

## Advances in regenerative therapy: A review of the literature and future directions

Edward H. Ntege

University of the Ryukyus, Japan

### Abstract

There is enormous global anticipation for stem cell-based therapies that are safe and effective. Numerous pre-clinical studies present encouraging results on the therapeutic potential of different cell types including tissue derived stem cells. Emerging evidences in different fields of research suggest several cell types are safe, whereas their therapeutic application and effectiveness remain challenged. Multiple factors that influence treatment outcomes are proposed including immunocompatibility and potency, owing to variations in tissue origin, ex-vivo methodologies for preparation and handling of the cells. This communication gives an overview of literature data on the different types of cells that are potentially promising for regenerative therapy. As a case in point, the recent trends in research and development of the mesenchymal stem cells (MSCs) for cell therapy are considered in detail. MSCs can be isolated from a variety of tissues and organs in the human body including bone marrow, adipose, synovium, and perinatal tissues. However, MSC products from the different tissue sources exhibit unique or varied levels of regenerative abilities. The review finally focuses on adipose tissue-derived MSCs (ASCs), with the unique properties such as easier accessibility and abundance, excellent proliferation and differentiation capacities, low immunogenicity, immunomodulatory and many other trophic properties. The suitability and application of the ASCs, and strategies to improve the innate regenerative capacities of stem cells in general are highlighted among others.

**Keywords:** Regenerative therapies; Tissue engineering; Tissue derived stem cells; Mesenchymal stem cells; Adipose-derived stem cells; Ex vivo culture-expanded stem cells; Scaffold free-cell sheet technology; Scaffold-based cellsheet technology

### INTRODUCTION:

Regenerative therapy is the therapeutic application of stem cells and/or progenitor cells based on their potential to stimulate repair mechanisms and restore function in damaged body tissues or organs. Stem cells (SCs) are defined by their self-renewal and differentiation capacity into one or multiple specialized cell types [5,6], as well as their unlimited regenerative potential through various trophic properties. SCs are classified into two broad categories according to their differentiation capacity and tissue of origin. SCs are referred to as totipotent, pluripotent, multipotent or unipotent cells depending on their differentiation ability. Totipotent cells are those capable of giving rise to a new organism as they can differentiate into all tissues including germline and extra-embryonic tissues. Pluripotent cells are able to generate all body cells including germ cells. Multipotent cells are capable of generating all or at least some tissue cell types. Unipotent cells are able to generate a single cell type. The classification according to origin is essentially divided into two main cell types: embryonic stem cells (ESCs) and tissue derived (somatic) stem cells. The tissue derived stem cells are further divided into: fetal stem cells isolated from fetal tissues such as placenta, amniotic fluid, Wharton jelly [18], umbilical cord blood [19], adult stem cells isolated from various tissues including bone marrow (BM-MSCs) [20], adipose tissue (ASCs) [8], dental pulp, skeletal muscle, skin, peripheral blood, liver, neural tissue, heart, and intestine. Recently, two more sub-divisions of tissue-derived stem cells have been reported: induced pluripotent stem (iPS) cells and induced tissue-specific stem (iTS) cells.

This review describes several important aspects of each SC category based on their origin, and offers greater emphasis on adult stem cells. The adult stem cells also known as multipotent mesenchymal stromal/stem cells (MSCs) have been extensively studied for over three decades for their therapeutic potential over a wide range of diseases. A plethora of preclinical studies have demonstrated the consistent ability of MSCs to promote tissue healing, reduce excessive inflammation and improve outcomes in a wide range of animal disease models. However, human clinical translation in advanced phases presents variable and discordant outcomes. Therefore, deciphering the reasons of dissonance is indeed paramount. The currently proposed factors contributing to the differences between animal model findings and clinical outcomes include inter alia differences in the preparation, potency, and functionality of MSCs in terms of tissue source, culture, and expansion. ASCs are particularly promising candidates for diverse clinical applications, owing to their excellent proliferation and differentiation capacity, low immunogenicity, and ability for immunomodulation. Here, the clinical suitability of MSCs is highlighted in detail while focusing more on current applications, benefits, challenges, and strategies to improve the therapeutic efficacy of stem cells.

#### Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent cells with the ability to differentiate into any mature cell types of the trilaminar germ lines. ESCs are obtained from the inner cell mass of the early (5–7 days post-fertilization) pre-implantation blastocyst. They were initially derived from mouse embryos in the early 1980s, and later from a number of different species including rat, rabbit, sheep,

pig, horse and human . Human ESCs are promising candidates for cell-based therapy given their distinctive properties such as; self-renewal, pluripotency and genomic stability . At the beginning of the 21st century, ESCs generated great interest in different fields namely regenerative medicine, immunotherapy, and drug discovery. However, application of these cells is challenged by the limited access to the tissues of origin. Moreover, they are currently considered high risk because of their potential to form teratomas, the difficulty in obtaining clinical grade quality cells and the restrictive ethical concerns .

### Overview of ASCs clinical application

The therapeutic utility of ASCs is based on autologous and allogeneic approaches. ASCs in cell therapy can be applied in a single step as non-expanded cells or in vitro expanded cells. In vitro expansion helps in selecting a more homogenous cell population to meet standard criteria for MSC identification, and precise determination of cell dosage to ensure high reproducibility of the results, but it is costly and may present with concerns described later herein. The autologous ASCs approach involves adipose tissue harvesting from an individual, isolation and ex vivo expansion of the ASCs before transplanting them to the same individual. This often increases patient hospital stay or visits, and in peril of donor site morbidity, the most important being scars, infection and loss of function .The allogeneic ASCs approach is based on the understanding that ASCs have a low immunogenicity in vitro, their secretome varies across different cell donors leading to highly variable outcomes, and are quickly available as an off-the-shelf product for immediate use . Various reports have shown that allogeneic ASCs have the ability to elicit humoral and cellular immune responses in vivo particularly at sites of inflammation, and hence are not fully immune privileged .However, a meta-analysis report of 82 preclinical studies has demonstrated that the approach is safe and as effective as autologous therapy. Moreover, allotransplantation is very useful in especially the elderly and/or those patients affected by co-morbidities where autologous ASCs may have reduced regenerative and therapeutic potential. Both approaches of ASCs-based therapy present some drawbacks and should to be considered on a case by case basis. Nonetheless, allogeneic ASCs seem to attract more clinical interest, as many patients can benefit from one or more donor cells having optimized characteristics and being selected for specific applications. Recently, MSCs-based clinical trials mainly at phase I and II, have been conducted for a variety of human diseases with increasing utility of ASCs cell type. Chu et al. in a recently published review have highlighted that the studies about ASCs human trials have been increasing year by year starting from 2007 and reaching its peak in 2015 with up to 187 clinical trials using adipose stem cells. Other reports have indicated a total of 282 registered trials in late 2018, although 22 (8%) of them utilized SVF and only 13 (5%) had progressed to advanced phases.

The clinical utilization of ASC-based therapy is mostly in medical and surgical conditions including aesthetic, orthopedic, immune system, cardiovascular, craniomaxillofacial, skin and connective tissue, nervous system, nutritional and metabolic diseases. Clinical application details regarding some of the registered clinical studies of ASCs for different diseases including Osteoarthritis,

Ischemic heart disease, Critical Limb Ischemia, Amyotrophic Lateral Sclerosis, Multiple System Atrophy, and Spinal cord Injury are summarized. The application of ASCs in aesthetic surgery is common in trials involving repair of soft tissue defects. The fat grafting procedure has long been employed to repair soft tissue defects. However, it presents with substantially variable successes in different surgical hands, and significant complications such as loss of volume, fibrosis, and necrosis. Therefore, ASCs are used to take advantage of their angiogenic, survival enhancement, proliferation, and differentiation properties for the improvement of graft viability. Pioneering work involving the use of SVF in combination with intact lipoaspirate of tissue in breast reconstruction or augmentation had positive outcomes such as improved volume retention, no evidence of fibrosis or adhesions, but also a few minor complications such as cyst formation or microcalcifications. Other reports have indicated significant improvement in the healing of post mastectomy radiated skin and dermis complications with both SVF and ASCs.

### Conclusion and further perspectives

Cell-based therapies involving stem cell products such as the MSCs are indeed promising approaches to the treatment of disorders for which current medical and surgical solutions are obscure. Because multiple factors negatively affect stem cells and/or progenitor cells, characterization of function is important to guide more efficacious cell-based therapeutic applications. The magnitude of impact and strategies to ameliorate the regenerative potential of cells remain elusive. For example, a narrative review of MSC function in CVD provided a systematic evaluation of a total of 41 studies examining CVD-related MSC (dys)function. Their findings revealed that CVD affects MSC characteristics and regenerative potential but many of these studies presented conflicting results. Remarkably though, this and many other studies in different treatment fields have contributed enormously to the comprehension of complex biological processes underlying regeneration regardless. This has enabled the current understanding that the classical concept of regeneration where an insulted tissue or organ can be repaired by the administration and engraftment of large numbers of cells – stem cells or not- is inadequate. Instead, as reported previously, regeneration should be considered as a global and balanced process involving the entirety of target tissue structures in addition to the cell types . Furthermore, to achieve the ultimate goal of building functional tissues or organs, the following priority areas of research are suggested: in-depth understanding of cell proliferation processes and their control mechanisms, development of means to identify and track cell subpopulation(s) capable of division in the insulted target tissue or organ based on ploidy and other characteristics, identification of markers of cell proliferation rather than of cell cycle activity, analysis of transcriptional pathways underlying target tissue cell dedifferentiation, replication, migration, and maturation.

The practical advantage of adipose tissue derived MSCs in clinical practice is their primary source – the adipose tissue being abundant and easy to obtain with minimal adverse effects. However, compared to ESCs and iPS cells, the multipotency of ASCs is limited. Moreover, their differentiation potential can be influenced by multiple factors such as anatomic location of fat, the donor's

---

gender, age and comorbidity, and the key molecular mechanisms of proliferation and differentiation remain unravelled. With promising data from the current research at the university of the Ryukyus hospital, and the guidance from related experiences elsewhere, carefully designed, and enriching studies are anticipated. Following completion of the assessment of safety and effectiveness of site-specific autologous ASCs instillations, the university hospital department of plastic and reconstructive surgery plans to conduct clinical evaluation of allogeneic ASCs in treating various diseases. Moreover, in the near future, pre-clinical studies will be considered as well, to assess the therapeutic impact of site-specific administration of combined cell types: regenerative and bonafide differentiated cells, using the scaffold based cell sheet technology.