research are being

translated into innovative technology and medical applications. Just about endless individual approaches in the recent decades have led to improved implant materials to be used in the clinics, as well as bioactive factors with proven efficacy, and MSCs combined with biomaterial and bioactive factors emerged as a powerful additional concept of enhancing bone formation in degenerated or injured tissues. Successful cell-based bone tissue engineering has been reported for isolated MSCs from bone marrow, followed by in vitro cultivation and expansion before seeding plastic adherent MSCs onto porous scaffold material in a 3D culture system (P. Bianco & Robey, 2001). Many laboratories have demonstrated that MSCs, when being loaded on hydroxyapatite scaffolds and implanted into NOD/SCID mice are capable of generating functional bone tissue (Krebsbach et al., 1997). Besides hydroxyapatite other natural or synthetic material has been tested to serve as innovative scaffolds such as plant-derived material, collagen type I, tri-calcium phosphate ceramics, or poly-DL-lactic-co-glycolic acid (PLGA)(Cancedda, Dozin, Giannoni, & Quarto, 2003). For treatment of osteogenesis imperfecta, a genetic bone disorder, bone marrow-derived MSCs have been infused whichfunctionally improved bone and cartilage formation (Pereira et al., 1995). Also an experimentally set traumatic alveolar bone defect in the maxilla of Sprague-Dawley rats could be successfully treated with non-pre-osteoinducted bone marrow-derived MSCs actually yielding considerably higher bone formation (Zhang et al., 2002). Most tempting were results challenging the immune modulatory potential of bone marrow-derived MSCs, as application resolved severe inflammation at the implantation site of ceramic ankle prosthesis, or in cases of aseptic loosening of the total ankle arthroplasty (Ohgushi & Caplan, 1999).

Bone is continuously remodeled by a concerted action of osteoclasts responsible for bone resorption, and osteoblasts accounting for osseous growth. Thus due to the ability of MSCs to form osteoblasts, MSCs have early on been tested in bone regeneration studies. Osseous healing features but one particular interesting result, which is scarless functional bony tissue. A commonly used method to investigate bone regeneration is the so-called ‘critical size defect’, which when left alone shows insufficient ossification (Schmitz & Hollinger, 1986). Employing this approach as a by now commonly

196 accepted standard to investigate defect healing, rapid progress has been achieved in the previous
197 decades (Fröhlich et al., 2008). Ectopic implantation of sheep bone marrow-derived MSCs loaded on
198 a hydroxylapatite carrier into a critical size defect within long bone improved bone formation and
199 healing to a much higher content when compared to controls (Kon et al., 2000).

200 In conjunction with cell-based approaches emphasis has been put on the quickly translate cell-based
201 skeletal tissue engineering strategies to the bedside (for a recent review see Panetta, Gupta, &
202 Longaker, 2010).

203 Similarly, regenerative methods for cartilage repair have been introduced in the clinic in 1999 by
204 performing autologous chondrocyte transplantation (Brittberg, 1999). By the same token, the
205 application of MSCs for cartilage regeneration is an evolving field. Many attempts have been
206 undertaken applying MSCs after forced in vitro chondrogenic differentiation and subsequent seeding
207 of the precursors onto specific scaffold materials prior to engraftment into cartilage defects.
208 Interestingly, when filling a cartilage defect for just 10 minutes with synovium-derived MSCs, enough
209 cells appear to settle in thereby resulting in efficient cartilage regeneration. Due to this type of
210 unsophisticated application, i.e. without preceding periosteal coverage of the wound and no further
211 use of scaffold material, this low-invasive method appears most promising (Koga et al., 2008).

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213 ***Heart***

214 Heart failure is often fatal. Presently, myocardial infarction is the most common cause for heart
215 failure with highest prevalence in the western societies. Heart is also thought to exhibit least
216 regenerative potential as growth of new vessels within infarcted areas appears to be greatly
217 suppressed and cardiomyocytes are supposedly arrested in the cell cycle after development. This
218 paradigm is shifting, and the myocardium is currently being targeted by various regeneration
219 strategies (Choi et al., 2010). Adult progenitor and stem cell treatment of diseased human
220 myocardium has been carried out for more than 10 years (Menasche et al., 2001). Since then various

precursor types and cells have been employed in studies in humans. Notably, hematopoietic stem cells or endothelial progenitor cells, although yielding considerable angiogenic effects, showed no satisfying results (Stamm, Nasseri, Choi, & Hetzer, 2009). Working along similar lines, MSCs and related cell types have been evaluated in preclinical models of disease as well as in more than 20 clinical trials, which employed adult stem cells (bone marrow stem cells, mobilized peripheral blood stem cells and skeletal myoblasts) for treatment (Wollert & Drexler, 2010). Thoroughly studied to date is also therapeutic treatment with MSCs. A general consensus from these trials is that MSCs or blood-derived stem cells do not replenish lost cardiomyocytes or vascular cells, yet it is the powerful paracrine effect of MSCs, which prevailingly acts on the heart tissue that triggers beneficial effects of tissue regeneration (Lai, Chen, & Lim, 2011).

Spinal cord injury

Severe trauma of the spinal cord may lead to functional impairment of nervous tissue, in particular structural damage of axons resulting in demyelination, and clinical consequences for patients who suffer from severe spinal cord injury (SCI) is partial or complete loss of motor and sensor function. Regeneration and functional restoration after SCI (Callera & do Nascimento) only rarely occurs as glial scar formation takes place driven by non-permissive environmental circumstances of enduring and overly prevailing inflammatory responses, and not to forget the fatal lack of neurogenic supporting factors in adult tissues. In the US, there are approximately 12,000 new patients per year who contract SCI, mostly as a result of physical injury accompanied with inflammation (Ichim et al., 2010). Currently there is no efficient treatment for SCI.

In this context MSCs could be turned into neurogenic precursors which express specific neuronal markers in vitro (Delcroix, Curtis, Schiller, & Montero-Menei, 2011; Deng, Petersen, Steindler, Jorgensen, & Laywell, 2006). After engraftment into rats, these precursors formed bundles of neurofilament-positive fibers in scar tissues (Hofstetter et al., 2002). Currently MSC-based

approaches are tested to warrant survival and remyelination of axons (Wright, El Masri, Osman, Chowdhury, & Johnson, 2010).

Also the immunosuppressive effects of MSCs are considered benevolent, in particular as they are thought to cease the characteristic symptoms of SCI by settling the inflammatory response, which in due course should reduce cavity formation and demyelination (Uccelli, Benvenuto, Laroni, & Giunti, 2011). In spite of future expectations and promising vistas, only vague anticipations have been gained of whether MSCs perform salubrious after transplantation into patients suffering from SCI (Ichim et al., 2010). It remains to be shown whether bone marrow-derived MSCs exhibit sufficient neurogenic transdifferentiation capacity in vivo, and whether they show high enough survival rates within the transplantation sites (Wright et al., 2010).

The dog is the most frequently used, and presumably also most appropriate animal model to investigate SCI. The “Olby scoring system” allows a highly accurate analysis of regeneration and recovery of damaged axons (Olby et al., 2001). Working along this line, the performance of autologous and allogenic MSCs has been evaluated in the SCI healing process. Notably, autologous bone marrow-derived MSCs showed improved functional recovery after SCI compared to allogenic cells (Jung et al., 2009). The interpretation of this finding is tempting as it is likely that it is more than the paracrine factors, which are produced by MSCs, playing a critical role: it is highly likely that MSC progeny exert the beneficial effect through integration into reconstituting nervous tissue. Also other animal models have been employed as experimental models in order to improve MSCs application to cure SCI, for instance to investigate different delivery methods of MSCs (Table 2). In a rat model undergoing a subtotal hemisection at cervical level 4, human bone marrow-derived MSCs have been grafted after SCI in order to specify the most efficient way of transplantation. In this study delivery via lumbar puncture compared superior over intravenous injection or the commonly used method of direct injection (Paul, Samdani, Betz, Fischer, & Neuhuber, 2011). An interesting way of improving regeneration of SCI is electrical stimulation or electrical acupuncture to invoke permissive physiological signals for the recovery of damaged nerve tissue, i.e. applying spike waves, resulted in

improved functionality and recovery in SCI rats by increasing the survival rate of the implanted bone marrow-derived MSCs (Wu et al., 2011).

It is generally believed that a very important factor in regenerating damaged neurons and axons is the action of neurotrophic factors secreted by MSCs (Wright et al., 2010). The role of neurotrophin 3 (NT-3) was investigated in this context using human umbilical cord MSCs overexpressing this bioactive molecule (Shang et al., 2011). In rats, NT-3 secreting MSCs brought about improved locomotor function and myelination. Furthermore, the neuropeptide pituary adenylate cyclase activating polypeptide (PACAP) is known to trigger cAMP production actually not only controlling axonal regeneration but also neurogenesis and protection. A combinatorial therapy of immortalized human MSCs and PACAP was thus performed (Fang et al., 2010). In vitro analysis on neurologic differentiation revealed a positive effect on neurodifferentiation when exposing human MSCs to PACAP and other neurogenic factors, such as dbcAMP, β-mercaptoethanol and retinoic acid. Notably in SCI rats, hind limb functionality greatly improved, which ranks this particular way of thinking and experimentation high in continuation of optimizing future strategy in this field.

Neurological disorders

MSCs are also considered potential assets for other neurological disorders. Multiple system atrophy (MSA) is paralleled by degeneration of nerve cells in specialized brain tissue, and thus besides movement and balance disorders MSA patients suffer also from a combination of autonomic failures. Intravenous infusion of GFP-tagged MSCs in a transgenic MSA mouse model resulted in protection of dopaminergic neurons in the substantia nigra pars compacta and also ameliorated neuroinflammation in this area (Stemberger et al., 2011). The potential of MSCs in the treatment of experimental autoimmune encephalomyelitis (EAE), a model for human multiple sclerosis has also been exploited (Uccelli et al., 2011). Intravenous infusion of bone marrow-derived MSCs improved the clinical course of EAE (Gerdoni et al., 2007; Zappia et al., 2005). In this model, the injected MSCs

297 firstly induced peripheral T cell tolerance to myelin proteins resulting in reduced migration of
298 pathogenic T cells to the CNS, and secondly and most notably, also homed themselves to the CNS
299 where they protected axons and stalled demyelination. In a later study, adipose-derived MSCs
300 demonstrated pronounced therapeutic potential following a bimodal mechanism by which the
301 autoimmune response was greatly suppressed in early phases of disease, while in animals with
302 established disease local neuroregeneration by endogenous progenitors was stimulated only later
303 (Constantin et al., 2009). Bone marrow-derived MSCs were also tested in an animal model
304 resembling Parkinson's disease thereby exerting neuroprotection on 6-hydroxydopamine-exposed
305 dopaminergic neurons supposedly enforcing anti-apoptotic mechanisms (Wang et al., 2011). In the
306 same rat model intranasal injection of MSCs resulted not only in the appearance of cells in the
307 olfactory bulb, cortex, hippocampus, striatum, cerebellum, brainstem, and spinal cord, they also
308 decreased the concentrations of a variety of inflammatory cytokines in the lesioned side comparable
309 to levels in the intact hemisphere, and they completely eliminated 6-hydroxydopamine-induced
310 apoptosis (Danielyan et al., 2011). This study most likely also represents one of the first non-invasive
311 applications of stem cells countervailing a neural disorder.

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313 **Potential pitfalls**

314 Many of the aforementioned clinical applications of MSCs bear considerable risks of undesirable
315 outcomes, primarily because there are currently no unique molecular identifiers to specify well-
316 defined subpopulations within the heterogenous MSCs set, which actually exert distinct superior
317 activities in a particular medical setting. Dealing with such functional implications, for instance
318 immune suppression triggered by MSCs, could be both benefit as well as detriment. Either
319 supporting or exogenously controlling these particular features needs to be decisively implemented
320 in the standards and guidelines of clinical therapies. By the same token, other phenotypic
321 characteristics of mesenchymal progenitors such as fate specification, tissue-specific homing

properties, migration and adhesion abilities are to be considered in this context, yet have so far not been studied in great detail. MSCs, although being a highly appreciated stem cell type for clinical application, do also interact with tumor cells leading either to tumor growth or suppression as reported in several well-documented cases (Klopp, Gupta, Spaeth, Andreeff, & Marini, 2011). Indeed prospectively designed long-term safety studies are imperative here, as more and better understanding of those mechanisms which govern MSC tumor interaction need to be gained in order to prevent potential tumor growth and/or metastasis (Si, Zhao, Hao, Fu, & Han, 2011). In the case of MSC-based therapies in SCI patients, direct injection of MSCs into the site of injury may also lead to further neurological impairment, thus highly refined surgical procedures have to be developed in parallel to enhancing the MSC regenerative potential.

Conclusion and future perspectives:

Over the last two decades the suitability and appropriateness of MSCs for clinical applications could be demonstrated, and highly specific therapeutic strategies have been introduced. Besides a myriad of studies carried out in the field of bone, cartilage and cardiac repair, stimulation of tendon regeneration, and also the MSC's potential efficacy for functional restoration of nervous tissue has been exploited, indeed resulting in tempting and promising data. Working along this line, a vastly increasing number of clinical trials using MSCs as the therapeutic agent, are actively pursued or currently being launching. Quite some trials were recently completed and corresponding study results have been reported (Table 3). There are however many more studies that have only recently been completed yet as of now results have not been reported as notified at http://clinicaltrials.gov (Table 4). These outcomes will hopefully shed new lights on the efficacy of MSC-based medical therapies and related approaches, thus certainly more scientifically rewarding information is to be expected for the nearest future.

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351 **Table 1: Tendon tissue engineering using MSC**

Tendon Tissue	MSC	Animal model	Type of study	outcome	reference
Achilles tendon	Synovium-derived MSCs	rat	Early remodeling of tendon-bone healing	Early Tendon-Bone remodeling is improved by the implantation of synovial MSCs	(Ju, Muneta, Yoshimura, Koga, & Sekiya, 2008)
Achilles tendon	BM-derived MSCs	rat	Growth factor enhanced tendon defect repair	BMP12 induced tenogenic differentiation of rMSCs in vitro and resulted in the formation of tendon like tissue	(Lee et al., 2011)
Superficial digital flexor tendon	BM-derived MSCs	horse	Repair of superficial flexor tendon in racing horses	The use of autologous undifferentiated MSCs was successful in regenerating damaged equine tendons without bone deposition	(Pacini et al., 2007)
Achilles tendon	Murine MSC line C3H10T1/2.	rat	Manipulation of molecular signaling to induce neotendon formation	MSCs overexpressing the Smad8 molecule and BMP2 improves regeneration of torn ligaments	(Hoffmann et al., 2006)
Patellar tendon	BM-derived MSCs	rabbit	Repair of tendon injuries using a cellularized biomatrix	Composites made from MSCs and collagen gel improve patellar tendon repair biomechanics compared to natural repair	(Awad et al., 2003)
Achilles tendon	BM-derived MSCs	rabbit	MSCs loaded collagen matrix for tendon repair	Application of a MSCs-collagen scaffold enhances tendon repair	(Young et al., 1998)

352 **Table 2: Animal studies using MSC in experimental spinal cord injury (SCI) models**

Spinal cord injury	MSC type	Animal model	Type of study	outcome	reference
Severe contusive SCI	BM-derived MSCs	rat	Intravenous administration	Functional recovery after SCI was improved in MSCs treated SCI models compared to sham control group	(Osaka et al., 2010)
SCI	BM-derived MSCs	beagle dogs	Cell transplantation autologous vs allogenic	Autologous MSCs transplantation superior over allogenic MSCs	(Jung et al., 2009)
SCI	BM-derived MSCs	rat	MSC expressing neural markers in vitro promote recovery after SCI	MSCs survived in damaged nerve tissue thereby forming nerve fiber similar to normal tissue with functional improvement of recovery	(Hofstetter et al., 2002)
SCI	BM-derived MSCs	rat	combined treatment applying electric stimulation together with MSC	Concomitant application of spike waves prolongs MSCs survival	(Wu et al., 2011)
SCI clip spinal cord injury	Umbilical cord blood derived MSCs	rat	Recombinant expression of NT3 by transplanted MSCs	NT-3 enhanced the therapeutic effects of MSCs transplantation	(Shang et al., 2011)

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357 **Table 3 Previously published studies testing MSCs under various clinical conditions**

Clinical condition	MSC	Patient number	Type of study	Outcome	reference
Jaw defect	BM-(bone marrow) derived MSCs	6 patients (3 male, 3 female)	Osteogenic induced MSCs loaded on a bone substitute are implanted into intra-oral defects	Bone formation by implanted MSCs was observed in 1 out of 6 patients	(Meijer, de Bruijn, Koole, & van Blitterswijk, 2008)
Multiple sclerosis	BM-derived MSCs	10 MS patients (7male, 3 female), and 8 healthy donors	Intravenous application of autologous BM MSCs as a novel safe and feasible approach in MSC patients	The feasibility and safety of this approach was guaranteed and there was no significant differences in brain imaging MTR measures between controls and patients	(Connick et al., 2011)
Stroke	BM-derived MSCs	12 patients (3 female, 9 male)	Intravenous injection of autologous MSCs in patients suffering from stroke	Intravenous infusion of BM derived autologous MSCs decreased lesion volume and no tumor or abnormal cell growth was detected by MRI analysis within one year	(Honmou et al., 2011)
Type 2 diabetes patients with islet cell dysfunction	PD -placenta derived) MSCs	10 (7 males)	Transplantation of PD MSCs as a new therapeutic approach helping to recover T2D patients with islet cell dysfunction	PD-MSCs treatment reduced their daily insulin requirements, controlled their blood glucose fluctuations and improved their quality of life	(R. Jiang et al., 2011)
Acute Myocardial Infarction	BM-derived MSCs	78 (62 men, 56±8 years)	Effect of MSCs mobilization by granulocyte-colony stimulating factor (G-CSF) on Acute Myocardial infarction	MSCs mobilized by G-CSF are not homing and reduce recovery of acute myocardial infarction (Explanation the inverse association between circulating MSCs and changes in ventricular function)	(Ripa et al., 2007)
Acute Myocardial Infarction	BM-derived MSCs	69 (66 male, 3 female)	The effect of autologous MSC transplantation on the function of the left	MSCs implantation improved cardiac function	(S. Chen et al., 2004)

			ventricular function		
Old myocardial infarction	BM-derived MSCs	8 (1 female, 7 males),mean age 49 (range:36-66 years)	Autologous MSCs were either applied into myocardium or into coronary arteries to improve recovery of patients suffering from old myocardial infarction	Cardiac function was improved. Application of MSCs to myocardium emerged to be a safe and feasible procedure	(Mohyeddin-Bonab et al., 2007)
End stage liver cirrhosis	BM-derived MSCs	8 (4male, 4 female, thereof 4 patients hepatitis B, 1 hepatitis C, 1 alcoholic, and 2 cryptogenic)	The effect of autologous MSCs on end stage liver cirrhosis patients	Injection of MSC improved liver function as verified by the Model for Liver disease score which significantly decreased in all patients	(Kharaziha et al., 2009)
Degenerative joint disease	BM-derived MSCs	One patient (Case Report)	Application of autologous MSCs into the knee of a patient suffering from degenerative joint disease	Patient treated with autologous MSCs depicts significantly higher cartilage and meniscus growth visualized by MRI	(Centeno et al., 2008)
Multiple Sclerosis/ Amyotrophic Lateral Sclerosis	BM-derived MSCs	15 (multiple sclerosis, MS), 19 (Amyotrophic Lateral Sclerosis, ALS)	Evaluation of the safety and feasibility of MSCs transplantation in patients with MS and ALS	Application of autologous MSCs into MS and ALS patients was found to be a safe therapeutically method and strongly induces immediate immunomodulatory effects. MRI analysis revealed possible dissemination of the MSCs from the lumbar site of inoculation to occipital horns, meninges, spinal root and spinal cord parenchyma.	(Karussis et al., 2010)
Hematologic malignancies (including primary immune deficiencies, lacking an HLA matched donor)	BM-derived MSCs	14 Patients (9 male, 5 female), 47 controls (28 male, 19 female)	In vitro expanded MSCs accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem cell	Co-transplantation of MSCs and haploidentical peripheral blood stem cells reduced the risk of graft failure in haploidentical HSC transplant recipients	(Ball et al., 2007)

			transplantation		
Liver Cirrhosis	BM-derived MSCs	4 (1male, 3 female)	Transplantation of autologous BM MSCs into patients suffering from end stage liver cirrhosis	The MELD score was improved at patient 1 and 4, furthermore life quality of all follow-up patients was meliorated	(Mohamadnejad et al., 2007)

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361 **Table 4: Recently completed, yet unpublished studies employing MSC (source of information: www.clinicaltrial.gov, July 2011)**

Study	Condition	Intervention/Phase	Primary outcome measures	ClinicalTrials.gov Identifier number and contact	Study completion Date
Autologous Adipose Derived MSCs Transplantation in Patient With Spinal Cord Injury	Spinal Cord Injury	Intravenous infusion of autologous adipose derived MSCs ("RNL-Astrostem"). Dose: 4 x 10e8 cells into eight male patients suffering from SCI. Phase I	Safety evaluation through physical examination, vital sign and laboratory test after "RNL-Astrostem" injected	NCT01274975 RNL Bio Company Ltd Principal investigator : SangHan Kim, MD Anyang Sam Hospital, Korea	February 2010
Safety and Efficacy of Intracoronary Adult Human MSCs After Acute Myocardial Infarction	Acute Myocardial Infarction	Intracoronary injection frequency : single dose of autologous bone-marrow derived MSCs Dosage : 1x1e6 cells/kg Duration: mean injection duration approximately 4 weeks after primary percutaneous coronary intervention Phase II/III	Absolute changes in global left ventricular ejection fraction (LVEF) as measured by SPECT 6 months after cell infusion	NCT01392105 Yonsei University FCB-Pharmicell Co Ltd. Principal Investigator: Seung-Hwan Lee, MD, PhD Yonsei University Wonju College of Medicine, Wonju Christian Hospital, Korea	May 2010
Induction Therapy With Autologous MSCs for Kidney Allografts	Renal Transplant Rejection	Kidney transplantation with MSCs infusion	Incidence rate of biopsy-proven acute rejection and early renal function recovery after MSCs	NCT00658073 Fuzhou General Hospital, China Study Director: Jianming Tan, M.D and Ph.D Fuzhou General Hospital, China	May 2010
Articular Cartilage Resurfacing With MSCs In Osteoarthritis Of Knee Joint	Osteoarthritis	Intra Articular Injection of Mesenchymal cells to the knee joint Phase I	Evaluation of the effect of MSCs transplantation in respect to patients pain relief	NCT01207661 Royan Institute Study Chair: Hamid Gourabi, PhD Head of Royan Institute, Iran	November 2010
Stromal Therapy of Osteodysplasia After Allogeneic Bone Marrow Transplantation	Osteodysplasia	Infusions of ex vivo expanded, gene marked donor bone marrow stromal cells following allogeneic bone marrow transplantation Phase I	Evaluation of the safety of the stromal cell infusion	NCT00186914 St. Jude Children's Research Hospital Drexel University Wayne State University Principal Investigator: Kimberly Kasow, DO St. Jude Children's Research Hospital, Tennessee, US	January 2008
Donor MSCs Infusion in Treating Patients With Acute or Chronic Graft-Versus-Host Disease After Undergoing a Donor Stem Cell Transplant	Cancer	Best dose of donor MSCs in treating patients with acute or chronic graft-versus-host disease after undergoing a donor stem cell transplant Phase I	Safety evaluation of MSCs infusion by monitoring patients for 6 hours for infusion related toxicity.	NCT00361049 Case Comprehensive Cancer Center National Cancer Institute (NCI) Principal Investigator: Hillard M. Lazarus, MD Ireland Cancer Center at University Hosptials Case Medical Center, Case Comprehensive Cancer Center, Ohio, US	November 2010

Autologous Transplantation of MSCs (MSCs) and Scaffold in Full-thickness Articular Cartilage	Knee Cartilage Defects; Osteoarthritis	Evaluation of the clinical results obtained with autologous MSCs expanded in culture for the treatment of full-thickness chondral defects in human knee Phase I	Knee cartilage defects after a time frame of 12 months	NCT00850187 Royan Institute Tehran University of Medical Sciences Study Chair: Hamid gourabi, PhD Chief, Iran	December 2010
Prochymal™ Adult Human MSC for Treatment of Moderate-to-severe Crohn's Disease	Crohn's Disease	Prochymal™ MSCs or adult MSCs infused into patients with moderate-to-severe Crohn's disease. Infusions will occur on two separate days, 7-10 days apart. Patients will be monitored for reduced Crohn's disease symptoms. Patients will receive high (8 million cells) or low dose (2 million cells) Phase II	Crohn's disease activity index	NCT00294112 Osiris Therapeutics, US	September 2008
Improvement of Liver Function in Liver Cirrhosis Patients After Autologous MSCs Injection: a Phase I-II Clinical Trial	Liver Failure; Cirrhosis	Infusion of autograft MSCs differentiated into progenitors of hepatocytes into the portal vein for the salvage treatment of patients with end-stage liver disease into portal vein under ultrasound guide Phase I /II	Liver function test and MELD (model for end stage liver disease) score	NCT00420134 Shaheed Beheshti Medical University Tarbiat Modarres University Study Chair: Mohammad Reza Zali, MD Research center of Gastroenterology and Liver Disease	June 2009
Autologous Implantation of MSCs for the Treatment of Distal Tibial Fractures	Tibial fracture	Cells will be isolated from the patient's bone marrow, loaded onto a carrier and implanted locally at the fracture site. Phase I/II	Safety, Number of patients reaching clinical union of fracture	NCT00250302 Hadassah Medical Organization Teva Pharmaceutical Industries Principal Investigator: Meir Liebergall, Prof. Hadassah Medical Organization, Israel	April 2011
Comparison of Autologous MSCs and Mononuclear Cells on Diabetic Critical Limb Ischemia and Foot Ulcer	Autologous Transplantation, Diabetic Foot	MSCs and MNCs transplanted into impaired lower limbs by intramuscular injection Phase I	Magnetic resonance angiography	NCT00955669 Third Military Medical University Study Director: Chen Bing, doctor Endocrinology and Metabolism Department, the South West Hospital of the Third Military Medical University, China	August 2010
Treatment of Non Union of Long Bone Fractures by Autologous MSCs	Nonunion fractures of long bones	Injection of mesenchymal cells in fractured zone Phase I	Radiological progression of bone fusion , callus formation in fracture zone	NCT01206179 Royan Institute Tehran, Iran, Islamic Republic of Study Chair: Hamid Gourabi, PhD President of Royan Institute, Iran	May 2011
Safety and Efficacy of Stem Cell Therapy in Patients With Autism	Autism	Participants with rehabilitation therapy plus combination of hCB-MNCs (human cord blood mononuclear cells) together with hUC-MSCs (human umbilical cord MSC) transplantation	Childhood Autism Rating Scale months after treatment Clinical Global Impression	NCT01343511 Shenzhen Beike Bio-Technology Co., Ltd. Shandong Jiaotong Hospital Association for the Handicapped Of Jinan	May 2011

		with a 6 months follow-up. Phase I/II	Scale (CGI)	Principal Investigator: Yongtao Lv Shandong Jiaotong Hospital, China	
Marrow Mesenchymal Cell Therapy for Osteogenesis Imperfecta: A Pilot Study	Osteogenesis Imperfecta	Autologous transplantation of CD3 lacking BM-derived MSCs to patients suffering from OI	Effect of the infusion of BM-MSCs lacking CD3+cells with respect to the growth rate of children with osteogenesis imperfect or bone mineral content of children with OI	NCT00187018 St. Jude Children's Research Hospital Principal Investigator: Gregory Hale, M.D. St. Jude Children's Research Hospital, Tennessee, US	August 2007

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