Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 22
Sofia ● 2015

Review Articles

Briefly about Bone Defects and New Strategies to Treat Them: Review

R. Alexandrova[1], A. Abudalleh[1], T. Zhivkova[1], L. Dyakova[2], B. Andonova-Lilova[1], O. Alexandrov[3], N. Saha[4]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences*
[3]*Health Service, Gorna Malina, Bulgaria*
[4]*Tomas Bata University in Zlin,* Czech Republic

Treatment of severe bone defects, resulting from trauma or resorption, remains a major challenge in orthopaedic surgery and traumatology. These pathological conditions significantly decrease the quality of life of people affected and have a high social and economic costs. In the presented minireview we summarize data of both current therapy for bone regeneration and the perspectives in the field of bone tissue engineering.

Introduction

Bone disease is a serious health problem that directly impacts on the quality of life of patients. It has been predicted that the percentage of persons over 50 years of age affected by bone disease will double by 2020, especially in populations where aging is coupled with increased obesity and poor physical activity. Bone and joint degenerative and inflammatory problems, bone fractures, low back pain, osteoporosis, scoliosis ant other musculoskeletal problems need to be solved by using permanent, temporary or biodegradable devices [28, 30].

Bone structure, role and properties

Bone is a highly complex and specialized form of connective tissue which is composed of an organic matrix strengthened by deposits of calcium phosphate crystals. The organic matrix is composed of collagen type I fibers (approximately 95%) and of proteoglicans and numerous non-collagenous proteins (5%). This organic matrix, calcified

by calcium phosphate minerals embeds bone cells (**Table 1**), which participate in the maintenance and organization of bone, namely osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts [1, 4]. The cellular origin of bone was recognised in the early 19th century, and the term "osteoblast" was first used by Gegenbaur in 1864 to refer to the "granular corpuscles found in all developing bone as the active agents of osseous growth" [16].

Table 1. The cellular components of bone

Cell type	Origin	Function and phenotype
OSTEOBLASTS	Mesenchymal stem cells	Can have one of four different fates: 1) become embedded in the bone as **osteocytes**, (2) transform into inactive osteoblasts and become **bone-lining cells** – found along the bone surfaces that are undergoing neither bone formation nor resorption, inactive cells that are believed to be precursors osteoblasts, (3) undergo programmed cell death (apoptosis), or in some situations (4) transdifferentiate into cells that deposit chondroid or chondroid bone. **Active osteoblasts** are mononuclear cells with cuboidal shape; rich in alkaline phosphatase; synthesize and secrete collagen type I and glycoproteins (osteopontin, steocalcin), cytokines, and growth factors into a region of unmineralized matrix (osteoid) between the cell body and the mineralized matrix; produce calcium phosphate minerals extra- and intracellularly within vesicles. **Inactive osteoblasts** are elongated cells, undistinguishable morphologically from the bone-lining cells
OSTEOCYTES	Osteoblasts	An important role of osteocytes and their network of cell processes is to function as strain and stress sensors, signals that are very important for maintaining bone structure
OSTEOCLASTS	Hematopoietic stem cells	Polynuclear cells responsible for bone resorption (by acidification of bone mineral leading to its dissolution and by enzymatic degradation of demineralized extracellular bone matrix; important for growth and development
CHONDROCYTES	Mesenchymal stem cells	Cells found in cartilage that produce and maintain the cartilaginous matrix

According to [4, 14, 27, 43].

Despite its hard structure, bone actually exists in a constant state of dynamic turnover known as bone remodeling even once growth and modeling of the skeleton have been completed [21].

Bone defects

Bone defects often result from tumor resection, congenital malformation (such as osteogenbesis imperfects, osteopetrosis), trauma, fractures, surgery, or periodontitis in dentistry, as well as from diseases, such as osteoporosis or arthritis [15, 24, 39].

Osteogenesis imperfecta or brittle bone disease is the most common of the inherited disorders primarily affecting bone. It occurs in 1 in 10 000 to 20 000 live births and is associated with mutations in type I collagen genes (*COL1A1* and *COL1A2*) in ~ 90% of the patients [37, 40, 41].

Osteopetrosis is a rare genetic condition (with both autosomal recessive and autosomal dominant forms) characterized by an increase of bone mass due to defective osteoclast formation and function and spontaneous fractures [8, 42].

Osteoporosis, a major public health burden that affects millions of people (especially women) around the world, is defined as "a skeletal disorder characterized by risk of fractures of the hip, spine, and other skeletal sites." It is a systemic skeletal disease characterized by low bone mineral density and micro-architectural deterioration of bone tissue, leading to bone fragility and increased in risk of fracture [11, 31].

Bone health may be impaired in many patients being treated for cancer. Primary tumors that reside in (osteosarcoma and Ewing's family tumours) or form metastases (such as breast, prostate, lung cancers, myeloma) to bone can result in compromised skeletal integrity [17, 44]. Several medical conditions and medications significantly increase the risk for bone loss and skeletal fragility. These include androgen-deprivation therapy for prostate cancer and aromatase inhibitor therapy for breast cancer, among others. Hypogonadism induced by many of these cancer treatments results in bone loss and increases the risk of osteoporosis and fractures. Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis [7, 32].

Fracture healing is a complex, unique physiological process of repair, where, unlike in other tissues, the majority of bone injuries recover without the formation of scar tissue, and bone is regenerated with its pre-existing properties largely restored [13]. However, there are cases of fracture healing in which bone regeneration is impaired due to various factors leading to pathologies such as delayed union or fracture non-union [3]. The unsuccessful bone regeneration still results in many people never recovering fully their function and quality of life; with all the social, financial and psychological implications [38]. For example there are data demonstrating that old people suffering from femoral fractures will die within a year (15-25%) or become dependent (50%) [11, 31].

The techniques used to repair damaged bones

When an area of damaged bone is too large for self-repair, the damaged bones must be repaired by using alternative materials, such as autografts, allografts and artificial materials.

Autografts, which are transferred from healthy parts of the bones of the same patient, are widely used because they show high performance. Autologous bone remains the "gold standard" for stimulating bone repair and regeneration, but its availability may be limited and the procedure to harvest the material is associated with complications (e.g. additional surgical trauma). On the other hand, allografts, which are transferred from other people, have problems related to not only limited availability but also with foreign body immune reactions and infections (for example risk of HCV or HIV transmission to the recipient). In addition, they have a poor degree of cellularity, less revascularisation, and a higher resorption rate compared to autologous grafts, resulting in a slower rate of new bone tissue formation [26, 28, 30].

As a result there is a need for the development of artificial bone substitute materials with improved characteristics that are safe for patients, (relatively) easily produced and can be supplied at any time and in any amount.

Biomaterials for bone implants

A variety of materials with different structure, composition and mechanism of action are available or under development to enhance the repair of bone defects (Some of them are presented in **Table 2**).

Table 2. Brief description of some biomaterials for bone implants

BIOMATERIAL	SHORT CHARACTERISTIC	
	ADVANTAGES	**DISADVANTAGES**
CERAMICS Based mainly on hydroxyapatite, since this is the inorganic compound of bone	Able to form bone apatite-like material or carbonate hydroxyapatite on their surface, enhancing their osseointegration; Hardness, high compression strength and excellent wettability that result in low incidence of biologically significant particle generation and clinically significant osteolysis. Able to bind and concentrate cytokines, as in the case of natural bone. Used as a bearing surface in total hip arthroplasty (THA) for more than 30 years	Brittleness and slow degradation rates; low fracture toughness and linear elastic behavior which make them prone to breakage under stress. There are data that the addition of carbon nanotubes remarkably improves the mechanical characteristics of alumina.
BIOACTIVE GLASS based on a random network of silica tetrahedra containing Si–O–Si bonds. The network can be modified by the addition of modifiers such as Ca, Na and P.	The first man-made material to bond to living tissues; biocompatible and osteoconductive (especially glasses with SiO_2 content < 60% in weight), bond to bone without an intervening fibrous connective tissue; in vivo, there is a dynamic balance between intramedullary bone formation and bioactive glass resorption.	Low mechanical strength and decreased fracture resistance that can be easily overcome by modifying the composition and application in low load-bearing areas.
METALS Mainly stainless steel and titanium alloys (i.e. Ti-6Al-4V)	Excellent mechanical properties, which makes them the most widely applied implant material used in bone surgical repairs	The lack of tissue adherence and the low rate of degradation results either in a second surgery to remove the implant or in permanent implantation in the body with the related risks of toxicity due to accumulation of metal ions due to corrosion
NATURAL POLYMETS Collagen and glycosaminoglycans	Biocompatibility and biodegradability, since they compose the structural materials of tissues.	Low mechanical strength and high rates of degradation (they are used in composites or in chemical modification by cross-linking. These changes make cause cytotoxic effects and reduce compatibility).
SYNTHETIC POLYMERS	The versatility of chemically synthesized polymers enables the fabrication of scaffolds with different features (forms, porosities and pore size, rates of degradation, mechanical properties) to match tissue specific applications	
COMPOSITES	Can combine a synthetic scaffold with biologic elements to stimulate cell infiltration and new bone formation. Each individual material has advantages for osteogenic applications, each also has drawbacks associated in certain properties (i.e. brittleness of ceramics) that can be overcome by combining different materials.	

According to [2, 12, 18, 30, 34, 46].

Historically, these materials belong to the following three generations:

First generation – Bioinert materials. They are represented by alumina (Al_2O_3) and zirconia (ZrO_2) and played an important role for substitution purposes due to their low reactivity. This is not surprising because at the very beginning the main goal of investigators was to achieve substitution with the lowest tissue response, perhaps because the only expected tissue response was inflammation and material rejection [25,45];

Second generation – Bioactive and biodegradable materials. To this group belong bioactive glasses – synthetic silica-based materials with bone bonding properties due to the formation of a carbonate substituted hydroxycarbonate apatite layer (similar to the apatite layer in bone) on the surface of the materials after immersion in body fluid [18, 19, 25];

Third generation – Materials designed to stimulate specific cellular responses at the molecular level [19, 33]. They appeared at the same time as scaffolds for tissue engineering applications started to be developed.

Bone tissue engineering

Tissue engineering approaches have recently been devised to repair large bone losses. Three main players take part in this technology: i) stem cells (for example mesenchymal stem cells) that are having the potential to form the organ of interest; ii) scaffold (where the stem cells will be transferred into) – a three-dimensional porous structures that serves as a template for cell interactions and the formation of bone-extracellular matrix to provide structural support to the newly formed tissue; iii) signals stimulating proliferation and appropriate differentiation of the stem cells [1, 25]. Scafold materials must meet a number of requirements including biocompatibility, adequate mechanical properties, biodegradability, etc. [25, 47]. Among the most important properties that they should possess are osteoinduction (the ability to stimulate the differentiation of osteoprogenitor cells into mature bone cells and then the formation of new bone), osteoconduction (the physical property of the graft to serve as a scaffold for viable bone healing - osteoblasts from the margin of defect that is being grafted, utilize the bone graft material as a framework upon which to spread and generate new bone), and osteogenesis (the ability of the graft to produce new bone, this process is dependent on the presence of vital bone cells in the graft) [29].

Mesenchymal stem cells

Adult mesenchymal stem cells (MSCs) are non differentiated multipotent cells with self-renewal capacity that have the potential to differentiate towards lineages of mesenchymal origin, including bone and cartilage. Although originally isolated from bone marrow, MSCs have since been obtained from many other tissues such as adipose tissue, synovial fluid, periosteum, umbilical cord blood and several fetal tissues [5, 9, 22, 23, 48].

MSCs have several advantages that are of interest for bone tissue engineering:

• These cells can be relatively easily obtained from different sources including fat tissue, bone marrow, cord blood;

• MSCs can be extracted from patient's own tissue than can prevent the development of immune rejection;

• In comparison to embryonic stem cells there are no ethical concerns for their application;

• MSCs do not develop into teratomas when transplanted, a consequence observed with embryonic stem cells and induced pluripotent stem cells [20].

There are some difficulties in working with these cells which should also be mentioned:

• Lack of commonly accepted surface markers, which can be used for the identification of MSCs. A set of minimal criteria for MSC was recommended, which includes the capability of adherence to plastic surfaces, the expression of CD73, CD90 and CD105 and the absence of major histocompatibility complex (MHC) class II surface molecules (HLA-DR), endothelial (CD31) and hematopoietic-specific antigens (CD34, CD45, CD14) [10, 23, 35]. In culture their cell surface antigens may vary depending on the isolation and expansion methods used [23].

• The small percentage of MSCs in some tissues such as bone marrow – the amount of BM-MSCs varies between 0.001% and 0.01% of the total mononuclear cell. Such low frequency of BM-MSC requires prolonged cultivation of the cells in laboratory conditions, thus increasing the risk of differentiation induction and epigenetic alterations [20]. In addition, age-related changes in number, proliferation capacity and differentiation potential of MSCs have been reported [6].

• The protocols for isolation and cultivation of MSCs are complicated and not suitable for routine clinical practice [20]. Human MSCs are sensitive to serum and oxygen deprivation, which resulted in cell death in vitro when applied in combination for 48 hours. This fact must be taken into consideration when working with them [36];

• The biological activity of these cells is not fully clarified;

• And finally – MSCs can be obtained from various tissues but there is still no definite answer to the question what is the best source for their isolation.

Conclusion

Tissue engineering is one of the most rapidly developing and promising areas of science and medicine. The first successful steps in this exciting direction have already been made. Before its entry into routine clinical practice, it is necessary to overcome a number of challenges including establishment of scaffolds with improved properties that meet the requirements for these type biomaterials, identification of the most appropriate stem cells, optimizing strategies for their isolation, cultivation and directed differentiation.

Acknowledgements: This work was supported by the a COST Action MP1301 and by Grant No T 02/5 from 2014, National Science Fund at the Bulgarian Ministry of Education and Science.

References

1. **Amini, A. R., C. T. Laurencin, S. P. Nukavarapu.** Bone tissue engineering: recent advances and challenges. – Crit. Rev. Biomed. Eng., **40**(5), 2012, 363-408.
2. **Arcos, D., I. Izquierdo-Barba, M. Vallet-Regi.** Promising trends of bioceramics in biomaterials field. – J. Mater. Sci: Mater. Med., **20**, 2009, 447-455.
3. **Audigé, L., D. Griffin, M. Bhandari, J. Kellam, T. P. Rüedi.** Path analysis of factors for delayed healing and nonunion in 416 operatively treated tibial shaft fractures. – Clin. Orthop. Relat. Res., **438**, 2015, 221-232.
4. **Barrere, F., C. A. van Blitterswijk, K. de Groot.** Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. – Int. J. Nanomedicine, **3**, 2006, 317-332.
5. **Bieback, K., S. Kern, H. Kluter, H. Eichler.** Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. – Stem Cells, **22**, 2004, 625-634.
6. **Bellantuono, I., A. Aldahmash, M. Kassem.** Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss. – Biochim. Biophys. Acta., **179**(2), 2009, 364-370.
7. **Cannata, D. H., A. Kirschenbaum, A. C. Levine.** Androgen deprivation therapy as primary treatment for prostate cancer. – J. Clin. Endocrinol. Metab., **97**(2), 2012, 360-365.

8. **Coudert, A. E., M. C. de Vernejoul, M. Muraca, A. Del Fattore.** Osteopetrosis and its relevance for the discovery of new functions associated with the skeleton. – J. Int. Endocrinol., 2015, ID 372156. doi: 10.1155/2015/372156.
9. **De Bari, C., F. Dell' Accio, F. P. Luyten.** Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. – Arthritis Rheum., **44**, 2001, 85-95.
10. **Dominici, M., K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, et al.** Minimal criteria for defining multipotent mesenchymal stromal cells. The In ternational Society for Cellular Therapy position statement. – Cytotherapy, **8**, 2006, 315-317.
11. **D' Amelio, P., G. C. Isaia.** Male Osteoporosis in the Elderly. – Int. J. Endocrinol., 2015, ID 907689. doi: 10.1155/2015/907689.
12. **El-Ghannam, A.** Bone reconstruction: from biocermamics to tissue engineering. – Expert. Rev. Med. Devices, **2**, 2005, 87-101.
13. **Einhorn, T. A.** The cell and molecular biology of fracture healing. – Clin. Orthop. Relat. Res., **355**, 1998, S7-S21.
14. **Franz-Odendaal, T. A., B. K. Hall, P. E. Witten.** Buried alive: How osteoblasts become osteocytes. – Developmental Dynamics, **235**(1), 2006, 176-190.
15. **Forlino, A., W. A. Cabral, A. M. Barnes, J. C. Marini.** New perspectives on osteogenesis imperfecta. – Nat. Rev. Endocrinol., 7(9), 2011, 540-557.
16. **Gegenbauer, C.** Untersuchungen zur vergleichenden Anatomie der Wirbeltiere. – Heft 1. Carpus und Tarsus. Leipzig*:* Schultergtel, 1864.
17. **Gaspar, N., D. S. Hawkins, U. Dirksen, I. J. Lewis, S. Ferrari , M. C. Le Deley, H. Kovar, R. Grimer, J. Whelan, L. Claude, O. Delattre, M. Paulussen, P. Picci, K. Sundby Hall, H. van den Berg, R. Ladenstein, J. Michon, L. Hjorth, I. Judson, R. Luksch, M. L. Bernstein, P. Marec – Bérard, B. Brennan, A. W. Craft, R. B. Womer, H. Juergens, O. Oberlin.** Ewing Sarcoma: Current Management and Future Approaches Through Collaboration. – J. Clin. Oncol., **33**(27), 2015, 3036-3046.
18. **Hench, L. L.** The story of Bioglass®. – J. Mater. Sci: Mater. Med., **17**, 2006, 967-978.
19. **Hench, L. L., J. M. Polak.** Third-generation biomedical materials. – Science, **295**(5557), 2002, 1014-7.
20. **Hilfiker, A., C. Kasper, R. Hass, A. Haverich.** Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation? – Langenbecks Arch. Surg., **396**(4), 2011, 489-97.
21. **Hill, P. A.** Bone remodelling. – J. Br. Orthod., **25**(2), 1998, 101-107.
22. **In't Anker, P. S., S. A. Scherjon, K. C. Kleijburg – van der Keur C, W. A. Noort, F. H. Claas, R. Willemze et al.** Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. – Blood, **102**, 2003, 1548-1549.
23. **Jacobs, S. A., V. D. Roobrouck, C. M. Verfaillie, S. W. Van Gool.** Immunological characteristics of human mesenchymal stem cells and multipotent adult progenitor cells. – Immunol Cell Biol., **91**(1), 2013, 32–39.
24. **Jimi, E., S. Hirata, K. Osawa, M. Terashita, C. Kitamura, H. Fukushima.** The current and future therapies of bone regeneration to repair bone defects. – Int. J. Dent., 2012, ID 148261.
25. **Jones, J. R., P. D. Lee, L. L. Hench.** Hierarchical porous materials for tissue engineering. – Philos. Trans A Math. Phys. Eng. Sci., **364**(1838), 2006, 263-81.
26. **Kalfas, I. H.** Principles of bone healing. – Neurosurg. Focus, **10**(4), 2001, 1-10.
27. **Karageorgiou, V., D. Kaplan.** Porosity of 3D biomaterial scaffolds and osteogenesis. – Biomaterials, **26**, 2005, 5474-5491.
28. **Kokubo, T., H. Kim, M. Kawashita.** Novel bioactive materials with different mechanical properties. – Biomaterials, **24**(13), 2003, 2161-75.
29. **Kumar, P., B. Vinitha, G. Fathima.** Bone grafts in dentistry. – J. Pharm. Bioallied. Sci., **5** (Suppl. 1), 2013, S125-S127.
30. **Le Guéhennec, L., P. Layrolle, G. Daculsi.** A review of bioceramics and fibrin sealant. – Eur. Cell Mater., **8**, 2004, 1-10.
31. **Lane, N., S. Morris.** New perspectives on parathyroid hormone therapy. – Curr. Opin. Rheumatol., **17**(4), 2005, 467-474.

32. **Mirza, F., E. Canalis.** Management of endocrine disease: Secondary osteoporosis: pathophysiology and management. – Eur. J. Endocrinol., **173**(3), 2015, R131-R151.
33. **Navarro, M., A. Michiardi, O. Castaño, J.A. Planell.** Biomaterials in orthopaedics. – J. R. Soc. Interface, **5**(27), 2008, 1137-58.
34. **Ogihara, N., Y. Usui, K. Aoki, M. Shimizu, N. Narita, K. Hara, K. Nakamura, N. Ishigaki, S. Takanashi, M. Okamoto, H. Kato, H. Haniu, N. Ogiwara, N. Nakayama, S. Taruta, N. Saito.** Biocompatibility and bone tissue compatibility of alumina ceramics reinforced with carbon nanotubes. – Nanomedicine (Lond), **7**(7), 2012, 981-993.
35. **Pittenger, M. F., A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, et al.** Multilineage potential of adult human mesenchymal stem cells. – Science, **284**, 1999, 143-147.
36. **Potier, E., E. Ferreira, A. Meunier, L. Sedel, D. Logeart-Avramoglou, H. Petite.** Prolonged hypoxia concomitant with serum deprivation induces massive human mesenchymal stem cell death. – Tissue Eng., **13**(6), 2007, 1325-31.
37. **Primorac, D., D. Anticević, I. Barisić, D. Hudetz, A. Ivković.** Osteogenesis imperfecta – multisystemic and life-long disease that affects whole family. – Coll Antropol., **38**(2), 2014, 767-72.
38. **Shekkeris, A. S., P. K. Jaiswal, W. S. Khan.** Clinical applications of mesenchymal stem cells in the treatment of fracture non-union and bone defects. – Curr Stem Cell Res Ther., **7**(2), 2012, 127-33.
39. **Scaramuzzo, L., L. Messuti, P. F. Manicone, L. Raffaelli, B. Rossi, P. Gallenzi, D. Berardi, G. Maccauro.** Clinical and histological modifications in osteopetrotic bone: a review. – J. Biol. Regul. Homeost. Agents, **23**(2), 2009, 59-63.
40. **Scaramuzzo, L., L. Raffaelli, M. S. Spinelli, G. Damis, G. Maccauro, P. F. Manicone.** Orthopaedic and dental abnormalities in osteogenesis imperfecta: a review of the literature. – J. Biol. Regul. Homeost Agents., **25**(3), 2011, 313-321.
41. **Shaker, J. L, C. Albert, J. Fritz, G. Harris.** Recent developments in osteogenesis imperfecta. – Faculty Rev., **681** 2015,. doi: 10.12688/f1000research.6398.1. eCollection.
42. **Sobacchi, C., A. Schulz, F. P. Coxon, A. Villa, M. H. Helfrich.** Osteopetrosis: genetics, treatment and new insights into osteoclast function. – Nat. Rev. Endocrinol., **9**(9), 2013, 522-536.
43. **Tuan, R. S., G. Boland, R. Tuli.** Adult mesenchymal stem cells and cell-based tissue engineering. – Arthritis Res. Ther, **5**(1), 2003, 32-45.
44. **Terpos, E., C. B. Confavreux, P. Clézardin.** Bone antiresorptive agents in the treatment of bone metastases associated with solid tumours or multiple myeloma. – Bonekey Rep., **4**(744), 2015, doi: 10.1038/bonekey.2015.113.
45. **Thamaraiselvi, T. V., S. Rajeswari. Biological Evaluation of Bioceramic Materials – A Review.** – Trends Biomater. Artif. Organs, **18**, 2004, 9-17.
46. **Välimäki, V. V., H. T. Aro.** Molecular basis for action of bioactive glasses as bone graft substitute. – Scand. J. Surg., **95**(2), 2006, 95-102.
47. **Williams, D. F.** On the mechanisms of biocompatibility. – Biomaterials, 29, 2008, 2941-2953.
48. **Zuk, P. A., M. Zhu, P. Ashjian, D. A. De Ugarte, J. I. Huang, H. Mizuno, et al.** Human adipose tissue is a source of multipotent stem cells. – Mol. Biol. Cell, **13**, 2002, 4279-4295.