Chapter 3. Cells

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Sources of cells for tissue engineering strategies

Regenerative medicine centers on the restoration of lost, damaged, or aging cells and tissues in the human body. For in vitro production of engineered tissue, cells are needed the use of cells to populate matrices and produce matrix resembling that of the native tissue. Foremost, the largest advances in the field have come from using autologous (taken from the patient) somatic cells, and used in combination with scaffolds to produce tissue for re-implantation. However, there are limitations to this strategy, because of the invasiveness of cell and tissue harvesting and the risk of cells being associated with potential disease. Consequently, focus has shifted to the use of stem cells, including embryonic stem (ES) cells, mesenchymal stromal/stem cells (MSCs) from various adult tissues (Fig 1). To date, various stem cell types have been explored in tissue regeneration in both animal models and human clinical studies, with varying degrees of success.

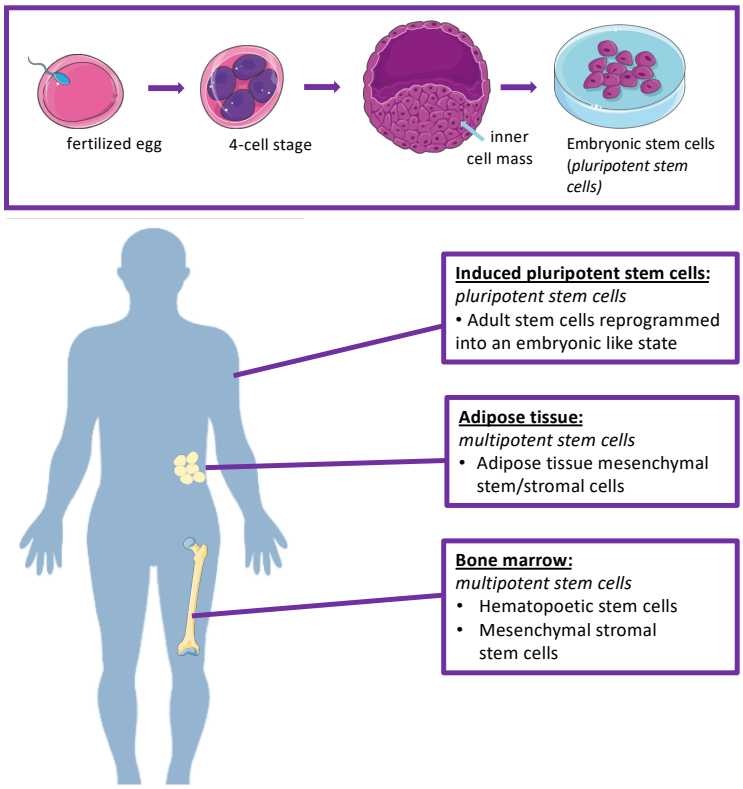

Fig 1. Human stem cell sources

Stem cells

Stem cells are cells capable of producing copies of themselves (self-renewal) or differentiating into specialized cell types. From the human cell perspective, several cell types are congregated under the same umbrella jointly called 'stem cells; 1) human embryonic stem cells (hESCs), 2) human induced pluripotent stem cells (hiPSCs), which are basically reprogrammed somatic cells, and 3) adult stem cells, which cover numerous types of cells of hematopoietic and mesenchymal origin (Fig 1 stem cells). MSCs and tissue-specific progenitors reside in the human body in most tissues during an individual's life and commonly have a limited expansion and differentiation [1].

Embryonic stem cells

Pluripotent stem cells can differentiate to all specialized cell types. The fact that hESCs are actually pluripotent comes from their ability to form teratomas[2]. The main source of stem cells comes from the inner cell mass of human embryo (hESC), but lately hiPSCs have gained a lot of interest in clinical cell therapy and regenerative medicine (Fig 1 stem cells). iPSC lines, are basically genetically reprogrammed somatic cells using transcription factors [3,4]. hESCs could potentially allow production of type-matched tissues for individual patients, either by stem cell banking or by using therapeutic

cloning. This property allows for the stem cells to form multiple tissue types but also emphasizes the significance of using a terminally differentiated cells lacking latent stem cell-like properties. When using both hESCs and hiPSCs there is a risk of mutations already in the laboratory, due to the lengthy in vitro culturing time and extensive cell manipulation [5]. In vivo reports of tumorigenicity have raised concern for safe in using these cells in clinical work [6].

The prospects of cell therapy using pluripotent stem cells (PSCs) has attracted much attention from both scientists and the general public, but such technology is not yet fully developed. While ESCs were for a long time anticipated as a cell source for regenerative medicine, use of these has been impeded by the risk of immune rejection and ethical issues. Induced pluripotent stem cells (iPSCs) have arisen as a cell source circumventing these problems nevertheless, despite their promising potential, many obstacles must be overcome before human iPSC-based therapy will emerge in clinics. For example, similarly to ESCs, application of iPSCs involves a risk of teratoma development. Further, iPSC therapy also involves genetic modification, which may give rise to various obstacles.
One of the critical steps of using hPSCs for regenerative medicine is to control the differentiation of the cells to the wanted tissue lineages. Differentiation of hESCs has been achieved using protocols modified from BM-MSC (bone marrow derived MSCs) protocols whereby hESCs can be directed to express features of bone, notably the accumulation of mineral [2].

Adult stem cells

As of date, the main stem cells applied in tissue engineering are tissue derived, so called adult stem cells, which can be obtained from most adult tissues. They can be transplanted into the same individual as the original cells or tissue was harvested from (autologous transplantation) avoiding risks of disease transfer or immunological reactions. These cells may also be transplanted into another individual (allogenic transplantation)[7].

MSCs are multipotent, nonhematopoietic adult stem cells, that can be isolated from bone marrow, umbilical cord, placental or adipose tissue. MSCs have the potential to differentiate into various cell types such as osteoblasts, chondrocytes, and adipocytes as well as endothelial, cardiovascular, and neurogenic cell types and are attaining standing as a therapeutic agent because of their expansion capacity and ethical acceptability. Further, in addition to their role in tissue regeneration, MSCs have compelling anti-inflammatory and/or immunosuppressive properties[8].

MSCs are of great interest scientifically and clinically owing to their potential in tissue engineering applications. The most commonly studied MSCs are derived from bone marrow (BM-MSC) and adipose tissue (adipose tissue stem/stromal cells; AT-MSCs). While both BM-MSCs and AT-MSCs have a roughly matched potential to differentiate into cells and tissues of mesodermal origin (i.e., fat, bone, cartilage), AT-MSCs have a distinctive benefit, as adipose tissue is a more easily accessible than bone marrow, greater amount of tissue is available for cell isolation. Several thousand clinical trials associated with the term 'stem cells' are currently registered in the World Health Organization International Clinical Trials Registry Platform (http://apps.who.int/trialsearch/). The vast majority of the trials are applying adult stem cell as therapeutics, but the registry also comprises the first pluripotent stem cells-based clinical trials, associated with eye diseases such as macular dystrophy or degeneration. Albeit the technology may in place to generate a wider range of therapies, safety issues are not completely understood, consequently the transition from bench to bedside advances with cautious steps [9].

In recent years, though, there has been somewhat of a paradigm shift in the field of applications of stem cells in regenerative medicine, the focus of the therapeutic effects has turned to paracrine activity of the cells rather than the engraftment and differentiation into functional cells [10]. The current believe is that the therapeutic effect of MSCs is owing to a 'hit-and-run' mechanism facilitated by the production of extracellular vesicles (EVs) or exosomes or secretion of trophic and immunomodulatory factors [9] (Fig 2 paracrine effects). In fact, the so called 'cell-free' therapies, mediated by paracrine factors or vesicles secreted by cells, in contrast to treatments based on whole cells, are easier to administer and safer due to lower quantities of membrane-bound proteins such as MHC molecules and their inability to directly form tumors [4,11].

Nevertheless, the mechanism by which MSCs act in a paracrine fashion are not fully understood. Thus, it is of interest to consider the possibilities that the complex paracrine regenerative actions of exogenously administered MSCs and other stem cells communicate by transferring information and regulatory genes mediated, to some degree, by released EVs and that EVs derived from cultured MSCs have the potential to constitute a safe, effective cell-free therapy[12].

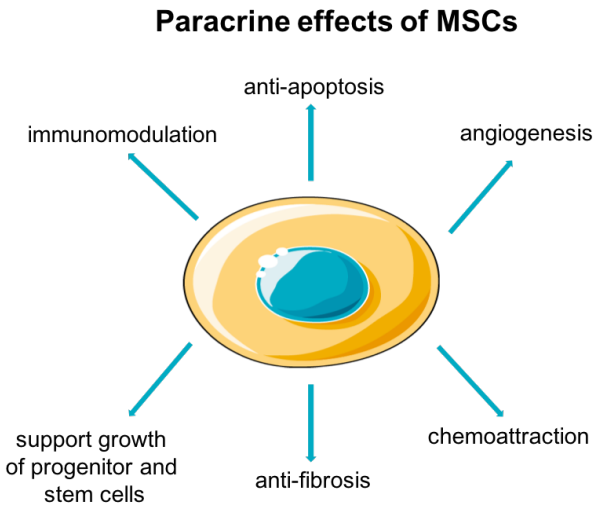

Fig 2. Paracrine effects of MSCs

Stem cell stimulation

Stem cell commitment to various lineages is controlled by many signals in the local tissue microenvironment, presented in Figure 3. The following section will focus specifically on the secreted factors.

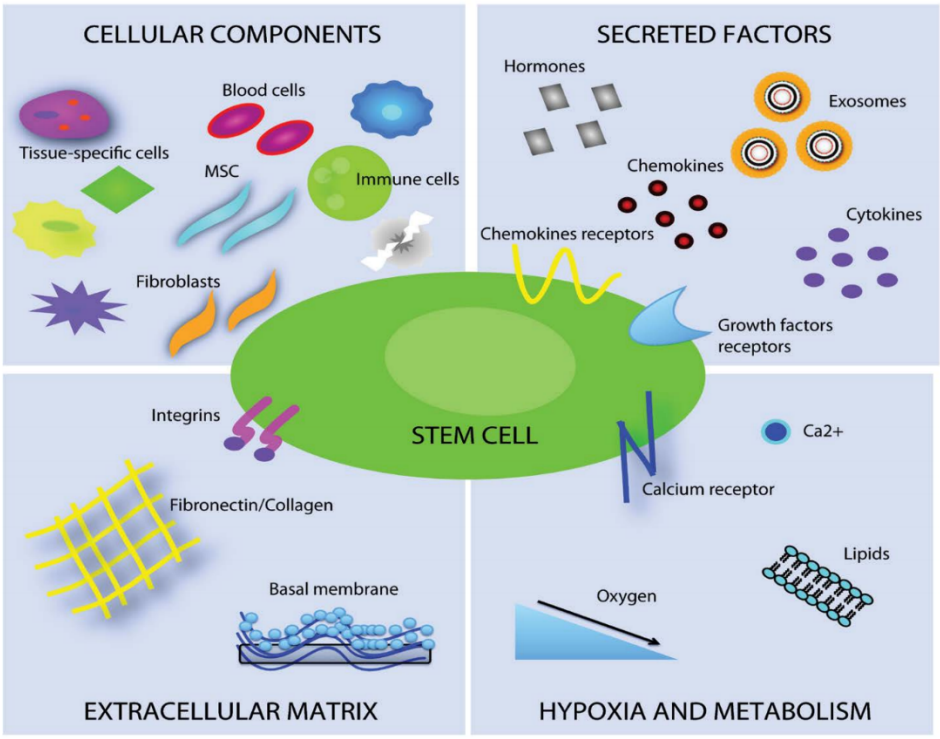

Fig 3. The cells communicate with its environment with structural, physical, chemical and cellular components which brings complexity to tissue engineering. Image from https://www.esciencecentral.org/ebooks/ebookchapter/resident-stem-cells-stimulation-new-promise-for-tissue-regeneration--165/3.

Growth factors

Bone Morphogenetic Proteins (BMPs) form a unique group of proteins within the transforming Growth Factor beta (TGF-beta) superfamily. BMPs play a central role in bone and cartilage development and bone metabolism, but they are playing crucial roles in all organ systems, and should therefore perhaps be named *body* morphogenetic proteins [13]. Currently, the BMP family is comprised of several members from BMP-2 to BMP-18.

While BMPs were originally discovered inducing bone formation, e.g. BMP-3 is reported to be a negative regulator of bone density. Some BMPs may not be important for bone formation, as conditional deletion of BMP-7 from limb showed no noticeable effect in a study by Wang and co-workers [14]. Moreover, contradictory results of the osteogenic potential of BMPs have been reported in vitro. In a study by Kyllönen at el., supplementation of BMP-6, BMP-7 and vascular endothelial growth factor (VEGF) and their combinations in two- and three-dimensional cultures using AT-MSCs showed no substantial augmentation of osteogenesis [15], while Li et al. reported a synergistic effect of BMP-6 and VEGF on the osteogenic differentiation of the same cells [16]. Using periodontal ligament cells, supplementation with BMP-2 or BMP-6 showed no enhanced osteogenesis [17].

The best studied in the context of osteogenesis, BMP-2, has been implied potency in bone formation, yet, the in vitro and in vivo reports have been contradictory. BMP-2 and BMP-7 received approval for clinical use, BMP-2 and -7 they quickly conquered ground in clinical therapy and are the most studied growth factors for bone tissue engineering. However, BMPs, like many other growth factors, also carry many of the limitations associated with protein therapeutics. For example, they are produced using recombinant DNA technology at elevated costs. Trace amounts of biologically active contaminants within the product may compromise their clinical use. Further, BMPs have been reported as eliciting unfavorable immune responses in patients. Another clearly relevant problem in their clinical use is the need for high doses have led to undesirable side effects in many patients [18,19]. In 2015, FDA published a safety communication on recombinant human BMP-2 use, with recommendation to cautiousness especially for pediatric patients in the use of BMPs until further safety evidence is available [20].

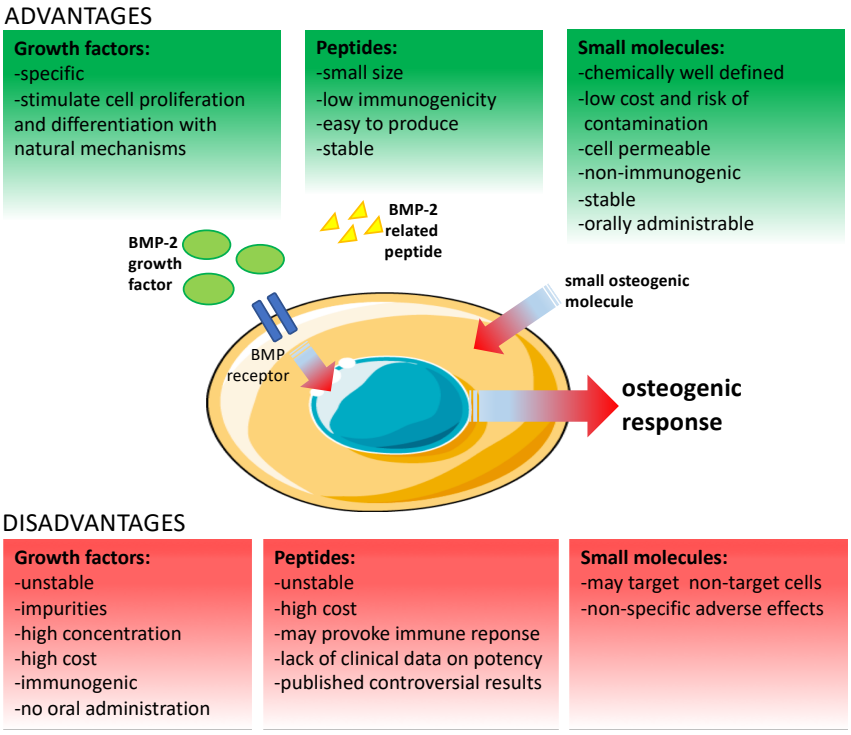

Fig 4. Advantages and limitations of growth factors, peptides and small molecules for bone regenerative medicine. Image modified from Balmayor et al. 2015[19].

New compounds for osteogenesis

Therefore, there is a need for new compounds capable of inducing cell differentiation and tissue healing with high efficiency and reduced side effects. These osteoinductive molecules should be easy and inexpensive to produce, stable and immunologically inert in the host organism, such as peptides and small molecules (Fig 4). Short peptides derived from therapeutic proteins, such as BMP-2, have been investigated extensively for tissue engineering applications. Compared with growth factors, peptides are smaller and are thus expected to be less immunogenic. They are able to interact with BMP receptors as the native protein, and activate different signaling pathways within the cell. Several patents exist on short osteogenic peptides, yet few pre-clinical and clinical studies have been conducted to investigate their use as osteogenic molecules. These peptides also display certain limitations such as high costs and short half-life [21].

Evading the drawbacks of the short peptides, small molecule drugs have recently surfaced as promising candidates for tissue regeneration. Similar to short peptides, the small molecules are non-peptide natural or synthetic molecules with low molecular weight, displaying low immunogenicity. These molecules can easily diffuse across the cellular membrane also due to their small size. In the particular case of small osteoinductive molecules, these compounds can induce the differentiation of

multipotent mesenchymal stem cells (MSCs) or osteoprogenitor cells to a more mature osteoblastic stage.

Despite their conceivable impact, the small molecules often do not reach the clinical arena. The main limiting factor hindering their translation to the clinics are the nonspecific adverse effects. Particularly in the bone regeneration field, 3D scaffolds are required for the treatment of large defects. Another critical problem is the absence of suitable and reproducible drug delivery systems that allows for controlled release of the small molecules[19,22]. Thus, several challenges hamper the small osteogenic molecules in their transit to the clinics.

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