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Cell- & Tissue-based Therapy

Adipose-derived stem cells for the regeneration of damaged tissues

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As the promise of stem cell-based therapies begins to be realised, and efforts to bring advances to the clinic mount, the source of these cells is increasingly important. The morbidity associated with harvesting stem cells from solid organs and the invasive nature of bone marrow biopsies may limit their practicality for wider clinical applications. An emerging body of literature suggests that adipose tissue may provide an abundant, readily accessible source of cells with similar potential to that described of other adult stem cells. This review will address advances in the use of adipose stem cells in fields as divergent as soft tissue reconstruction and cerebral infarction recovery. Numerous challenges will also be discussed; however, rapidly accumulating advances suggest that adipose stem cells may be as effective as they are abundant.

Keywords: adipose, differentiation, regeneration, stem cells, stromal cells, tissue engineering

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1. Introduction

Stem cells, first identified in embryonic tissue and later in numerous adult tissues, possess the unique capacity to differentiate into a range of tissue types. However, although embryonic cells are the most flexible of stem cell lines, they bring with them a myriad of ethical issues. Stem cells isolated from adult tissue sidestep this issue entirely. They are a fibroblast-like reserve of cells that lie quiescent in mature tissues, but can home to and repair injury when necessary [1]. To date, adult stem cells have been found in such divergent sites as bone marrow, peripheral blood, CNS tissue, liver, pancreas, muscle, skin, lung, intestine, heart and fat [1-3]. For clinical applications, an ideal source of stem cells would be abundant, accessible and readily harvested with minimal risk to the patient. Stem cells isolated from adipose tissue may provide exactly this set of specifications.

Researchers studying the development of adipose tissue have long worked with a fibroblastic cell line, known as preadipocytes, isolated from adipose tissue digestion [4-8]. These cells, so named for their ability to differentiate into mature adipocytes, have recently become a focus of mesenchymal stem cell (MSC) researchers following the recognition of their broader multipotentiality [3,9]. Since those initial reports, adipose stem cells (ASCs) have demonstrated a diverse plasticity, including differentiation into adipo- [3,9-15], osteo- [3,9,12,15-19], chondro- [3,9,12,18,20,21], myo- [3,9,22], cardiomyo- [23-26], endothelial [27], hepato- [28], neuro- [9,12,29-33], epithelial [34] and haematopoietic [35] lineages, a repertoire not dissimilar to that described for bone marrow-derived MSCs (BM-MSCs) [36,37].

When ASCs are compared with BM-MSCs, further similarities have been demonstrated in regards to their growth kinetics, cell senescence, gene transduction efficiency [38], as well as CD surface marker expression [9,39,40] and gene transcription [39]. Noted exceptions include the lack of expression of CD62L [39] as well as the presence of CD49b [9,39], molecules thought to be involved in cell

homing to bone marrow or adipose tissue compartments. Although independent ASC preparations yield a reproducible cell population based on adherence and cell and protein profiling, the delineation and interpretation of a specific phenotype remains elusive and ethereal [39]. Both ASCs and BM-MSCs are stromal populations isolated from a fatty compartment based on their ability to adhere to plastic. Adipose tissue, in contrast to bone marrow, is routinely extracted in cosmetic procedures with minimal risk to the patient. From this tissue, average yields in the range of 200,000 – 290,000 cells/g of tissue [41,42], or 404,000 cells/ml of lipo-aspirate [43], can be isolated, making adipose tissue an abundant and accessible source of stem cells for investigations of cell-based therapies. It is impossible to say precisely what volume of tissue will be necessary until defined treatment parameters for given clinical indications evolve; however, a modest tissue harvest of 100 g may yield as many as 20 million – 40 million cells.

2. Mesenchymal applications

2.1 Adipogenesis

One of the most intuitive uses of ASCs is for the replacement of adipose tissue itself. Large soft tissue defects are a common problem following trauma, burns and oncological resections, such as mastectomy. In addition, thousands of patients annually seek cosmetic treatments to smooth wrinkles, fill in cheeks and otherwise augment natural subcutaneous adipose compartments. Numerous strategies for autologous fat transplantation have been tried, but nearly all are characterised by extensive resorption and loss of volume [44-46]. Mature adipose is a fragile, lipid-laden tissue particularly susceptible to ischaemia [44]. This may explain the characteristic resorption seen in all but the smallest diffusible fat transfers. For restoration of larger defects, vascularised surgical flaps or artificial implants are the best options at present.

In order to develop more physiological alternatives for soft tissue reconstruction, several laboratories have investigated the possibility of creating tissue-engineered cell-seeded scaffolds for the generation of *de novo* adipose tissue. Numerous studies have reported the *in vitro* differentiation of ASCs along adipogenic lineages, including the accumulation of intracellular lipid droplets, and the expression of characteristic proteins and enzymes [3,9-15]. Initial successes have been described using these cells to seed artificial scaffolds subsequently implanted subcutaneously in mice and rats [47-49]. In each trial, cell-seeded grafts showed significant neovascularisation of the implant, as well as penetration of the preadipocytes or ASCs into the scaffolding, and their differentiation into mature lipid-laden adipocytes. Mature adipocytes, however, only penetrated 0.35, 1.80 and 2.5 mm into the implants, respectively [47-49].

The influence of variables such as porosity [48,50-52], biomaterial composition [48,51] and seeding technique [51] have been examined for their effects on adipogenic differentiation and tissue formation. Significant differences in efficacy based on these factors suggest that optimisation of the constructs may further improve adipogenesis. New trials combining slow basic fibroblast growth factor (bFGF)-releasing gelatin microspheres, collagen scaffolds and preadipocytes have shown significant improvements in the total volume of *de novo* adipose tissue [53]; however, at a size of 5×5×3 mm, this technique requires further enhancement in order to allow for clinically significant tissue regeneration. Arguably the most unexpected results came in the form of *de novo* adipogenesis found following subcutaneous injection of bFGF-enriched Matrigel™ in mice [54]. In this study, enriched Matrigel with and without preadipocytes was injected into mice. Following bFGF-induced neovascularisation, endogenous fibroblastic cells invaded acellular Matrigel and proceeded to differentiate into mature adipocytes. *De novo* fat pads created from cell-seeded and acellular Matrigel closely resembled each other and remained stable for at least 10 weeks. It is unclear what role prior cell seeding versus endogenous cell recruitment may play in attempts to increase the volume of *de novo* adipose tissue that can be generated; however, appropriate environmental signals appear to be key.

The long-term maintenance of engineered grafts may prove to suffer from similar difficulties as seen clinically with free fat transfers. In an extension of his initial study [49], Patrick *et al.* looked at preadipocyte-seeded polylactic co-glycolic acid (PLGA) scaffolds implanted subcutaneously in rats. As with previous studies, he found mature adipocytes and neovascularisation within his implants. Measured over time, however, adipose tissue formation peaked at 2 months, with complete resorption by 5 months [55]. This corresponded to the degradation profile of the collagen scaffold, which may be necessary in order to maintain the structural integrity of the new tissue. Alternatively, resorption may have been a result of implanting constructs in an anatomic compartment typically devoid of adipose tissue. This method may prove to be more successful in a more physiological setting.

Using an alternative injectable strategy, Halberstadt *et al.* [56] implanted subcutaneously in sheep 2 ml of alginate-RGD hydrogel fragments coated with preadipocytes. Well-defined adipose tissue formation was demonstrated, which was not seen when acellular fragments were injected. Another novel strategy reported by Schoeller *et al.* [57] included the creation of an intramuscular capsule by prior implantation of a silicone tube. Following capsular formation and removal of the tube, preadipocytes suspended in fibrin glue were injected into the cavity. Within 4 weeks, well-organised adipose tissue, characterised by mature adipocytes with thin, fibrous septa, was found within the capsule. This fat cylinder retained both its volume and histological structure over the following year. It remains unclear whether this resilience was due to the initial nutritive support from the fibrin, prior capsule formation, or the more highly vascularised bed inherent in skeletal muscle. Developing strategies to reconstruct larger tissue defects,

however, remains a formidable challenge due to the inherent ischaemic conditions within larger transplants and the time necessary for the establishment of an extensive vascular network.

2.2 Osteogenesis

The *in vitro* osteogenic potential of ASCs has been documented in multiple studies [3,9,12,15-19]. In efforts to utilise this potential for tissue-engineered bone repairs, many laboratories have begun seeding osteogenically differentiated ASCs onto various scaffolds and biomaterials. Trials using seeded polyglycolic acid (PGA) [15] and atelocollagen [17] scaffolds demonstrated the maintenance of an osteogenic phenotype for at least 8 weeks when implanted subcutaneously. Later studies comparing the role of the specific biomaterial examined hydroxyapatite/tricalcium phosphate (HA-TCP) and Collagraft® scaffolds seeded with osteogenically differentiated cells. HA-TCP scaffolds successfully formed osteoid in 80% of samples at 6 weeks following subcutaneous implantation. By comparison, Collagraft supported differentiation in only 20% of implants [58]. Concurrent studies at an independent laboratory focused on HA-TCP constructs and ASCs, and confirm osteogenic differentiation *in vivo* [19].

In an effort to augment this potential, ASCs have been transfected with an adenoviral vector containing bone morphogenic protein (BMP)-2 cDNA [59,60]. Transduced cells showed rapid expression of an osteoblastic phenotype and matrix production without the need for culture in a standard induction media. When seeded onto collagen sponges and implanted into severe combined immunodeficient mice, transduced (but not non-transduced) ASCs produced bone visible by radiography and histology. In a follow-up femoral reconstruction model, ASC-seeded scaffolds were implanted into critical-sized femoral defects in rats. Constructs using BMP-2-impregnated scaffolding or BMP-2-expressing cells successfully healed femoral defects; however, control cells without this growth factor failed to promote healing [61].

In an alternative approach, Cowan *et al.* [62] used ASCs without prior genetic manipulation to heal critically sized calvarial defects in mice. Cells were seeded onto apatite-coated PLGA scaffolds to take advantage of the osteoinductive properties of apatite and the mechanical resilience provided by the PLGA polymer scaffold. It is unclear why non-transfected ASCs were capable of healing calvarial, but not femoral defects; however, strong paracrine signalling from the underlying dura mater and the osteoinductive properties of the apatite coating may have played a role in these divergent outcomes. Further successes have been observed in a rat cleft palate model. Osteogenically differentiated ASCs seeded onto polylactic acid scaffolds led to near-complete palatal repair at 12 weeks when implanted into the defect. In comparison, no bone formation was seen with acellular scaffolds and those seeded with undifferentiated cells [63].

The first case of autologous ASC use for osseous repair has been reported in the treatment of a calvarial defect in a 7-year-old girl [64]. Following a complex clinical course characterised by progressive bony resorption of skull fragments, a porous sheet seeded with milled bone and fresh ASCs was implanted into the defect. Despite the use of an otherwise limited amount of autologous bone, ossification visualised by computed tomography scanning was sufficient to allow for the discontinuation of the patient's protective helmet. Although the role of ASCs in relation to that of the milled bone graph is unknown, it is promising to note that a positive clinical outcome was achieved without evidence of tissue pathology. Although evidence to date suggests that ASCs may one day be useful in the treatment of difficult osseous repairs, further investigations are needed to determine their ultimate safety and efficacy in the clinic.

2.3 Chondrogenesis

Clinical cartilage repair has remained an elusive goal for some time. Autologous [65] and allogeneic [66] chondrocyte transplants have been used successfully, but are limited by donor site morbidity and the slow repairs seen, respectively, with these approaches. Recognition of the chondrogenic differentiation potential seen in many stem cells has led to the exploration of an alternative source of cells. Chondrogenic potential, described *in vitro* in ASCs, includes evidence of cell condensation into nodules, and the production of an extracellular matrix rich in proteoglycans and collagen type II [3,9,12,18,20,21]. This phenotype can be maintained *in vivo* for a minimum of 12 weeks when differentiated cells within alginate constructs were implanted subcutaneously in nude mice [20].

A direct comparison of the *in vitro* chondrogenic potential of ASCs and BM-MSCs examined several criteria [67]. By histological and morphological measures, as well as gene expression under monolayer culture, no differences between the two cell lines were found. In order to enhance differentiation, cells were subsequently studied in high-cell density three-dimensional fibrin spheroids. Under these conditions, BM-MSCs, but not ASCs, advanced their molecular phenotype to one comparable to that seen in mature cartilage tissue. The discrepancies observed under high-density culture remain unexplained; however, further *in vivo* experiments using these ASC spheroids were successful at generating cartilage-like tissue [60]. Induced spheroids were implanted between two muscle bellies in immunodeficient mice. At 6 weeks, the implants were harvested and found to have produced a cartilage-like tissue consisting of cells within lacunae surrounded by a gel-like extracellular matrix in the absence of any fibrous network. This histology, as well as the expression of collagen type II, aggrecan and glycosaminoglycans, is consistent with a hyaline-like cartilage phenotype.

Although this study demonstrates an *in vivo* potential for differentiation intermuscularly, no physiological models of cartilage repair have yet been tested. Cartilage, particulary articular, primarily serves a structural and mechanical function in the body. To this end, one laboratory is investigating what

effects scaffold material [65,68], oxygen tension [65] and media composition [65] have on the biomechanical properties of ASC-seeded constructs. Clear differences are seen depending on the combination of factors used; however, nothing yet approaches the mechanical properties of mature cartilage. Complete differentiation and mature tissue formation may be dependent on *in vivo* stimuli that remain difficult to simulate in culture. When ASC/fibrin spheroids were studied *in vitro* and *in vivo*, the more mature hyalar cartilage-like phenotype was described only after implantation [60]. Thus far, results suggest that future *in vivo* models may demonstrate a potential for ASCs to enhance the healing of debilitating osteochondral diseases. Repairs with the resilience necessary for weight-bearing joints, however, will probably be more difficult to develop.

2.4 Cardiac repair and neovascularisation

There are a multitude of cardiac drugs on the market, targeted at everything from arrhythmias and vessel tone to heart rate and contractile force. In combination, these drugs augment the heart's ability to pump, but do little to repair damaged tissue. Congestive heart failure, a common clinical condition that results from acute ischaemic events and diffuse progressive weakness, is essentially a failure of the myocardium. Existing pharmaceutical treatments can temporarily compensate for this effect, but necessarily drive the heart harder and, in the process, further weaken the muscle.

Cell-based cardiomyoplasty has emerged with the hope of providing a true repair of the damaged tissue. Recent reviews have focused on the use of BM-MSCs [69,70]. Since the first studies in 1999, BM-MSCs have demonstrated their ability to home to and engraft in injured myocardium, express cardiac markers and assume a morphology consistent with cardiac differentiation. In addition, transplanted cells have been incorporated into developing vasculature, possibly enhancing the revascularisation of compromised tissue. Treated animals demonstrate improved cardiac function based on numerous clinical measures. Furthermore, studies successfully utilising allogeneic and xenogeneic transplants indicate a possibility for development of 'off-the-shelf' treatments using prescreened cells [69,70].

Several recent reports suggest that ASCs may emerge as an equally promising source for cardiac therapeutics. *In vitro* differentiation, including morphological changes, spontaneously beating foci and the expression of cardiac-specific markers, has been demonstrated by multiple laboratories [23-26]. Early *in vivo* work with a murine model of myocardial cryoinjury demonstrated positive engraftment of ASCs at the site of injury. Donor-derived cells costained for troponin I, myosin heavy chain and Nkx2.5 in the absence of MyoD or CD45, a pattern consistent with cardiomyocytic differentiation [71].

Two functional studies using *in vivo* infarction models were recently presented at the 2005 meeting of the International Fat Applied Technology Society (IFATS). The first, a 60-min left coronary artery occlusion model, found improvements in cardiac function at 12 weeks in treated rats. Analysis showed improved ejection fraction and contractility, and a reduction in left ventricular remodelling and compensatory hypertrophy [72]. The second model consisted of a 30-min left anterior descending artery occlusion followed by direct myocardial injection of cells. They found a significant improvement of cardiac function, as measured by ejection fraction, cardiac output and stroke volume, at 1 month. At 2 months this effect had diminished in animals treated with cultured cells; however, those treated with fresh cells demonstrated significant improvements at this time point [73].

The therapeutic potential of ASCs may be further enhanced through their effects on neovascularisation. ASCs in culture are known to secrete proangiogenic factors, including vascular endothelial growth factor (VEGF), hepatocyte growth factor and transforming growth factor-β [74]. Under hypoxic conditions, they have shown enhanced secretion of VEGF and of an unidentified antiapoptotic factor [74]. *In vitro*, endothelial differentiation of ASCs has likewise been described in Matrigel cultures. In addition to the characteristic tube-like structures, differentiated ASCs also expressed endothelial markers, such as CD31, Flk-1$^+$ and von Willebrand factor [27,75]. *In vivo* results from a hind limb ischaemia model demonstrated increased capillary density and perfusion in limbs treated with ASCs, further supporting the cells' angiogenic potential [74,76]. Based on immunohistochemistry, transplanted cells incorporate into vessel walls and directly contribute to the structure of the new vasculature [76]. Recent histological and immunostaining results presented at the IFATS meeting in 2005 suggest that ASCs may assume a pericyte-like position within the vessel wall and express cell surface markers characteristic of pericytes [77,78]. Although the exact mechanisms of the proangiogenic response of ASCs are unclear, their ability to secrete cytokines, as well as their direct incorporation into new vasculature, could potentially prove therapeutic for the salvage and/or repair of ischaemic tissues.

More research is needed to understand the mechanisms by which ASCs impart their cardioprotective or cardioregenerative effect on injured tissue. Little is known about their capacity to engraft fully within native tissues or to effect sustained myocardial repair. The optimisation of timing, method of delivery and preparation of cells are yet to be clarified; however, if work with BM-MSCs sets any precedent, this will continue to be an interesting and promising avenue of research.

2.5 Myogenesis

Cell-based therapies for muscular disease evolved out of interest to restore dystropin levels in patients with Duchenne muscular dystrophy (DMD). Initial efforts using myoblast transfer demonstrated short-term benefits, but were ultimately limited by poor cell survival, immune rejection and poor migration of transplanted cells. A subpopulation of myogenic cells with greater survival and proliferative rates in DMD models has since been identified as muscle-derived

stem cells (MDSCs). This discovery has led to new investigations not only in skeletal muscle disease, but also in osteogenic and haematopoietic applications [79-81].

MDSCs are extremely rare, and efforts to find alternative sources of cells have begun. Recent work into the regenerative potential of BM-MSCs has been promising. Cells injected into cardiotoxin-damaged muscle were shown to engraft and incorporate into regenerating myofibres. Additional cells assumed a satellite cell-like distribution and were subsequently recruited when the muscles were reinjured. Further trials in a transgenic murine model lacking dytropin found the successful incorporation of injected MSCs into myofibres and the expression of human dystropin for at least 2 weeks post-transplant [82]. These results are suggestive of the capacity of MSCs to repair damage following acute injury as well as in a degenerative model. Emerging results with ASCs point to the possibility of a similar therapeutic potential.

ASC myogenic differentiation has been induced *in vitro*, as demonstrated by the expression of characteristic markers and the formation of multinucleated myotubules [3,9,22]. In the first *in vivo* report, F Bacou [83] injected ASCs into the anterior tibialis muscle of rabbits following cardiotoxin-induced injury. In concordance with prior work using satellite cells [84], treated muscles were found to be heavier, have an increased fibre area cross-section and exert greater maximal force. Although these results were statistically significant, it remains to be seen whether they will result in clinically noticeable improvements. In this acute model, the maximal force exerted only increased from 15.8 to 17.0 N between treated and untreated muscles. Degenerative diseases, on the other hand, are characterised by slow but progressive accumulation of damage. Cell-based therapies that may replenish the exhausted supply of satellite cells [82] may be particularly suited to prevent this decline. Significant work is needed to establish the methods necessary to treat progressive diseases; however, the demonstration that ASCs have myogenic potential both *in vitro* and *in vivo* is encouraging.

2.6 Hepatogenesis

For patients with hepatic failure, unlike other forms of organ failure, there are no dialysis or bypass machines to take over. In both acute and chronic failure, the only option is a liver transplant. Cell-based therapies are being investigated to fill this void. An extensive investigation into the differentiation of BM-MSCs has demonstrated strong evidence of hepatic differentiation, including *in vitro* production of albumin, glycogen storage, urea secretion, low-density lipoprotein (LDL) uptake and phenobarbital-inducible cytochrome P450 activity [85]. Further studies examining the fate of human BM-MSCs injected intrahepatically into rats have demonstrated positive differentiation, including α-fetoprotein and albumin expression by both immunostaining and reverse transcription polymerase chain reaction. Chromosome analysis by fluorescence *in situ* hybridisation of > 1400 cell nuclei found no evidence for cell fusion between murine and human cells, suggesting true hepatic differentiation of the transplanted cells [86].

Early evidence with ASCs shows promise that adipose tissue may be a similarly suitable source of stem cells for such applications. It is known that when injected intravenously, many ASCs will engraft in the liver. Partial hepatectomy increases that percentage [87]; however, it is unclear if this is a result of stem cell homing or simply increased perfusion to a damaged organ. More suggestive of their potential, however, is the recent *in vitro* differentiation of ASCs, including the characteristic production of albumin and urea, LDL uptake and morphological changes consistent with mature hepatocytes [28]. A subsequent *in vivo* model tracked human ASCs injected into mice 2 days following exposure to a known hepatotoxin. Donor cells engrafted in the liver assumed a hepatocyte-like morphology and began expressing human albumin [28]. Although these data suggest that transplanted cells may differentiate *in vivo* into mature hepatocytes, they did not address the rate of recovery of liver function. It remains to be seen whether this engraftment and differentiation potential will translate to enhanced tissue repair and recovery of organ function.

3. Non-mesenchymal applications

3.1 Neurogenesis

The first reports of ASCs as multipotent stem cells focused on the differentiation of cells towards mesodermal lineages [3,40]. Several laboratories, however, have found evidence for ectodermal differentiation of ASCs towards neuronal lineages, suggesting a range of plasticity much broader than originally thought. Initial reports of neuronal induction identified markers of early neural precursors and morphological changes consistent with a neural phenotype [9,29]. Further trials have successfully induced expression of an even broader array of mature neuronal and glial markers [30-33]. Controversy, however, exists as to whether these morphological and cytological data indicate true neural differentiation or are a result of cytoplasmic retraction and antigen concentration [88]. Additional evidence, including the acquired susceptibility to NMDA-induced excitotoxicity [33] and the expression of a delayed-rectifier type K^+ current [29], may be indicative of a more functional neuronal phenotype; however, no one has yet been able to demonstrate neuronal depolarisation or synaptic functioning in differentiated cells cultured *in vivo*.

The first *in vivo* test for the therapeutic potential of ASCs looked at their effects when injected intraventricularly in rats following middle cerebral artery occlusion. ASCs survived globally, with increased engraftment at the site of injury compared with controls. Immunochemical staining further demonstrated expression of the neural lineage markers microtubule-associated protein-2 and glial fibrillary acidic protein in some engrafted cells. Behavioural tests of the motor and sensory systems showed clear improvements in those treated with ASCs after infarct [31]. Investigation of ASCs in

the setting of traumatic brain injury is also underway. At the 2005 meeting of the American College of Surgeons, Okonkwo *et al.* presented a promising pilot study involving the injection of human ASCs subcortically in a rat model of percussive brain injury [89]. Investigations are underway to confirm the fate of these cells after injection, and their influence on the cognitive defects seen using this model (D Okonkwo, pers. commun.).

It is uncertain whether transplanted cells contribute to the direct replacement of lost neurons or provide a supportive role for existing *in situ* stem cells and injured neurons. In a coculture model, Kang *et al.* studied the interactions between neural stem cells (NSCs) and ASCs. In comparison to laminin-coated dishes, ASC feeder layers showed an ability to support the differentiation and survival of NSCs over 14 days in culture. Direct cell–cell contact was implicated in this effect, as demonstrated by reduced efficacy seen with conditioned media and transwell membranes [90]. *In vivo*, functional improvements seen after injection of stem cells may be a result of this apparent supportive ability. Engraftment studies have demonstrated that ASCs cross the blood–brain barrier [87], but it is unclear what their role is once they arrive. Despite this incomplete understanding, clear precedence exists for functional improvements in ischaemic [91] and traumatic [92] brain injury, as well as spinal cord injury [93], with the use of BM-MSCs. It is not unreasonable to believe that ASCs will be found to demonstrate similar results.

3.2 Emerging applications

In addition to the established mesenchymal and neural fields, a few recent studies have suggested emerging applications that are yet to be further investigated. One such study used a coculture model of keratinocytes and ASCs. In isolated culture, keratinocytes rapidly undergo apoptosis; however, when cultured in direct contact with ASCs, they survive and develop epidermal ridge-like structures [94]. This potential ability to support epidermal regeneration may be useful to enhance re-epithelialisation and wound closure in chronic and/or surgical wounds. Many more studies will be necessary to confirm this preliminary result. Evidence of pancreatic potential was published for the first time in 2006 [95]. In this report, ASCs cultured under defined conditions demonstrated an upregulation of the pancreatic developmental transcription factors Isl-1, Ipf-1 and Ngn3. Quantitative assays further showed the induction of increasing levels of the pancreatic hormones insulin, glucagon and somatostatin over 3 days. These findings remain to be corroborated; however, they may suggest a promising future for ASCs in the treatment of diabetes.

Another laboratory is investigating the potential of ASCs to be employed in bioartificial renal assist devices [34]. In an early success, they have demonstrated a potential for epithelial differentiation characterised by the increased expression of cytokeratin-18 and decreased expression of vimentin. Although still early, this research may be a step towards the induction of a kidney epithelial phenotype similar to that seen in the proximal and distal tubules. Based on successes treating acute renal failure with BM-MSCs [96], these results may not be that surprising.

In a fourth avenue of research, the haematopoietic potential of fresh ASCs was examined [35]. The stromal vascular fraction of adipose tissue was characterised by flow cytometry and plated in methylcellulose culture. A population of haematopoietic progenitor cells was identified based on the coexpression of CD34 and CD45, as well as an ability of some cells to give rise to haematopoietic colonies in culture. Further *in vivo* studies examined the potential of fresh ASCs and bone marrow cells to reconstitute the marrow of lethally irradiated mice. Although bone marrow transplant elicited a more rapid recovery, fresh ASCs achieved the same survival rate. It was suggested that ASCs acted primarily through support of the rebound of endogenous haematopoiesis rather than direct differentiation into bone marrow elements; however, a small percentage of the reconstituted marrow was of graft origin. It is possible that these cells are a result of haematopoietic contamination of fresh isolates; however, if so, it is a consistent and reliable source of contamination that may be clinically useful. Direct differentiation of ASCs towards haematopoietic lineages may be possible, but their ability to support haematopoietic recovery by other means may be equally or more valuable. Additional work discussed below suggests that their ability to improve engraftment and suppress rejection may be an avenue more readily utilised to enhance haematopoietic recovery following traditional bone marrow transplants.

3.3 Immune modulation

Another emerging application for ASCs may relate to their immunomodulatory effects when transplanted into an allogeneic host. The immunobiology of BM-MSCs has been extensively characterised *in vitro* and *in vivo*. A recent review of these findings describes the background science and ongoing trials using MSCs [97]. Differentiated and undifferentiated MSCs in culture do not express major histocompatibility complex (MHC) class II antigens or costimulatory molecules, and are known to suppress T cell proliferation. Surface expression of MHC class II antigens can be induced; however, the cells still fail to elicit a T cell response. Tests using a costimulatory antibody were equally non-reactive. The exact mechanism of their immunomodulatory effect has yet to be elucidated; however, based both on their non-reactivity and their suppression of an externally induced immune response, several clinical trials have already begun. The combined experience using MSC/HSC cotransplant includes 93 patients across eight diseases and has been associated with numerous positive outcomes without the development of adverse events. Based on these early results, MSCs may prove to be most applicable for the prophylaxis and treatment of severe graft-versus-host disease (GVHD), and for the enhancement of haematopoietic engraftment in bone marrow transplants [97].

It is unclear how predictive prior work with MSCs will be in terms of the immunomodulatory potential of ASCs; however, several reports suggest a number of similarities. As with MSCs, ASCs do not express HLA-DR type II proteins [40,43]. They have also been found to suppress mixed lymphocyte reactions and inhibit T cell proliferation induced by a third cell type and by mitogenic factors [98,99]. Traditional wisdom for the use of human cells in animals has supported the use of immunocompromised animals to reduce the potential for rejection; however, that assumption has been called into question by these studies. The most recent work on ASCs found that cells lost their immunogenicity by the second passage in culture, and that cultured cells were specifically immunosuppressive even when present only at very low percentages [99]. In a trial using albino rats injected intraventricularly, ASCs failed to induce glial scarring or any other inflammatory response based on histology [31]. This may be due in part to the semiprivileged immune compartment enjoyed by neural tissue or the attenuated immune response of albino rats; however, similar precedents have been seen with other human cells [100-103]. It remains to be seen if ASCs prove equally non-reactive outside the neural setting.

Positive effects on engraftment and haematopoietic recovery have also been demonstrated with the cotransplant of ASCs. Sublethally irradiated mice were transplanted with a combination of peripheral blood stem cells and either ASCs or BM-MSCs. In this study, ASCs promoted engraftment and haematopoietic recovery at a rate comparable to MSCs [104]. Further successes have been published in regards to the treatment of advanced Crohn's disease. Following an initial success using ASCs to treat a recurrent rectovaginal fistula [105], a five-patient Phase I clinical trial was designed to evaluate the safety and feasibility of autologous ASCs for the treatment of Crohn's fistulae [106]. Patients who had failed medical therapy and at least two prior surgical attempts at closure were treated using ASCs through direct injection and the use of a fibrin glue cell suspension. Of eight fistulae treated, 75% demonstrated complete healing within 8 weeks. The biological mechanisms of these successes are unknown; however, Crohn's disease is a chronic inflammatory condition. The apparent therapeutic effect of ASCs within this clinical setting, as well as the absence of adverse outcomes, is promising indeed.

Although more research is necessary to determine the full extent of these immunomodulatory effects, the apparent ability of ASCs to escape immune recognition themselves and to suppress the immune response directed towards additional cell types may prove useful in multiple chronic inflammatory diseases, as well as for the attenuation of acute rejection and GVHD. Allogeneic therapies may also warrant further study, but significant *in vivo* work will need to be done in regards to the immune profile of ASCs following *in situ* differentiation and the possibility of chronic rejection.

3.4 Gene therapy

In contrast to many of the direct tissue repair applications discussed thus far, ASCs are also being investigated for their use in targeted gene delivery. These cells have been stably transduced using adeno-, retro-onco- and lentiviral vectors, of which lentiviral vectors have demonstrated the highest efficiency [107]. Using this vector, investigators were able to document stable gene expression over long-term passage and following terminal differentiation of the cells. These characteristics are vital if ASCs are to be used for permanent gene expression in a mature tissue. *In vivo*, gene therapy has already been tested on a limited basis. BMP-expressing ASCs enhanced osteogenic repair in critically sized femoral defects in rats, whereas unmanipulated cells failed to do so [61]. In a second study using a murine stroke model, brain-derived neurotrophic factor-expressing ASCs improved behavioural outcomes beyond the level seen with ASCs alone [31]. Large-scale investigations of ASCs as gene delivery vehicles have yet to be tested; however, to date, results are encouraging.

4. Conclusion

ASCs provide an abundant and readily accessible source of multipotent stem cells. Mounting evidence continues to grow supporting their application for tissue regeneration in settings as diverse as osteogenic repair and acute stroke. One common hurdle facing clinical researchers is the use of undefined, immunostimulatory and potentially infectious bovine serum for cell expansion [108-111]. The authors' laboratory has recently developed new serum-free and low human serum media formulations for the culture of ASCs in a targeted effort to address this issue [112]. Significant *in vivo* trials, however, still remain to confirm and optimise treatments for clinical use. Adult stem cells in general and ASCs in particular have been used clinically without complication; however, their safety in larger trials has not been proven. One report of ASCs expanded over 4 – 5 months in culture reported spontaneous cell transformation and a potentially oncogenic phenotype [113]. Although the extensive culture duration associated with the emergence of this phenotype is unlikely in the clinical setting, it does raise a word of caution when developing clinical trials. Regenerative applications using fresh or early passage cells will probably continue to advance and make available a broad array of autologous and, perhaps, allogeneic medical treatments.

5. Expert opinion

Multipotent stem cells have been found in a wide range of adult tissues, indicating a recurrent biological theme of resident quiescent cells that are available for the repair of inevitable tissue ageing and/or damage. When stem cells isolated from divergent tissues are compared, they routinely demonstrate more similarities than differences. In fact, differences that are detected are often mutable characteristics

that vary with environmental stimuli. Distinctions that had been drawn between differentiated and undifferentiated cells, haematopoietic and mesenchymal progenitors, and mesodermal and ectodermal lineages continue to be blurred, suggesting that a cell's present phenotype is perhaps more a product of its environment than any inherent identity.

Cell-based therapy presents the unique opportunity of using millions of self-regulating units that respond to and communicate with their environment with a dynamic sensitivity that will never be possible using pharmaceuticals. The complexity inherent in those interactions is not one that can be understood through deconstruction of its parts. Cell-based therapy is, by its nature, an emergent property stemming from the ability of a living cell to respond to the changing paracrine and autocrine signalling within its environment. As such, it seems logical that these cells and their applications will be best understood and studied as an interactive population in the setting of microenvironments and niche milieus that strive to emulate the *in vivo* condition.

Although safety is paramount, *in vivo* efficacy in the repair of ischaemic, toxic and traumatic injury is no less significant when details of the underlying science remain unclear. Ultimately, nomenclature and methods will need to be improved so as to enable precise reproducibility and predictability within and across laboratories. Once biological mechanisms are better understood and treatment parameters defined, the therapeutic potential of stem cells may be vast indeed. The 'exogenous' use of ASCs may simply saturate an area so as to allow for the repair of an injury that would otherwise exhaust the limited supply of *in situ* 'endogenous' cells. Similar results might be achieved using cells isolated from any number of tissues, but the practicality and appeal of adipose tissue – along with the expanding base of science associated with its regenerative potential – make it an ideal source of cells for an entirely new paradigm in medical therapy.

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