



Adipose-derived stem cells: a review of osteogenesis differentiation

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ABSTRACT

This review article provides an overview on adipose-derived stem cells (ADSCs) for implications in bone tissue regeneration.

Firstly this article focuses on mesenchymal stem cells (MSCs) which are object of interest in regenerative medicine. Stem cells have unlimited potential for self-renewal and develop into various cell types. They are used for many therapies such as bone tissue regeneration. Adipose tissue is one of the main sources of mesenchymal stem cells (MSCs). Regenerative medicine intends to differentiate ADSC along specific lineage pathways to effect repair of damaged or failing organs. For further clinical applications it is necessary to understand mechanisms involved in ADSCs proliferation and differentiation.

Second part of manuscript based on osteogenesis differentiation of stem cells. Bones are highly regenerative organs but there are still many problems with therapy of large bone defects. Sometimes there is necessary to make a replacement or expansion new bone tissue. Stem cells might be a good solution for this especially ADSCs which manage differentiate into osteoblast in *in vitro* and *in vivo* conditions.

KEY WORDS: mesenchymal stem cells, regenerative medicine, adipose tissue

Mesenchymal stem cells

Mesenchymal stem cell (MSCs) are non-hemopoietic, multipotent, adult stem cell. MSCs have ability to self-renew and differentiate into multiple tissues, including bone, cartilage, fat, and other tissues of mesodermal origin. They are present in blood, adipose tissue, bone,

skin and Wharton's jelly (Maleki et al. 2014).

The multidirectional therapeutic potential of MSCs has generated increasing amount of research in all over the world. It caused lack of homogenous methods in isolation, cell culture and

identification of mesenchymal stem cells. It has forced The International Society for Cellular Therapy (ISCT) and International Federation for Adipose Therapeutics (IFATS) to creating a minimal criteria to define MSCs. Based on it human MSCs identified by adherence to plastic and expression of

cell surface markers including CD90, CD73, CD105 and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface proteins. There also must differentiate to osteoblasts, adipocytes and chondroblasts in vitro condition (Dominici et al. 2006, Fathi et al. 2016, Bourin 2013)(Tab. 1).

Table 1. Minimal criteria for defining mesenchymal stem cells.

	Feature
cell culture	adherence to plastic
differentiation potential	osteoblasts, adipocytes and chondroblasts
≥ 95% population of cell with expression surface markers	CD90, CD73, CD105
≤ 2 % population of cell with lack of surface expression	CD45, CD34, CD14 or CD11b, CD79a, CD19, HLA-DR

It was proven that MSCs have inflammatory, immunomodulatory functions and they can penetrate into inflammatory sites. They secrete factors such as: TGF- β , IL-6, -7, -8, -10, 11,-12, -14, inducible nitric oxide synthase (iNOS) and hemoxygenase (HO). MSCs can modulate immunological responses through T-cell-mediated (Takano et al. 2014, Rahimzadeh et al. 2014) They release also multiple angiogenic and growth factors: VEGF, HGH or IGH-1 (Fathi et al. 2016, Soulnier et al. 2010). It suggest that they are improving neovascularization and promote angiogenesis process (Abudusaimi et al. 2011).

Thus MSCs have been applied in various diseases connected with bone damages, for example: rheumatoid arthritis (Takano et al. 2014), avascular necrosis of the femoral head (Abudusaimi et al.2011) and large mechanical defects.

Adipose derived stem cel

Adipose tissue is one of the most richness source of stem cells. Adipose derived stem cells (ADSCs) are plastic-adherent cells, which are characterized by a variety of cell surface markers

(Undale et al. 2009). They were first described. in 2001 as a population of cells derived from adipose tissue with the potential of differentiation (Zuk et al. 2001). Isolation method based on digested it with collagenase Type I, and separated the cellular components by centrifugation (Gimble et al. 2003). The number of cells after isolation is connected with amount of adipose tissue. The lowest amount of tissue is 0,1-2mg but the number of stem cells depend on tissue and their volume is associated with destination of ADSCs (Cheng et al. 2011).

ADSCs are able to differentiate into a number of mesenchymal cell types, including osteoblasts, chondrocytes and adipocytes (Undale et al. 2009, Fernandez et al. 2015).

Compared to other types of stem cells, ADSCs have many advantages. ADSCs can be easily obtained from a donor. Adipose tissue donation is the easiest and less invasive for patients in comparison e.g. bone marrow biopsy. Procedure of liposuction can provide a lot of tissue and cells. Moreover isolation of stem cells from bone marrow is less effective and cells often are contaminate (Chen at al.2013, Fathi et al. 2016, Dai et

al. 2016). Comparison of mesenchymal stem cells obtained from different tissues (fat tissue, bone marrow, placenta) showed that adipose stem cells did not differ morphologically from bone marrow cells. These cells have similar expression of the main marker genes (Musina et al. 2005).

It has been shown that from 1 gram of fat tissue may be isolated from 0.5×10^4 to 2×10^5 stem cells. Differences in the amount are connected with a gender, age, body mass index of donor but also medical record, type of adipose tissue (white or brown) and its location (Bajek et al. 2008, Olkowska et al. 2008). Research suggested that cells from younger donor are grown and differentiating better than from old donors (Bunnell et al. 2008, Musina et al. 2005).

Moreover there is not ethical problems with using ADSCs which is a huge issue for embryonal stem cell (Dai et al. 2016).

The transcriptome analysis with microarray technique of ADSC were reveals their more adipogenic potential than osteogenic in compared to bone marrow stem cells (BMSC). They also have larger capability to lipid synthesis. It suggest that ADSCs indicate better ability to differentiation into adipocytes than osteoblast. The differences were related to MSCs location. However ADSCs show lower immunogenicity than stem cells in bone marrow. Moreover tissue harvesting is easy, quick and efficient and thus they seem to be a better alternative as a stem cells source in compared another tissues (Bionaz et al. 2015, Monaco et al. 2012).

However, different methods of isolation of adipose tissue have influence on expression profile of genes characteristic of ADSCs. Comparison of adipose tissue collected during the surgery and adipose tissue collected by

liposuction have shown the bigger amount of cell in case of liposuction and the population of these cells was more homogenous. As a result, processes of differentiation of both types of cells to mesoderm (cartilage, osteoblasts and adipocytes) it was more efficient in cells isolated from lipoaspirate than biopsy (Gnanasegaran et al. 2014). There is not many results about collecting stem cells from different adipose tissue places. It suggested that difference in number of cells between subcutaneous adipose tissue from the arms in compared to abdomen and breast. The amount of adipose derived stem cell is connected with location, type and species. (Kolaparthi et al. 2015).

The comparison of isolation adipose derived stem cells ADSC manual and automatic methods difference in cells activity did not observed. The number and viability of cells were similar in both cases (Doi et al. 2013).

Methods of differentiation of stem cells

Standard method for initiating osteogenic differentiation in stem cell culture is application some components which induce this process. Culture of MSCs in osteogenic medium causes manifestation of osteoblasts markers (Birmingham et al. 2012). Basic substances with proved action on osteogenesis in stem cells are: dexamethasone (Dex), ascorbic acid (Asc) and β -glycerophosphate (β -Gly). For osteogenic differentiation at least 21 days of treatment this substances are necessary (Langenbach et al.2013).

Mechanism of induces osteogenesis process by dexamethasone is multidirectional. Dex induces differentiation into osteoblast by activating Wnt/ β -catenin pathways. It indicated that by Four And A Half LIM Domains 2 (FHL2) upregulation which influence on expression of *RUNX2*.

Consequently expression of collagen type I alpha 1 (*COL1A1*) is also upregulated. Moreover dexamethasone regulates the function of *RUNX2* via the activity of molecule TAZ which is transcriptional coactivator with PDZ-binding motif and mitogen-activated protein kinase (MAPK) phosphatase (MPK-1).

Bone morphogenetic protein (BMP) signaling also change the activity *RUNX2* which was connected with initiating osteogenesis process. Binding BMPs to their receptors causes of phosphorylation SMAD proteins (SMAD 2, SMAD 5 and SMAD8) which subsequently bind with SMAD 4 and after translocation to nucleus regulates the expression of osteogenic transcription factors as *RUNX2*, *OSX* and *DLX*. Experiments showed that optimal concentration of dexamethasone in medium culture is 10nm (Langenbach et al. 2013).

Ascorbic acid induce differentiation of stem cells through the enhancement secretion of collagen type I into the extracellular matrix (ECM). It is a cofactor for hydroxylate proline and lysine which they are required for transformation pro-collagen into active form. β -glycerophosphate is a phosphate source in mineralization process and it induce expression of genes connected with osteogenesis through phosphorylation of kinases (Langenbach et al. 2013).

Numerus studies showed the relevant impact of vascular endothelial growth factor on the osteogenesis (Behr et al. 2011, Clark et al. 2015). VEGFA promotes the differentiation of progenitor cells into the direction of factors associated with angiogenesis, but also it impacts on the cells from bone tissue. VEGFA is one of the most important mediators of angiogenesis, cell migration and mineralization (Clark et al. 2015). It is extremely important element of

osteogenesis due to bone vascularization during its expansion and repair (Behr et al. 2011). It was also noticed that the strong relationship occurs with VEGF factor and the bone morphogenetic proteins pathway. BMPs induces the intracellular signals which causes the differentiation of progenitor cells into osteoblasts (Zhang et al.2012). The treatment of ADSC with VEGF and BMP6 in vitro caused the increase in expression of alkaline phosphatase, genes associated with osteogenesis e.g. collagen type 1 (*COL1A1*), the osterix transcription factor and gene which encodes the homeotic protein *DLX5* (distal-less homeobox 5) and also cells mineralization (Zhang et al.2012, Clark et al. 2015, Li and Madhu et al.2015, Li and Liu et al. 2015). Similar effect was observed both in the case of simultaneous treatment of ADSC with VEGF and BMP2, 4 and 9 (Zhang et al.2012, Li and Liu et al. 2015). Besides the use of VEGF and fibroblast growth factor (*FGF-2*) caused only the initiation of angiogenesis (Clark et al. 2015).

Experiments suggest also application of active form of 1,25(OH)₂ D₃ vitamin as the induction factor of osteogenesis (Kato et al. 2015). It was shown that there is possibility of generation both the osteoblasts and the cells similar to osteocytes from induced pluripotent cells inter alia by the supplementation with 1,25(OH)₂ D₃ vitamin (Kato et al. 2015).

Another factor showing the impact on differentiation of stem cells derived from adipose tissue is the hypoxia. It was noticed that the culture of ADSC in the conditions with the low level of oxygen (1-2%) strengthens the survivability and proliferation of the cells. This state intensifies the potential of differentiation into osteoblasts and also strengthens the expression of genes responsible for the maintenance of stemness: *OCT4* (octamer-binding transcription factor 4),

NANOG (transcription factor), KLF4 (Kruppel-like factor 4) (Valorani et al. 2012, Xu et al. 2014).

The increase of genes associated with angiogenesis, adhesion and the growth factor release was also documented. Probably, it is the effect of physiological presence of low concentration of the oxygen at the stem cells niche (Valorani et al. 2012, Xu et al. 2014). Another study showed that the hypoxia weakens the proliferation ability of MSC but does not influence on the phenotype and seems to maintain them in the more immature stage than at the standard culture. This state caused the increase in pluripotent gene expression: SOX2 (SRY sex determining region Y-box 2), NANOG, OCT-4 (Ranera et al 2012).

The hormone participating in the regulation of glucose homeostasis in organism is the glucagon-like peptide type 1 (GLP-1). The use of GLP-1 in

culture medium cause the increase in mRNA expression of markers specific to osteoblasts, the activity of alkaline phosphatase and mineralization of calcium. This hormone is especially important in insulin secretion by glucose-dependent pathway and has the anti-diabetic impact in the treatment of type 2 diabetes. Additionally, patients with diabetes have a higher risk for bone fracture and osteoporosis, which proves about close relationship which osteogenesis and the activity of GLP-1 hormone and the disruption of carbohydrate economy (Lee et al. 2015).

Estrogens acts also an important role at the formation of bone structure. The characteristic changes in the level of estrogens in the perimenopausal period causes osteoporosis, therefore numerous study suggest the use estrogens in the osteogenesis of the stem cells (Gao et al. 2015, Veronesi et al. 2015) (Tab. 2).

Table 2. Methods of differentiation of stem cells.

Differentiating factors	References
dexamethasone (Dex), ascorbic acid (Asc), β -glycerophosphate (β -Gly)	Langenbach et al.2013
vascular endothelial growth factor (VEGF)	Behr et al. 2011 Clark et al. 2015 Li and Madhu et al.2015 Zhang et al.2012
bone morphogenetic protein (BMP)	Zhang et al.2012 Clark et al. 2015 Li and Madhu et al.2015 Li and Liu et al. 2015
transforming growth factor (TGF- β)	Li and Liu et al. 2015
active form of 1,25(OH) ₂ D ₃ vitamin	Kato et al. 2015
hypoxia	Valorani et al. 2012 Xu et al. 2014 Ranera et al. 2012
glucagon-like peptide type 1 (GLP-1).	Lee et al. 2015
estrogens	Gao et al. 2015 Veronesi et al. 2015

Mechanism of osteogenesis stem cells

Treatment of large bone defects and incurable fractures is difficult clinical problem. Adipose tissue stem cells have ability to regenerate damaged bone

tissue. However, there are necessary coexistence of efficient processes of angiogenesis and osteogenesis extending in order of their use (Behr et al. 2011).

The process of the osteogenesis of the stem cells derived from adipose tissue is regulated by the transcription of numerous genes. It should be noted, that in case of adipogenesis and osteogenesis the receptor proteins are activated by the PPAR (peroxisome proliferator-activated receptors). At the side of osteogenesis, these proteins acts as the negative regulator. The central role in this process is acted by the Wnt and PI3K/AKT and also the MAPK (mitogen-activated protein kinases) pathways (Bionaz et al. 2015, Chen and Shi et al. 2013). During the osteogenesis lots of growth factors such as bone morphogenic proteins (BMP), fibroblast growth factor (FGF), transforming growth factor beta (TGF β), platelet-derived growth factor (PDGF) and the vascular endothelial growth factor (VEGF) are secreted (Li and Madhu et al. 2015). The regulation of the majority of these factors is based on noncoding activity of micro RNA (miRNA) (Oshita et al. 2011, Chen et al. 2013).

The impact study of the interleukin family (IL-1) on the MSC confirmed its induction of the osteogenesis of human mesenchymal stem cells. It was proved that IL-1 activates the Wnt pathway i.e. the Wnt-5a gene and its orphan receptor of tyrosine-protein transmembrane receptor (ROR2). Similar effect was noted in the presence of cytokines such as interleukin-6 family (IL-6) and the tumor necrosis factor alpha (TNF α), besides with weaker effects of differentiation (Sonomoto et al. 2012, Tanaka 2015).

The differentiation of stem cells *in vitro* into the cells of bone tissue is multi-stage. In the first step, which is ongoing from five to fourteen days the expression of alkaline phosphatase (ALP) both at the level of transcriptome and proteome. ALP is known as reliable marker of early osteoblast differentiation (Clark et al.

2015). Additionally, at the early stage, the expression of collagen type 1 also increases and then the ALP level decreases. At the next stage which is ongoing from about fourteen to twenty-eight day increases the expression of osteocalcin an osteopontin (Birmingham et al. 2012)

To fully knowledge of differentiation mechanisms of stem cells into the osteoblasts will allow to maximize the use of this process in regenerative medicine.

3D culture of ADSC in regeneration of bone defects

ADSCs have shown promising results in many diseases. However positive results of experiments in two-dimensional plate culture are not meaningful, because of not sufficient condition of environment, without cell-cell and cell-environment interaction. The development of tissue and biomaterials engineering in last years resulted in a significant improvement of regenerative medicine and three-dimensional scaffolds are used more widely Three-dimensional cells culture techniques initiate cellular microenvironment similar to *in vivo*. 3D scaffolds have many advantages in compared to 2D culture. It observed that they can enhance the cell viability during proliferation (Dai et al.2016,)

Scaffolds are produced using biomaterials from selected components; they must be biocompatible and do not cause immune reaction. Their mechanical, chemical properties and microstructural patterns must be adapted to cell line. Scaffolds can be also biodegradable and non-biodegradable. It depends on its destination. Many studies indicate that ADSCs culture in 3D scaffolds can be alternative treatment in orthopaedic tissue repair (Dai et al. 2016). Nowadays traditional autologous and allogeneous bone grafts are replacing

by different biomaterials. It is caused by lack of donor, potential disease transmission and severe immunogenic responses (Zhang et al. 2013).

Polylactic acid polymer scaffolds have the advantage of being degradable, porous and easily moldable. It was found that polypyrrole-coated polylactide scaffolds can provide higher alkaline phosphatase (ALP) activity levels, which benefit the early osteogenic differentiation of ADSCs (Dai et al. 2016). Experiments show that PLA scaffolds escalate angiogenesis and osteogenesis of adipose derived stem cells but with co-culture with osteoblast or endothelial cell. It helps to create cell-cell interaction (Shah et al. 2014). These scaffolds provide properly growth and osteogenic differentiation of adipose derived stem cells (Lu et al. 2014). Chitosan is one of the substance which is examined for using in regenerative

medicine of bone damage . It can be used as 2D or 3D scaffolds which have many advantages like: porosity, non-toxic and biocompatibility, high adsorption capacity and biodegradability (Busilacchi et al. 2013, Dash et al. 2011).

Conclusions

Adipose tissue is rich source of stem cells. ADSCs have multidirectional potential to differentiation inter alia into bone tissue. Development of regenerative medicine help in treatment large bone defects and their metabolism disorders. Unfortunately stem cells still must be examined for the safety of potential patients. Problems are inefficient differentiation, optimization of osteogenic medium and also comorbidities influence on proliferation and metabolism of stem cells.

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Streszczenie

Komórki macierzyste to komórki posiadające zdolność nieograniczonych podziałów oraz umiejętność do wielokierunkowego różnicowania. Mezenchymalne komórki macierzyste (MSC) to somatyczne komórki występujące w tkankach i narządach dorosłego organizmu takich jak: szpik kostny, tkanka tłuszczowa oraz mięśnie. Ulegają one różnicowaniu w kierunku komórek pochodzących z jednego listka zarodkowego jakim jest mezoderma. To pozwala na wykorzystanie ich w regeneracji chrząstki, kości lub wypełnienia ubytków tkanką tłuszczowa między innymi w chirurgii plastycznej.

Obecnie głównym źródłem z którego pozyskiwano MSC był szpik kostny, jednak coraz szersze zastosowanie wykazuje tkanka tłuszczowa. Komórki z niej pochodzące wykazują takie same właściwości jak te pochodzące z szpiku kostnego, a procedura izolacji jest dużo mniej inwazyjna dla pacjenta. Bardzo często natomiast ich ilość jest nieporównywanie większa. Stąd też niniejsza praca porusza temat wykorzystania MSC z tkanki tłuszczowej w regeneracji tkanki kostnej.