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Musculoskeletal tissue engineering: Adipose derived stromal cell implementation for the treatment of osteoarthritis

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Abstract

Osteoarthritis (OA) is a progressive degenerative joint disease which results in chronic degeneration of articular cartilage and sclerosis of bone. While tendons and ligaments may heal to a limited extent, articular cartilage has poor intrinsic regenerative potential, and critical-sized bone defects and pathological fractures cannot regenerate spontaneously. OA represents a significant burden of disease globally, affecting 240 million people in the world. The objective of tissue engineering is to recapitulate the natural healing cascade and developmental process by transplanting stromal and progenitor cells which can act directly or indirectly. As the ultimate goal of regenerative medicine is to avoid *in vitro* expansion of cells and its associated complications, the adipose-derived stromal cell (ASC) is an attractive progenitor cell for tissue engineering for treatment of OA. While clinical studies are still in their infancy, ASCs together with novel scaffold materials represent promising treatment options for patients suffering from OA. How ASCs exert their regenerative potential is a topic of debate, whereby it may be a result of direct differentiation of ASCs into the desired regenerating tissue, and/or through paracrine activity. With the advancement of material science, it is increasingly possible to enhance engraftment of ASCs

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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through the use of biomaterials or to direct progenitor cell fate by activating biophysical signals through designed material microstructures. There are currently over 180 completed or ongoing registered early stage clinical trials involving ASCs, with 17 completed studies reviewed herein detailing the use of ASCs in OA. In order for ASC therapy to become an “off-the-shelf” option for treating OA, several strategies are currently being explored such as ASC cryopreservation and use of allogeneic ASCs. Newer approaches, such as exosome therapy, allow for the use of acellular ASC-derived therapies and are also currently the focus of ongoing investigations.

Keywords

Adipose derived stromal cells; Adipose derived stem cells; Tissue engineering; Exosomes; Scaffolds; Osteogenesis

1. Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease which results in chronic degeneration of articular cartilage and sclerosis of bone. While tendons and ligaments may heal to a limited extent, articular cartilage has poor intrinsic regenerative potential, and critical-sized bone defects and pathological fractures cannot regenerate spontaneously. OA represents a significant burden of disease globally, affecting 240 million people in the world and accounts for 2.4% of years lived with disability worldwide, most notably among 9.6% of men and 18% of women over 60 years of age [1–3]. OA prevalence continues to increase globally as life expectancy and obesity rates rise [1]. OA can manifest as a spectrum of debilitation. While mild OA causes intermittent pain with minimal effect on daily living, severe cases are characterized by progressive irreversible structural deficits with progressive loss of function and an inability to work, often associated with psychological sequelae as well as increased mortality [1–4].

Tissue engineering (TE) can be defined as the implementation of a combination of cells, engineering materials, and biochemical factors to improve or divert biological processes [5]. A shared TE objective is to recapitulate the natural healing cascade and developmental process by transplanting stromal and progenitor cells or by endogenous manipulation of resident cells to augment their native regenerative potential [6]. Thus, by engineering and delivering tissues and/or cells with regenerative capacity, TE offers the potential to treat MSK disease, such as OA.

One must consider many issues in the regeneration of functional tissue in TE. A readily abundant source of cells, harvested with minimal donor site morbidity, and with the potential to express the phenotype of the desired tissue is required in addition to a biocompatible matrix to deliver and/or support cells in tissue regeneration [5]. In comparison to differentiated cells, the application of stem and progenitor cells is favorable because of their increased proliferation capacity, successful culture with a large number of passages, broader differentiation potential, and ability to promote vascularization [7]. In regenerative medicine, as we endeavor to harness the body’s own cells for treatment, innovation continues to advance stem cell-based therapies. Stem cells have been identified in many adult tissues [8–17], which contribute to both maintenance and regeneration. Postnatal (or

"adult") stem cells display tissue-specific differentiation patterns, proliferate in response to specific physiological cues, and are necessary for growth, homeostasis, and tissue regeneration [18].

The number and type of stem and stromal cells being investigated in human clinical trials for the treatment of OA continues to increase. Many approaches focus on mesenchymal stromal cells derived from bone marrow (BM-MSCs) and those derived from adipose tissue, known as adipose derived stromal cells (ASCs). As an overarching goal of regenerative medicine is to forgo *in vitro* expansion of cells and its associated complications, ASCs are an ideal cellular tissue engineering building block. Each mL of bone marrow contains 6,000 to 60,000 BM-MSCs, while over 200,000 ASCs can be isolated from 1 g of adipose tissue. Thus, when considering sources of progenitor/stromal cells, ASCs are more abundant and widely available source in comparison to BM-MSCs [5,19,20]. In this review, we will focus on the implementation of ASCs in MSK TE for the treatment of OA both in pre-clinical and clinical studies and the advantages and disadvantages of such treatment modalities, prior to describing future strategies (Fig. 1).

1.1. Adipose tissue and characterization of ASCs

The main function of adipose tissue is to store free fatty acids in the form of triglycerides that are then released during starvation or physical activity [21]. Adipose tissue is the largest endocrine tissue of humans, regulating the body's metabolism and immune system. There are two widely accepted sub-types of adipose tissue, white and brown adipose tissue. These differ considerably in function, metabolic activity, morphology, and distribution – white adipose tissue is the predominant fat in adults, whereas brown adipose tissue is the main adipose tissue of fetuses and newborns but declines with increasing age. In addition, lesser known discrete tissue-associated adipose depots have been described in the dermis, bone marrow, and mammary glands [22]. White adipose tissue is involved in lipid storage and is active in immune-endocrine responses, while brown adipose tissue is a key regulator of thermogenesis, whereby heat is generated by uncoupling of mitochondrial respiration as a result of sympathetic nervous system innervation.

White adipose tissue is composed of adipocytes, together with a mixture of endothelial, stromal, and immune cells and is where ASCs may be found. Zuk and colleagues first characterized ASCs and described their harvest from human subcutaneous adipose tissue in 2002 [10]. Since their characterization, these cells have been studied extensively by tissue engineers [10]. ASCs are described as a mesenchymal stromal cell (MSC) population that are harvested from the adipose tissue stromal vascular fraction (SVF) and share many regenerative properties as other MSCs. The International Society for Cellular Therapy reports that the term "ASC" only applies to the subset of progenitor cells that meet the following three minimal criteria: (i) plastic adherence, (ii) expression of $CD34^+/CD45^-/CD31^-/CD13^+/CD73^+/CD90^+$, and (iii) trilineage differentiation potential, as stated in a conjoined effort with the International Federation for Adipose Therapeutics (IFATS) in 2013 [23]. Contrastingly, the SVF is heterogenous containing fibroblasts, vascular smooth muscle cells, endothelial cells, lymphocytes, macrophages, and ASCs. ASCs, due to their capacity

to differentiate into multiple cell lineages, are attractive building blocks to repair, maintain, or enhance various tissues.

Adipose tissue represents a viable alternative donor site to bone marrow due to its abundance, and reduced donor site morbidity. In addition, cells harvested from adipose tissue are exempt from ethical concerns pertaining to other stem cell sources, such as embryonic stem cells [24]. ASCs are harvested through liposuction and processed with washing, digestion, and centrifugation [24]. In the United States, over 250,000 liposuction procedures are performed annually, with a mean of 3 L of lipoaspirate discarded after each procedure [25]. Theoretically, by processing 3 L of discarded lipoaspirate, one could collect up to 6 billion ASCs from a single patient after a single passage [26].

1.2. Harvest of adipose tissue

Given the great interest in the regenerative capacity of ASCs, several studies have tried to discern the best method for fat harvest to ensure optimal preservation of these cells. In addition to traditionally used suction-assisted liposuction (SAL), more complex methods of liposuction have been developed to prioritize surgical removal of adipose tissue, mostly by increasing the ease and speed of liposuction, while optimizing patient results. These methods have *not* been developed to maximize cellular preservation for use in regenerative medicine. Ultrasound assisted liposuction (UAL), laser assisted liposuction (LAL), and mechanical assisted liposuction are examples of these new methods. Panetta et al. amongst others, reported that there was no difference in the biological properties and differentiation capacity of ASCs obtained from SAL versus UAL [27–30]. However, Chung and colleagues demonstrated that although ASCs derived from SAL and LAL successfully underwent osteogenic and adipogenic differentiation in pre-clinical studies, the cell yield, viability, proliferation, and frequency of ASCs in the stromal vascular fraction (SVF) were significantly less with LAL *in vitro* [31]. Furthermore, less efficient *in vivo* osteogenesis was also appreciated in ASCs derived from LAL relative to SAL in animal studies [31]. Thus, various liposuction methods can be used to harvest ASCs, however SAL and UAL yield higher quality ASCs than LAL for use in tissue engineering.

1.3. Isolation of ASCs

The protocol described by Zuk and colleagues continues to be the most widely used method of ASC isolation [10]. Here, lipoaspirate samples are extensively washed in equal volumes of phosphate-buffered saline and then digested at 37° Celsius for 30 min with 0.075% collagenase for tissue dissociation. When enzymatically digested, lipoaspirate yields a heterogenous mixture of endothelial cells, monocytes, lymphocytes, myeloid cells, pericytes, pre-adipocytes, smooth muscle cells, and mesenchymal cells [32]. SVF culture expansion then allows for selection of ASCs which are plastic adherent [32] (Fig. 2).

Enzymatic digestion-based methods are mainly applicable in experimental purposes and are less favorable in clinical practice. The use of proteolytic enzymes (e.g. trypsin-EDTA solution, dispase, or collagenase) may reduce cell viability and alter surface antigen expression [33]. Digestion with animal-derived enzymes, such as collagenase, while efficient, is considered more than “minimally manipulated” by the US Food and Drug

Administration as it can alter cellular characteristics [34]. Furthermore, preparation of cells using xenogeneic components may result in immune reactions when the resultant cells are used clinically [35–39]. Many groups are now turning to non-animal derived, manufactured enzymes to abate the risks of these xenogeneic components. However, manufactured enzymes are also flawed and several reports suggest that they also alter cellular phenotype [34,40], again resulting in more than minimal manipulation.

1.4. Optimizing the potential of ASC-based therapy: evidence from preclinical studies

The method by which ASCs exert their regenerative potential is a topic of ongoing debate. Several reports indicate that the improvement achieved with ASC application does not correlate directly with the levels of cellular engraftment and downstream differentiation, instead suggesting that paracrine activity could account for the beneficial effect of ASC-based therapy [41–48]. As Zuk and colleagues reported, rather than direct differentiation of ASCs into the desired regenerating tissue, it is entirely possible that the ASC “simply directs tissue formation from the sidelines” [49–51]. ASCs are known to secrete numerous factors and cytokines, including NGF, BDNF, VEGF, HGF, and multiple interleukins, thus leading to the “secretome” theory of ASCs [45,50–53]. In animal studies, ASCs have been proposed to improve tissue repair through both direct differentiation of injected cells and by the effect of their secretome (Fig. 3).

Specific subtypes of human ASCs have also been identified which demonstrate increased osteogenic potential. For example, surface expression of BMPR1b and CD90 have been shown to have an enhanced ability to form bone both *in vitro* and *in vivo*, while low expression of CD105 has been shown to promote osteogenesis in animal studies [54–56]. Given the need for isolation using fluorescence activated cell sorting, these methods require further innovation before promise can be seen in clinical studies.

ASCs have been implemented together with biomaterials to improve ASC engraftment, drive differentiation of ASCs to downstream progenitors, mimic the extracellular microenvironment, and offer mechanical support while coordinating the healing process [57]. ASCs can be incorporated into biomaterial scaffolds. Ideally, a scaffold should be biodegradable/absorbable, elicit minimal immunological and inflammatory response, and biocompatible. Gradual substitution of the scaffold by autologous tissue should also occur [47]. Two fundamental properties of a scaffold are porosity and stiffness. Stiffness provides adequate support for the engineering of bone and cartilage, whereas porosity results in vascular ingrowth. Thus, when considering scaffold design, the properties of porosity and stiffness need to be carefully considered. In addition, scaffolds can be osteo-inductive or osteo-conductive. Osteoconductive scaffolds act as inert support that guides bone extracellular matrix formation and cellular proliferation to regenerate bone. Thus, they can support ASCs in MSK TE but do not drive differentiation to specific downstream fates. Contrastingly, osteoinductive biomaterials can attract progenitor cells and drive downstream differentiation to osteoblasts, leading to *de novo* bone formation. In MSK TE, ASCs have been combined with scaffolds derived from combinations of both organic and inorganic sources such as decellularized matrices, inorganic ceramics (e.g. hydroxyapatite, glass ceramics, tricalcium phosphate) and synthetic biodegradable polymers such as polylactic

acid (PLA) and polyglycolic acid (PGA) [47,58]. Calcium phosphate cements, for example, have intrinsic osteoinductive and osteoconductive features without the addition of osteogenic factors [47,59]. Using 3-dimensional (3D) printing, various biomaterials can be built in a layer-by-layer manner to result in an extracellular matrix scaffold with the goal of promoting regeneration of both functional bone and cartilage.

As ASCs are multipotent, an important strategy that has been pursued for TE is to determine how to drive specific ASC downstream lineage differentiation [58]. One such strategy is to increase osteogenic potential by the use of biomaterials and/or matrix proteins to recreate a niche micro-environment that can enhance differentiation of specific osteogenic cellular subpopulations. Natural polymers, such as fibrin, hyaluronic acid, and collagen are widely used in MSK TE. Fibrin, a biopolymer of the monomer fibrinogen involved in cell-matrix interactions, inflammation, blood coagulation, and wound healing, is an attractive biomaterial for this purpose. Fibrin use has repeatedly been shown to promote osteogenic differentiation, while also promoting vascularization [58,60,61]. It can also be loaded with osteoinductive agents to stimulate osteogenesis, as evidenced by functional bone regeneration when implanted in rat tibial defects [60]. Hyaluronic acid-based scaffolds support cell migration and differentiation and have been shown to be effective when combined with ASCs as an intra-articular injection in the prevention of OA disease progression and promotion of cartilage regeneration in sheep [46,62].

Engler et al. demonstrated that varied material physical properties could actively dominate biological behaviors of stem cells when they reported that varied matrix elasticity could guide specific lineage differentiation of MSCs (Fig. 4) [63]. This discovery that “pre-committing” stem cells to a specific lineage via appropriate material physical conditions established a solid scientific foundation for regenerative material design and application [63,64]. Simply put, soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic [63]. Cartilage, especially articular cartilage, is constantly exposed to hydrostatic pressure. Ogawa and colleagues applied hydrostatic pressure (HP) to ASCs to mimic the native environment of chondrocytes and reported increased chondrogenic differentiation of ASCs in a three-dimensional collagen scaffolds following treatment with cyclic HP relative to control [65]. In addition, Safshekan et al. investigated the effects of both intermittent HP on chondrogenic differentiation of ASCs with or without induction medium. They respectively treated cell pellets with chemical induction or HP or combined chemical/mechanical stimuli for seven consecutive days and demonstrated that the chemical/mechanical group exhibited higher gene expression of chondrogenic mediators (e.g. *Sox9*, *Collagen* II) than the other two groups [66]. Thus, combined chemical and mechanical stimulation can optimize chondrogenic differentiation.

Osteochondral TE is challenged by the difficulty in the simultaneous regeneration of hyaline cartilage and subchondral bone. Thus, strategies combining biomaterials that can conduct/induce bone and cartilage formation have been explored. For example, scaffolds combining hyaluronic acid, chitosan, and PLGA were developed that possess two different regions – one supporting hyaline chondrogenesis and another zone with bonded BMP-2 supporting osteogenesis. These osteochondral scaffolds were then seeded with ASCs, resulting in

regeneration of rabbit osteochondral defects [67]. In addition to combining different scaffold polymers to induce cartilage and bone regeneration, strategies implementing differential concentrations of calcium have also been used for osteochondral TE. For example, Mellor and colleagues reported that human ASC seeded-stacked polylactic acid nanofibrous scaffolds containing either 0% or 20% tricalcium phosphate nanoparticles resulted in *vitro* site-specific osteogenesis (high calcium concentration) and chondrogenesis (basal calcium concentration) [68].

With the advancement of material science, it is increasingly possible that stem cell fate can be directed by activating biological and mechanical signals through specially designed material microstructures [69]. Designed biophysical signals include, but are not limited to structure micropatterning, material rigidity and elasticity, materials coated with extracellular matrix proteins, and mechanotransduction [70]. Surface micropatterning creates topography to guide cellular alignment, shape, and adhesion and can then be integrated with different matrices by 3D bioprinting, thus generating micropatterned 3D structures to guide stem cell based tissue regeneration [64]. For example, a difference in the scale of scaffold topography may improve tenogenic differentiation from ASCs for incorporation into tendon and tendon bone interfaces [64]. Zhou and colleagues demonstrated that micro-scale topography resulted in improved neo-tendon formation and stable tenogenic marker expression when compared to nanoscale topography [70]. Thus, smart scaffolds and matrices can promote downstream differentiation of ASCs for incorporation into MSK TE.

1.5. Clinical trials: ASCs in osteoarthritis

For the purpose of tissue regeneration, adipose tissue is often administered as unpurified SVF, which is obtained by enzymatic lipoaspirate digestion, centrifugation and removal of the adipocyte (floating) fraction (Fig. 2). As previously elaborated, this results in a heterogeneous cell suspension that contains ASCs together with hematopoietic cells, fibroblasts, and extracellular matrix components, the so-called SVF. The patient's SVF may be combined with other materials, such as hyaluronic acid and/or platelet-rich plasma (PRP), which have been proposed as both a source of growth factors and a physical scaffolding to support the ASCs [71–73]. The resultant mixture is then introduced into the target joints via percutaneous intra-articular injection and is reported to support cartilage regeneration both through direct differentiation into chondrocytes, and through secretory/paracrine effects to support regenerative activity of tissue-resident cells [71–73]. The use of ASCs in clinical treatment of OA is still in its infancy and in the sections below, we will highlight the history of ASC use in clinical studies of OA and discuss additional ongoing studies.

The first case series of OA using ASCs was published in 2011. Here, Pak reported MRI-evident cartilage regeneration, as well as improvement in pain and functional metrics, in two patients with knee OA who were treated via intraarticular injection of autologous ASCs resuspended in hyaluronic acid, platelet-rich plasma (PRP), and calcium chloride [74]. Since then, additional studies have further investigated applications of ASCs in OA patients and reported similar results [75,76]. Early studies focused on establishing the safety and feasibility of ASC treatment for OA. In 2014, a dose-escalation study by Jo et al. found no treatment-related adverse events even at the highest dose of ASCs (1.0×10^8 cells) and

observed regeneration of histologically “hyaline-like” joint cartilage within 6 months of treatment [77]. Similarly, in the context of knee OA, a 2016 study by Fodor and Paulseth [78] of 6 patients receiving SVF injection and a 2016 dose-escalation study by Pers et al. in 18 patients all reported no serious adverse events, with the longest study reporting patient follow-up up to 2 years [79]. Notably, in a 2020 study retrospectively examined outcomes for 34 knee OA patients treated with autologous ASCs and observed that pain scores tended to improve earlier than outcomes related to activities of daily living and sports/recreation. The authors also reported a significantly greater improvement in patients with more severe cartilage lesions, suggesting that ASC treatment may be most beneficial to patients with more severe disease [80].

There are currently over 180 completed or ongoing clinical trials of ASCs registered with the National Institutes of Health (NIH, www.clinicaltrials.gov - a database of privately and publicly funded clinical studies conducted around the world). Of the 180 clinical trials of ASCs, 45 target OA with 12 completed studies to date and 13 studies in the active enrollment phase as reported by the NIH [81]. Of the studies registered with the NIH, five are not yet recruiting and therefore do not have listed locations. Among the other studies that are recruiting, active, completed, or unknown, the most heavily represented countries are the United States (13 studies), China (8 studies), and South Korea (7 studies), France (3 studies), and Taiwan (3 studies). All other countries had less than three trials listed. The majority of studies focus on OA of the knee (n = 33), but additional studies focus on OA of the hip (n = 2), wrist (n = 2), spine (n = 1), or hand (n = 1). Some studies evaluate treatment of multiple joints: there are two studies of knee, hip, and shoulder, one study of knee, hip, ankle, shoulder, and wrist, and one study of spine, knee, and hip. Two studies do not identify a target joint for treatment. The majority of the published results from these studies detail the use of intra-articular injection in knee OA, mostly in the form of cells without the incorporation of additional biomaterials. Notably, most of these studies remain in early stages (phase I and II), and further investigation is required to fully characterize therapeutic effects, optimal treatment regimen, and potential long-term adverse sequelae of ASC treatment [81]. Early results have however been very favorable, with nearly all studies to date reporting significant clinical improvement in OA patients treated with autologous ASCs. Table 1 details the results of 17 completed studies – 12 completed studies which were identified using the NIH registry (www.clinicaltrials.gov) and 5 additional studies which were identified following a literature review of PubMed (search terms: osteoarthritis, ASC, clinical trials).

Multiple controlled studies have identified significant benefits of ASC treatment, either as a monotherapy or adjunct to other treatments/procedures, such as microfracture surgery, for OA patients [82,83]. A 2016 study by Kim et al. compared treatment with bone marrow stimulation (i.e., microfracture surgery) and ASC injection to treatment with microfracture alone in 62 patients with ankle OA [82,83]. They demonstrated that ASC treatment significantly improved outcomes, as measured by both symptom severity and arthroscopic assessment [82]. Similar results were found in a 2016 retrospective study by Kim and Koh [82], though a notable limitation is that patients in this study individually decided whether or not to receive ASC treatment. A 2019 study by Hong et al. studied 16 knee OA patients, each of whom were randomized to receive SVF injection in one knee and hyaluronic

acid control injection in the contralateral knee [84]. SVF-treated knees had significantly improved functional, visual, and radiographic scores compared to HA-treated knees at 12 months [84]. Overall, while further randomized, controlled trials are needed with larger patient cohorts and longer-term follow-up, based on both clinical examination metrics and patient-reported outcome measures, all studies to date have suggested that intra-articular injection of ASC/SVF is a promising strategy for treating OA.

1.6. Challenges to clinical implementation

While early clinical studies have shown largely favorable results for ASCs' ability to improve symptoms in patients with OA, key obstacles must be cleared prior to widespread clinical implementation. Perhaps the most significant issue is the ease of preparation of autologous ASCs. As described above, isolation of SVF typically requires enzymatic digestion of lipoaspirate with collagenase, which is considered "significant manipulation" of tissue and thus, requires FDA approval and equipment that meets rigorous FDA standards [85], which are beyond the means of many medical centers. A 2018 study by Roato et al. focused on circumventing this issue by using autologous concentrated adipose tissue, prepared by centrifuging lipoaspirate without enzymatic digestion [86]. While the authors did observe symptomatic improvement in patients treated with this "lightly manipulated" adipose tissue, they did not directly compare outcomes to patients treated with SVF isolated in the standard fashion. In addition, while no serious adverse events occurred, most patients reported limited knee mobility in the month following injection, which may be related to the large volume injected – 35 mL of processed adipose tissue – compared to the typical ~5 mL of SVF reported in other studies [86].

Emerging studies have also suggested that SVF may not be the ideal means to deliver ASCs in OA treatment. Yokota et al. retrospectively compared outcomes for patients treated with fresh SVF (prepared directly from lipoaspirate without intervening cell culture which is the means of ASC delivery typically used in clinical trials) versus cultured ASCs (ASCs selected from lipoaspirate by adherence to plastic, then expanded in cell culture - the form of ASCs used in most preclinical studies) [87]. Patients treated with cultured ASCs had accelerated resolution of symptoms and a more significant decrease in pain. Furthermore, patients treated with SVF had more frequent minor complications (including knee effusion), collectively suggesting that culture-expanded ASCs may be superior to SVF for treating OA. While this study was limited by a lack of blinding or randomization, these results are not surprising as SVF is a heterogeneous mix of cells including inflammatory cells and of which only 1–10% of cells are ASCs [88]. In light of these findings, non-enzymatic approaches have been proposed to optimize ASC isolation. Mechanical ASC isolation may be performed whereby adipose tissue is emulsified using syringes, a technique which results in a higher yield of multipotent CD34+/CD31−/CD45−/CD13+/CD73+/CD146− ASCs from lipoaspirate in comparison to collagenase digestion [89]. Another proposal relies on ASCs migrating out of the undigested solid adipose tissue in culture, thought to arise as ASCs are drawn out of the tissue to nutrient rich media [34]. This method yields ASCs with healthier morphology in comparison to collagenase treatment but cannot be used in same day harvest and administration, as it requires a minimum of 7 days *ex vivo* culture [34,90]. One such method, termed "ceiling culture", allows adipose tissue fragments to adhere to the

top inner surface of a culture flask, which is filled completely with medium allowing for culture and expansion of ASCs without tissue digestion [57]. This technique is flawed due to limited surface for attachment and migration of ASCs out of the adipose tissue. To overcome this limitation, Yang et al. implemented use of a highly hydrated fibrin matrix to securely support the adipose tissue and provide increased surface area for attachment and migration of ASCs [91].

ASC purification comes with further innate challenges. The additional processing steps required to purify ASCs increased the risk of contamination as well as substantially increasing expense and regulatory hurdles. As a result, very few medical centers have the required approved equipment to perform this cell expansion step. In comparison, SVF isolation typically takes only 60–90 min and can be performed in the operating room using automated devices, making it significantly more accessible and translatable [85]. Additionally, while ASCs are typically thought to inherently "home" to sites of tissue damage, there is some evidence that stem cells may lose this homing ability with increased passages in cell culture, potentially representing an additional limitation of extended ASC culture [92]. Furthermore, cell culture typically relies on serum additives (most commonly from xenogenic sources, e.g., fetal bovine serum) to provide a source of necessary growth factors and nutrients. The use of animal products however does lead to concerns for immunogenicity. While several alternatives (such as human PRP) are being actively explored and show promise in *vitro* studies, they remain to be validated for clinical applications [93].

In order for ASC therapy to be realized as a pre-packaged, "off the shelf" option for OA management, several strategies are currently being explored. First, the ability to cryopreserve ASCs for later use would significantly expand their potential use. While traditional cryopreservation conditions (typically, in media comprising 90% dimethyl sulfoxide and 10% fetal bovine serum) pose concerns for cell toxicity, inhibition of stem cell activity, and immunogenicity, recent studies have explored alternative methods using decreased dimethyl sulfoxide concentration and altered media composition that may improve the stemness/differentiation capacity and/or viability of ASCs following freezing and thawing [94,95].

An additional challenge is the fundamental limitation of relying on autologous ASCs. Stem cell properties are known to vary between different individuals; for instance, it has been well established that ASCs from older donors have decreased viability/proliferation and differentiation potential compared to those derived from younger individuals [96, 97] and that stem cell functions are impaired in ASCs from diabetic individuals [98,99]. Given the demographics of OA, which is most common in the elderly, it is likely that ASCs from OA patients in general may have reduced therapeutic chondrogenic regenerative potential than ASCs from healthy, younger individuals. As such, there is significant interest in developing therapies employing allogeneic ASCs such that ASCs could be derived from young, healthy donors, expanded, and then frozen, to be readily available for clinical use. There are obvious benefits to such an approach; for instance, donors could be pre-screened to optimize ASC viability, and large numbers of ASCs could be grown from a single healthy individual for use in multiple recipients. Allogeneic ASC-based therapeutics are currently in pharmaceutical production although no such products have been specifically developed

for OA [100]. However, while ASCs are classically considered minimally immunogenic and in fact may exhibit immunosuppressive/anti-inflammatory properties [101–104], clinical studies have suggested that allogeneic ASC transplantation may result in an anti-donor immune response and lead to sensitization, particularly in patients requiring multiple rounds of treatment [105].

Finally, while early clinical data from patients treated with ASCs have been promising and have shown minimal short-term adverse effects, it is critical to thoroughly establish the long-term safety and efficacy of ASCs for the treatment of OA. To date, studies have been limited to 1–2 years of follow-up. Given the potential for a chronic inflammatory response over time (particularly with degradation of any animal-derived scaffolding materials [93]), as well as inevitable concerns regarding tumor tropism and tumorigenicity [85] (though no increased risk has been found in cancer-free patients receiving ASC transplantation [106]), future studies will need to robustly demonstrate the safety profile of ASCs at later timepoints. More rigorous studies (i.e., randomized, controlled, blinded, prospective clinical trials) with larger patient cohorts are also needed to fully establish the clinical benefits and guidelines for treatment with ASCs.

1.7. Future perspectives

In addition to ASCs showing promising results in early clinical studies in tissue regeneration by direct treatment, *ex vivo* genetic modification of ASCs could further enhance their intended therapeutic effect [107]. In gene therapy, viral and non-viral vectors are modified to encode a specific protein and consequently introduced into host cells, such as ASCs. The host cells are thus triggered to produce and release a therapeutic agent at concentrations reflective of physiological function, rather than supraphysiological levels as occurs following exogenous morphogen application [107]. In MSK TE, gene therapy using vectors is mostly applied to enhance bone regeneration by triggering host cells to produce bone morphogenetic proteins [107–119] or factors stimulating angiogenesis, e.g. VEGF [108,118]. Using virus-mediated gene transfer encoding bone morphogenetic protein (BMP)-2, BMP-7, BMP-4, or a combination thereof, ASCs have been modified to increase expression of osteogenic proteins *in vitro* [108–111] and enhanced osteogenesis *in vivo* in small [109,112–114] and large [116,120] animal models. For chondrogenic differentiation, manipulation of insulin like growth factor-1, transforming growth factor-β1, and BMP-2 expression in ASCs led to increased deposition of cartilage specific matrix, enhanced expression of proteoglycans, and increased collagen type II production in calf-derived MSCs [121,122].

While viral vectors are cost-effective and efficient, safety risks such as toxicity and high immunogenicity have been observed, and thus non-viral gene therapeutics have also been explored. Non-viral gene therapy implements plasmid DNA (pDNA), a circular double-stranded DNA found in bacterial cells, such as *E. coli* [123]. pDNA-transfected cells expressing BMP-2 have led to increased ALP activity, matrix mineralization and expression of osteogenic markers *in vitro* [117–119,123,124]) and enhanced osteogenesis and increased levels of osteocalcin and collagen I *in vivo* [123,124]. In contrast to viral vectors, pDNA has no mutagenic potential, triggers less immune response and delivers the therapeutic in

a sustainable manner with no systemic side effects. pDNA approaches are limited however by poor transfection efficiency [108, 109,117–119,123–125]. Nonetheless, this form of gene therapy represents a promising approach to further enhance the physiological function of ASCs.

Finally, safety concerns regarding direct transplantation of ASCs still exist, and in order to circumvent these risks, research has focused on further exploration of paracrine signaling mechanisms of MSCs [51,117, 126]. An accumulating body of work has revealed that the positive effects of MSCs on tissue repair are not facilitated only by direct differentiation into parenchymal cells that repair and replace tissues, but rather by stimulation of recipient cells in recipient tissues via paracrine signaling [127,128]. Exosomes are biomolecular nanostructures, naturally secreted by MSCs and ASCs, and play key roles in paracrine signaling [129]. They are 40–150 nm in size and originate from multivesicular bodies. These nanostructures contain microRNA, messenger RNA, proteins, and lipids and have been demonstrated to directly modulate gene transcription and target cell signaling pathways (Fig. 5) [51,130–133].

Given the safety concerns regarding direct application of ASCs, exosomes represent a promising alternative for targeted drug delivery as they are non-teratogenic with a low immunogenic potential. The use of ASC-derived exosomes in tissue engineering is an opportunity to circumvent cell-based therapy, negating the risk of neoplastic transformation. MSC-derived exosomes have been shown to increase matrix mineralization and expression of osteogenic genes and proteins *in vitro* [133–138] and increased bone formation in animal models *in vivo* [134–140]. Increased osteogenic potential has been mostly ascribed to specific bone-related microRNA contained in exosomes, such as miR-218, miR-196a or miR-21 [141–144]. Many technical challenges still need to be overcome in the development of exosome-based therapeutics (145). The large-scale production of high quality exosomes is the most important factor in their therapeutic application [146]. Traditionally isolated using ultrafiltration, exosome isolation has proved challenging. Tangential flow filtration, rather than ultracentrifugation, has been proposed as the ideal method for industrial scale manufacturing of exosomes and has been implemented with good manufacturing practice in validated processes [145,147]. However, further investigations into standardization of exosomes and their effects are required before they can be implemented in clinical practice.

2. Conclusion

OA is a progressive degenerative joint disease which results in chronic degeneration of articular cartilage and sclerosis of bone. OA represents a significant burden of disease globally, affecting 240 million people in the world and accounts for 2.4% of years lived with disability worldwide. The high prevalence of OA has led to significant advancement and investment in the field of tissue engineering to augment healing and improve traditional surgical interventions. ASCs together with novel scaffold materials are under investigation as potential treatment options for patients suffering with OA. While progress has been made in pre-clinical and clinical studies to date, more rigorous studies are required to fully characterize the therapeutic effects, optimal treatment regimen, and potential long-term adverse effects of ASC treatment for OA. As the field develops greater understanding of

material science, we can potentially drive ASC differentiation without the use of exogenous factors, instead opting for material cues to prime cellular differentiation. In order for ASC therapy to progress toward an “off-the-shelf” option for treating OA, several strategies are currently being explored such as ASC cryopreservation and the potential use of allogeneic ASCs. Newer approaches, such as exosome therapy, allow the use of acellular ASC-derived therapies and are currently the focus of ongoing investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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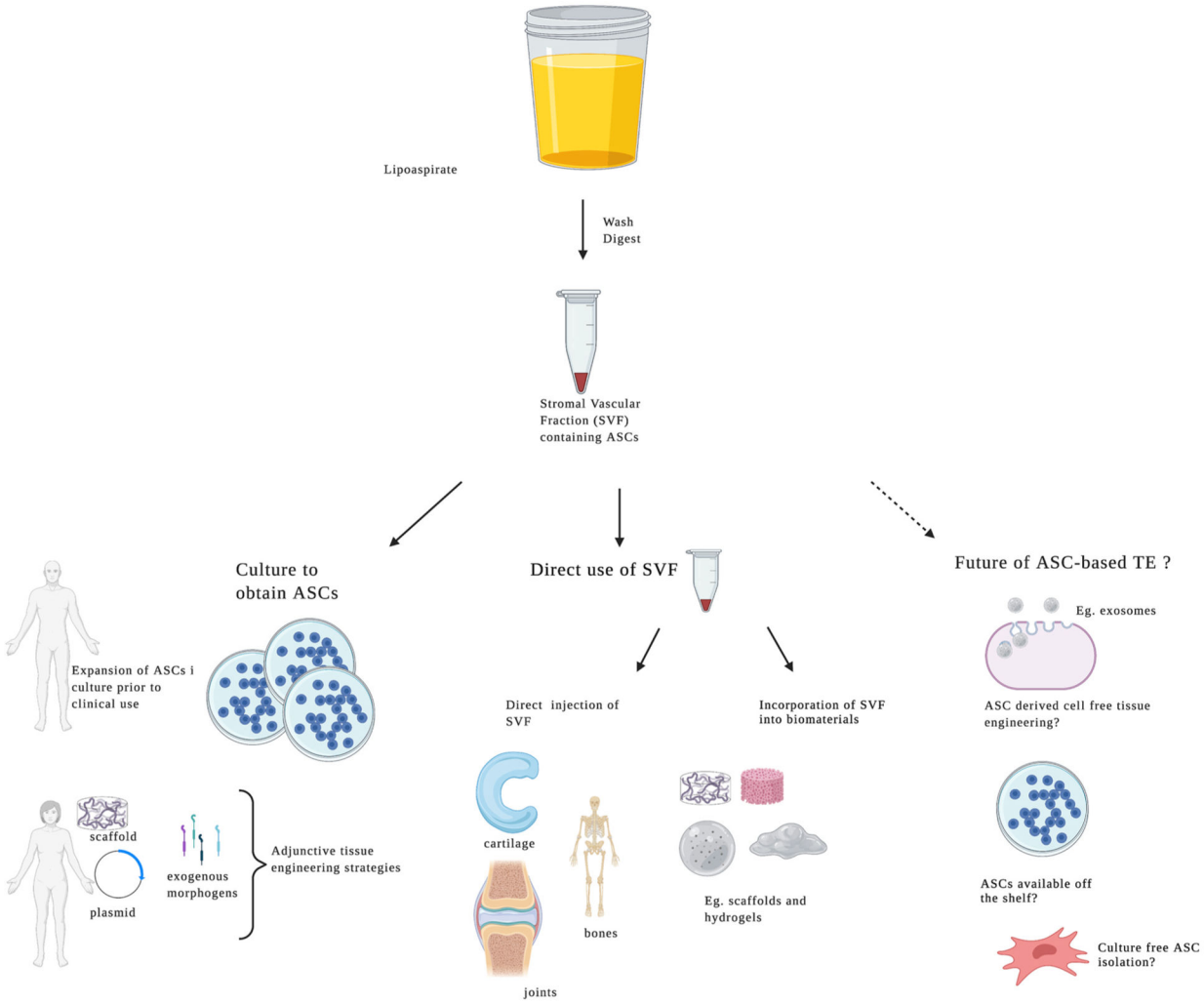

Fig. 1.

Implementation of ASCs in MSK Tissue Engineering

Lipoaspirate can be washed and digested to isolate the stromal vascular fraction (SVF), which contains ASCs. The SVF can be cultured to obtain ASCs, which can be augmented with plasmid DNA, or application of exogenous morphogens or incorporated into scaffolds to direct subsequent lineages, or it may be used directly in clinical applications to optimize regeneration of cartilage, ligaments and/or tendons, joints and bone. Figure created using BioRender.com.

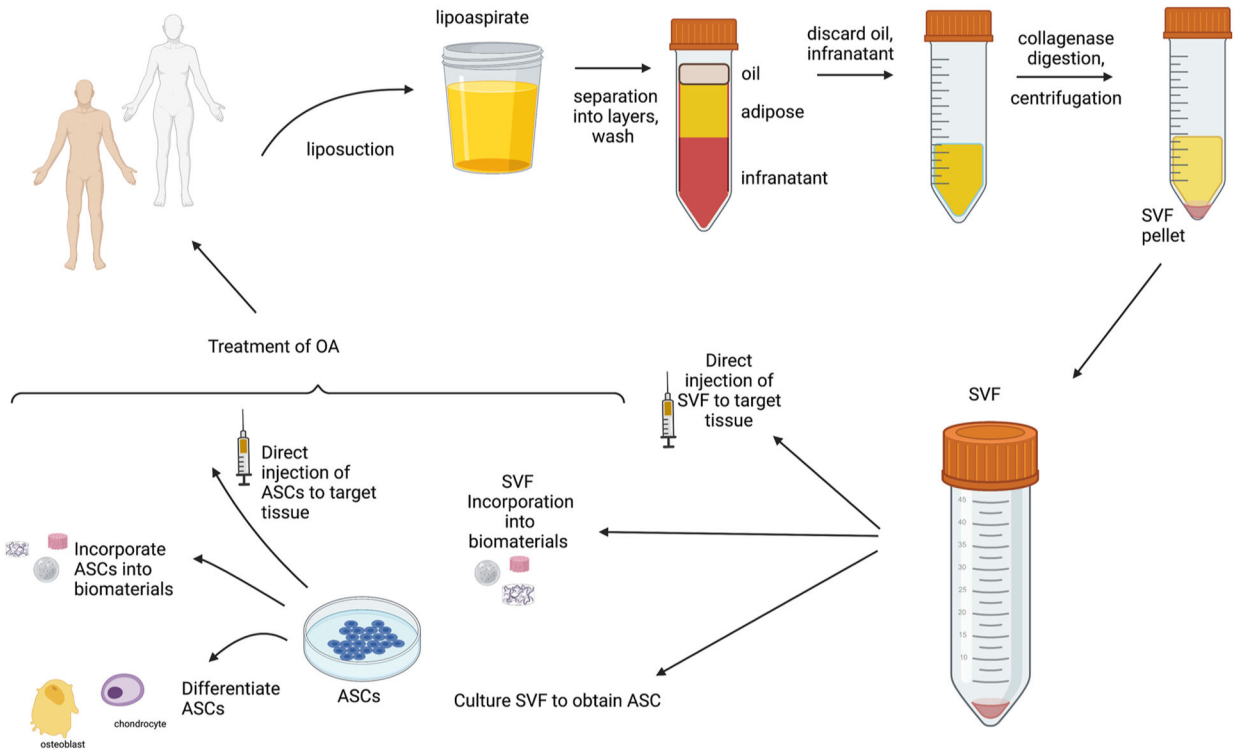

Fig. 2.

Harvest of ASCs for Tissue Engineering in OA

Adipose tissue is harvested by liposuction and the resultant lipoaspirate is washed and allowed to separate with removal of oil and infranatant layers (mixture of blood and tumescent solution). The adipose layer is then subjected to collagenase digestion and centrifugation to lead to the isolation of the SVF [148]. The SVF can be used in clinical applications in multiple methods: eg. by direct injection, incorporation into biomaterials or placed in culture for isolation of ASCs. ASCs can also be used in clinical applications in multiple methods: eg. by direct injection, incorporation into biomaterials or injection following differentiation into downstream progenitors. Figure created using BioRender.com.

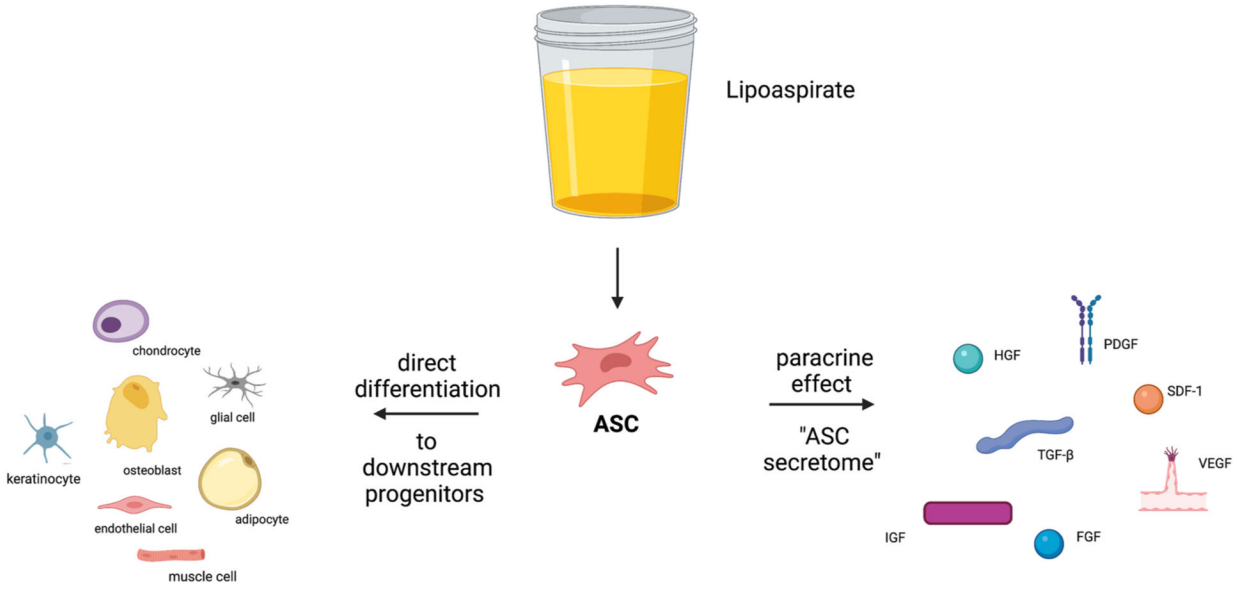

Fig. 3.

The role of ASCs in Tissue Engineering.

The method by which ASCs exert their regenerative potential is a topic of ongoing debate. Several reports indicate that the improvement achieved with ASC application does not correlate directly with the levels of cellular engraftment and downstream differentiation, instead suggesting that paracrine activity could account for the beneficial effect of ASC-based therapy [41–48]. Figure created using BioRender.com.

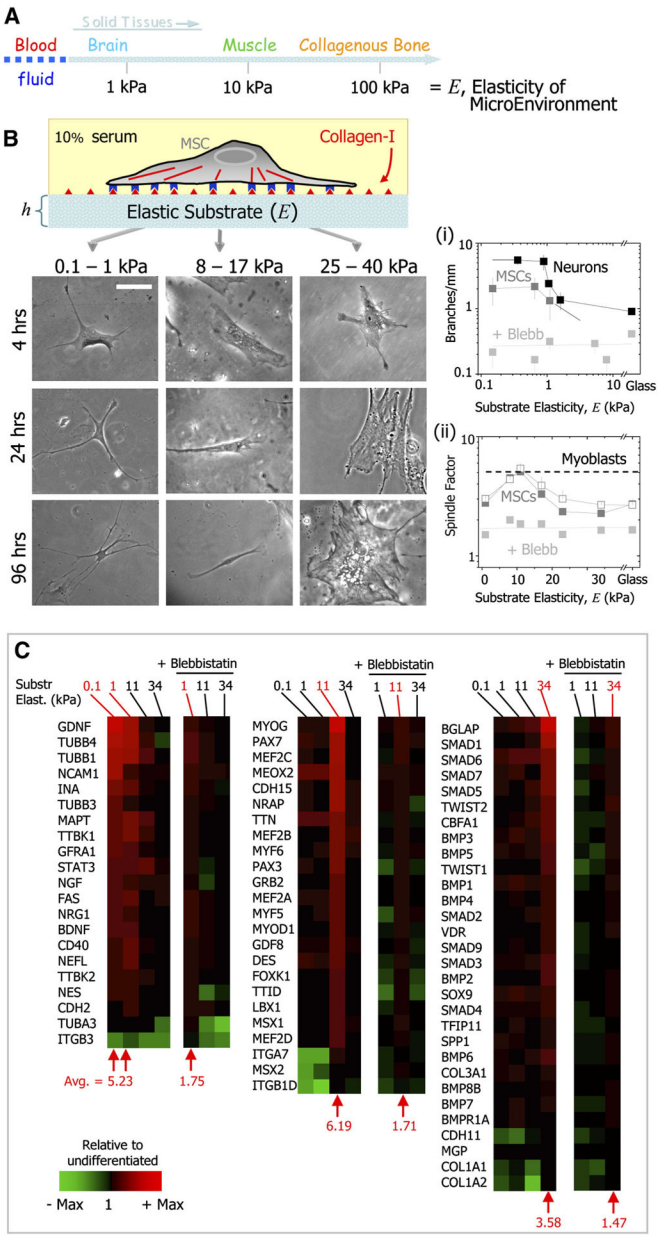

Fig. 4.

Matrix Properties Can Direct Stem Cell Differentiation.

(A) Solid tissues exhibit a range of stiffness, as measured by the elastic modulus, E.

(B) The *in vitro* gel system allows for control of E through cross-linking, control of cell adhesion by covalent attachment of collagen-I, and control of thickness, h. Naive MSCs of a standard expression phenotype are initially small and round but develop increasingly branched, spindle, or polygonal shapes when grown on matrices respectively in the range typical of E-brain (0.1–1 kPa), E-muscle (8–17 kPa), or stiff crosslinked-collagen matrices (25–40 kPa). Scale bar is 20 mm. Figure from *Engler* et al. reproduced with permission [63].

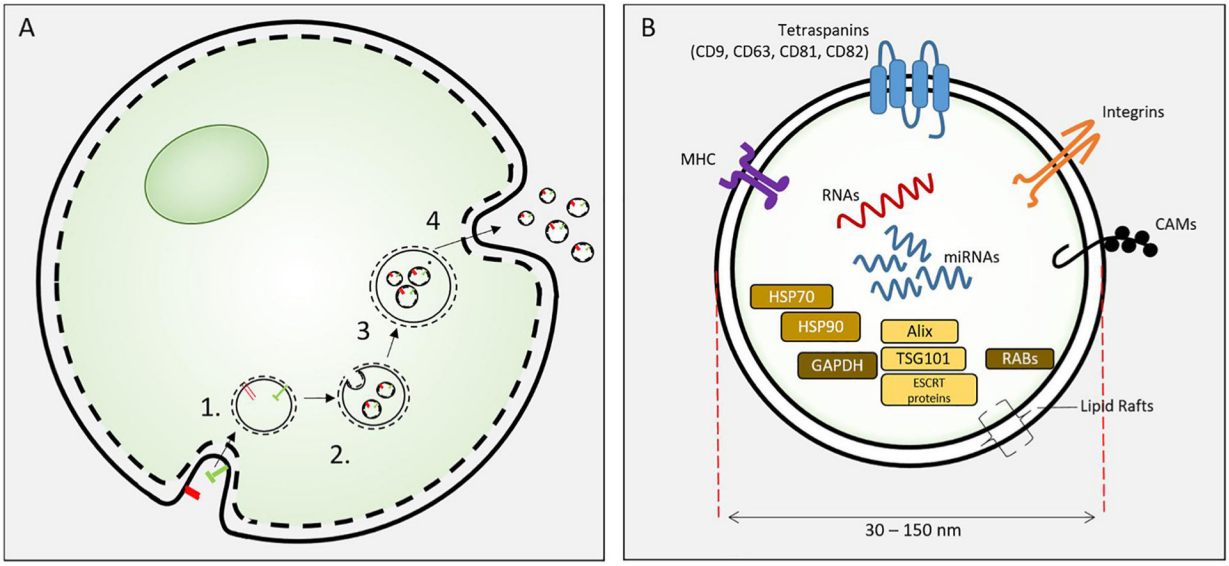

Fig. 5.

Exosome biosynthesis.

(A). Exosome biosynthesis. Exosomes are formed from endosomes [1] by an inward budding process to form intracellular vesicles [2]. These mature as multivesicular bodies [3] that fuse with the cell plasma membrane to release exosomes. (B) Exosomes are 30–150 nm extracellular vesicles containing specific proteins, RNAs and lipids. Proteins include HSP70, 90, GAPDH; proteins involved in synthesis (e.g. Alix, endosomal sorting complexes required for transport (ESCRT) proteins, TGS101), and membrane associated or transmembrane proteins (RABs, Annexins, CAMs, Integrins, Tetraspanins, MHC I and II) and other cytosolic proteins. Figure from *Cooper* et al. reproduced with permission [146].