Applications of Mesenchymal stem cells in bone regeneration

SHANG Linjing

1. Abstract

This review summarized the application of mesenchymal stem cells in the treatment of bone regeneration such as bone or cartilage defects, osteoarthritis and other diseases. According to the context below, researchers used multiple approaches to culture mesenchymal stem cells from different sources. Some of them have achieved good clinical efficacy, meanwhile the rest are still in the research stage. For these studies, the clinical trials and long-term efficacy verification need further research. In this paper, some pros and cons are also be demonstrated and finally considering all the references mentioned, opinions of future promotion would be came up with as well.

Key words: Mesenchymal stem cells (MSCs); Clinical application; Bone regeneration

2. Introduction

Mesenchymal stem cells are a type of pluripotent adult stem cells originating from mesoderm cells, which can be differentiated into osteoblasts, adipocytes, and

chondrocytes etc under different conditions. When this kind of cells were originally extracted from human bone marrow, some authors tended to define as murine mesenchymal stem cells used rat model to figure out the therapy protocols cellular and genetic for the human mesenchymal stem cells (Meirelles et al.,2003). Meanwhile, because of its ability to differentiate, researchers consider these cells are capable of treating many diseases.

Bone regeneration, one of this diseases, especially large area bone defect, was a most difficult surgery for clinical doctors. Compared to amputation or more complicated Ilizarov method (Ilizarov G.A., 1989), using mesenchymal stem cells has great advantages and mostly doctors inject the solution containing mesenchymal stem cells or its differentiated cells to treat the bone regeneration which is relatively simple and effective (Bianco, P et al.,2013).

Since mesenchymal stem cells were originally isolated from bone marrow of patients, although there is no immune rejection, a limitation still has to take into consideration especially when it comes to young patients who have rare bone marrow or loss due to trauma. Fortunately, it was identified that other tissues, such as adipose tissue,skeletal muscle and tendon etc.,are also the potential sources of mesenchymal stem cells (Phinney, D.G, 2012), which means there are multiple sources to obtain mesenchymal stem cells so that it is possible to apply this kind of cells to clinical practice of bone regeneration.

According to the references collected, mesenchymal stem cells are mainly used in the aspects of bone defect,Cartilage defect, Spine and intervertebral disc, Avascular

necrosis of femoral head, Arthritis (RA rheumatoid arthritis, OA osteoarthritis). The specific cases would be illustrated in the next part below.

3. Application of bone regeneration

3.1 Bone defect

The figure 1 showed that a little girl who suffered severe head injury resulting in a closed multifragment skull fracture after an accidental fall. To be notable that, it is very difficult for repairing bone defect, especially in children, let alone that was skull defect. For she was a seven-year-old girl, it was hard to obtain mesenchymal stem cells directly from bone marrow. According some authors reported that other tissues (eg. Adipose tissue) are alternative sources of mesenchymal stem cells (Zuk et al.,2002), which is also called Adipose-Derived mesenchymal Stem Cells(ADMSCs).

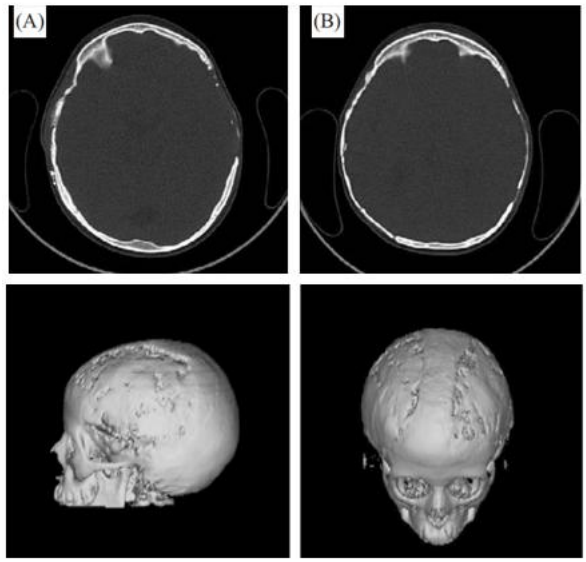

Figure 1. (A)skull bone defects preoperatively
(B) three months postoperatively

Since the human body is rich in adipose tissue, ADMSCs is one of the popular method to obtain mesenchymal stem cells. References illustrated that these stem cells can grow and proliferation stably in vitro (Mizuno et al.,2002).

Therefore, in this case, doctors chose to acquire mesenchymal stems cells from her adipose tissue and finally a total of 42 grams adipose was excised from her left gluteal area (Lendeckel et al.,2004) .

As for how to deal with these adipose tissues, according to Zuk's article, they were cut into small pieces and transfer to sterile blood bag (Zuk et al., 2001). Subsequently, after waiting two hours, doctors washed them with phosphate-bufferedsaline and 0.075% LiberaseTM to obtain mesenchymal stem cells.

At the progress of surgery, approximately 15 milliliters cancellous bone was extracted from the iliac bone and ground evenly to repair the defects. Due to the limited quantity of cancellous bone, adipose derived mesenchymal stem cells were also be used. To enhance the regeneration process, the preformed protective membrane was fixed with absorbable tacks as the scaffold for the injected solution of stem cells from adipose tissue.

In conclusion, the whole treatment repaired approximately 120cm^2 skull defect, apparently compared with the two CT images before and after, the effect was very significant. At that moment, fibrin glue was also used during surgery as a double insurance for fixing stem cells in the right place to proliferate and regenerate.

In addition, mesenchymal stem cells are used to reconstruct craniofacial bone defect as well. According Mesimäki reported, their team operated for a patient and

during a period of time they operated a total of three operation for this patient (Mesimäki et al.,2009).It was undeniable that they repaired his craniofacial bone defect which means this method also can be used in oral and maxillofacial surgery.

At the first surgery, about 60 milliliters serum was obtained and subcutaneous abdominal fat was extracted as well from patient who was a 65 year old male. The former was harvested stem cells subsequently, the later also was isolated in vitro.

Due to the complex structure of the oral cavity, at this bone regeneration, the bone regeneration not only requires to reconstruct bone tissue, but also cartilage tissue even vascular system so that doctors were supposed to ensure vascularization of new bone tissue. Therefore, adipogenic and chondrogenic differentiation cultures were also be used to extracted mesenchymal stem cells and fixed with a 4% PFA priorto staining procedures. After nine months of three operations and 12 months of follow-up, the patient has regenerated the bone tissue and vascular system.

3.2 Cartilage defect

As mentioned above, mesenchymal stem cells have been proved that are capable of differentiating hypertrophic chondrocytes, which requires FGF-2 to expand into cartilage in vitro (Solchaga et al.,2005).Different articles also have shown that the use of serum-free medium(Auletta et al.,2011), passage from three to six generation (Felka et al.,2010), three-dimensional culture (Cournil-Henrionnet et al.,2008), hypoxia incubator (Gardner et al.,2013) and mechanical stimulation (Adesida et al.,2012) can enhance the chondrogenic differentiation potential of mesenchymal

stem cells.

Admittedly, cartilage is a kind of connective tissue with supporting function, and it mostly exists between joints. There was a case using mesenchymal stems cell to cure the Congenital pseudarthrosis of tibia (CPT) which also belonged to a little patient who was suffered from the Congenital pseudarthrosis and Neurofibromatosis type I. Both of them are the most common congenital diseases and they tend to occur in patients simultaneously.

Figure 2. (a) before the surgery
(b) after 3 months

Figure 2 is shown the tibial of little patient, except the mesenchymal stem cells, different studies have proved their approaches to treat Congenital Pseudarthrosis. A complete removal of the diseased periosteum, combined with the transplantation of bone or free periosteum and tibia and fibula intramedullary rod was utilized (Thabet et al.,2008). In 2012, 11 cases of CPT were reported to treat with intramedullary rod combined with cortical bone transplantation, of which 9 cases were healed(Shah et al.,2012). There were also cases that was treated successfully with Ilizarov external fixator and antegrade intramedullary

rod (Agashe et al.,2012). Gouron et al. Reported a masquelet technique for bone defect reconstruction (Gouron et al.,2011).

As for mesenchymal stem cells, some authors suggested that its transplantation may be a promising treatment for CPT as well. The source for MSCs were from iliac crest bone marrow which was also called IC-MSCs (Granchi et al.,2010). The solution were injected into the bone marrow cavity of tibia and around the pseudarthrosis of tibia. After following up for 10 months, bone formation was observed, the results showed that the number of osteoclasts in cortical bone was twice as much as that before, in addition, the bone mineral content was improved after transplantation(Tikkanen et al.,2010). In 2012, Research team of Granchi improved their method and combined IC-MSCs with platelet rich fibrin(PRF) and freeze-dried bone(Granchi et al.,2012). Applying this combined treatment method, this patient in figure 2 with tibial pseudarthrosis healed just three months(Giuliani et al.,2013).

However, further studies are required to confirm that mesenchymal stem cells transplantation is more effective than other surgical treatment methods.

3.3 Spine and intervertebral disc

Undeniable that, because of long-period sit, most people have very weak back muscles, which leads to low back pain, that is because spine and intervertebral disc are damaged during the daily life.

As the matter of fact, Damage Intervertebral disc degeneration (IVDD) and Herniation are the major causes of low back pain(Luoma et al.,2000), which may need an operation and cause intervertebral disc defect after that. The differentiation of mesenchymal stem cells on the hydrogel would repair defect. According authors' study, Annulus fibrosus (AF) defect is a well known cause for the initiation and progression of Damage Intervertebral disc degeneration(Buser et al.,2019).

The traditional treatment is discectomy, which may lead to disc herniation again(Battie et al.,2014). At the same time, discectomy often requires vertebral fusion to avoid spinal instability, which eventually leads to reduced spinal mobility (Fjeld et al.,2019).

In author's study, decellular matrix was used to remove cellular components from tissues and retain extracellular matrix(Bejleri et al.,2019) which supports the structural and biological functions necessary for cell adhesion, proliferation and differentiation (Xu et al.,2014).

Therefore in this research authors chose mesenchymal stem cells to differentiate AF cells and divided it into 4 groups (Peng et al.,2020). Meanwhile, considering the quantity of extraction of extracellular marix, to enhance the effect, they combined

different groups with nanofibers respectively, which is similar to the structure of extracellular matrix so that it can imitate the structure and biological function of ECM.

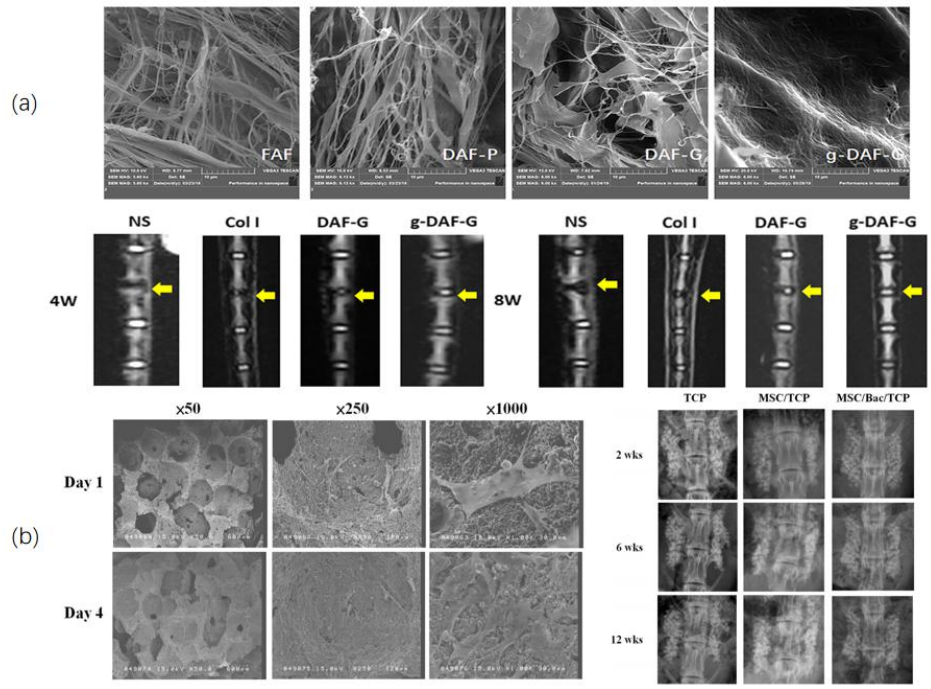

Figure 3. Spine and intervertebral disc

(a) Case of intervertebral disc in 4 groups

(b) Case of spine in 3 groups

Figure 3(a) shows that the structure of AF cells in scanning electron microscopy, they compared Fresh AF cells (FAF), decellularized AF powder (DAF-P), decellularized AF Hydrogels (DAF-G) and genipin-crosslinked decellularized AF Hydrogels (g-DAF-G). It was notable that, genipin was extracted from the natural compound geniposide with low toxicity and high biocompatibility. Therefore, it was chosen as a crosslinking agent for the AF-derived hydrogel, which was an unique combination of this paper.

As figure shows that, after eight weeks, AF cells,which were differentiated by mesenchymal stem cells, in rat model have finish the bone regeneration of

intervertebral disc, and combined with genipin would have a better result. It was proved that g-DAF-G could mimic the mechanical property of native AF tissues better than DAF-only hydrogel (DAF-G).

Another typical application is Spinal fusion which is used to treat patients who suffer from disc herniation or spinal canal stenosis without functional disorders caused by spondylolisthesis. After sugery, original spinal motion segment can no longer move and grow into a whole part. It is usually used when the stability of spine is damaged or orthopedic is required, and internal fixation is needed. However, spinal fusion has many complications, and secondary surgery tend to be high cost (Younger et al.,1989).

Considering these pros and cons, many authors suggested mesenchymal stem cell therapy could be used for spinal fusion. In figure 3(b), single level posterolateral spinal fusion was used to treat New Zealand rabbits model to assess the therapy for human. In order to verify the effect of three-dimensional tricalcium phosphate (TCP) scaffold and transgenic MSCs combined with baculovirus mediated growth factor expression on the success rate of spinal fusion, three groups of comparative experiments were set up(Fu et al.,2015).

Some authors have confirmed that mesenchymal stem cells were cultured with dexamethasone, ascorbic acid and b-glycerophosphate and differentiated into osteoblasts(Bruder et al.,1998 & Solchaga et al.,1999). Meanwhile, it was identified that recombinant human bone morphogenetic protein-2 (rhBMP-2) improves the osteogenic potential and the success rate of bone regeneration (Hanada et al.,1997,

Noshi et al.,2000, Noshi et al.,2001). Therefore in the paper, BMP-2 synthesis of genes were modified and combined with mesenchymal stem cells to express protein for promote bone formation.

Ho et al.have illustrated that mesenchymal stem cells can be transduced by baculovirus (Bac) with up to 76% efficiency, which is a DNA virus that widely utilized for recombinant protein production(Ho et al.,2006).

According studies above, Fu et al divided the experiment into 3 groups: TCP/ TCP+MSCs/ Tcp+MSCs+Bac and prepared TCP mesoporous scaffold for loading mesenchymal stem cells to delivery. Using SEM to observe the structure in figure 3(b), TCP scaffold have built successfully and stem cells have seeded onto the scaffold. And then, rabbits were operated and implanted three different scaffolds individually. Comparing the rate of spine fusion, TCP+MSCs+Bac ranked first and followed by TCP+MSCs and TCP respectively so that they can conclude that the former one harvested the most significant result among three groups with the significance level (p value) was less than 0.001.

3.4 Osteoarthritis and rheumatoid arthritis

Osteoarthritis is a kind of cartilage disease, which may lead to the loss of articular cartilage(Buckwalter et al., 2005).Some studies have pointed out that mesenchymal stem cells can not only differentiate into a variety of cells, but also have anti apoptotic effect and indirectly regulate inflammatory response(Kalaszczynskaet al.,2015). In clinical treatment, it can directly replace the

damaged cells through its paracrine effect. Therefore, mesenchymal stem cells transplanted into the joints of patients with osteoarthritis can produce paracrine reaction and induce it to repair inflammatory tissue.

Similarly to the cartilage defect mentioned above, patients with OA may suffer from bone defect as well. Studies proved that mesenchymal stem cells have great osteogenic potential and play an important role in tissue regeneration and articular cartilage maintenance(Pak et al.,2014). Therefore, authors tend to use two types of mesenchymal stem cells, one was derived from adipose tissue(ADMSCs), another was derived from Wharton's jelly(WJMSCs) to combine with Extracorporeal shockwave therapy (ESWT) to treat Osteoarthritis, examine whether these two types cells increased the efficacy of ESWT and compare the efficacy of them (Hsu et al.,2020).

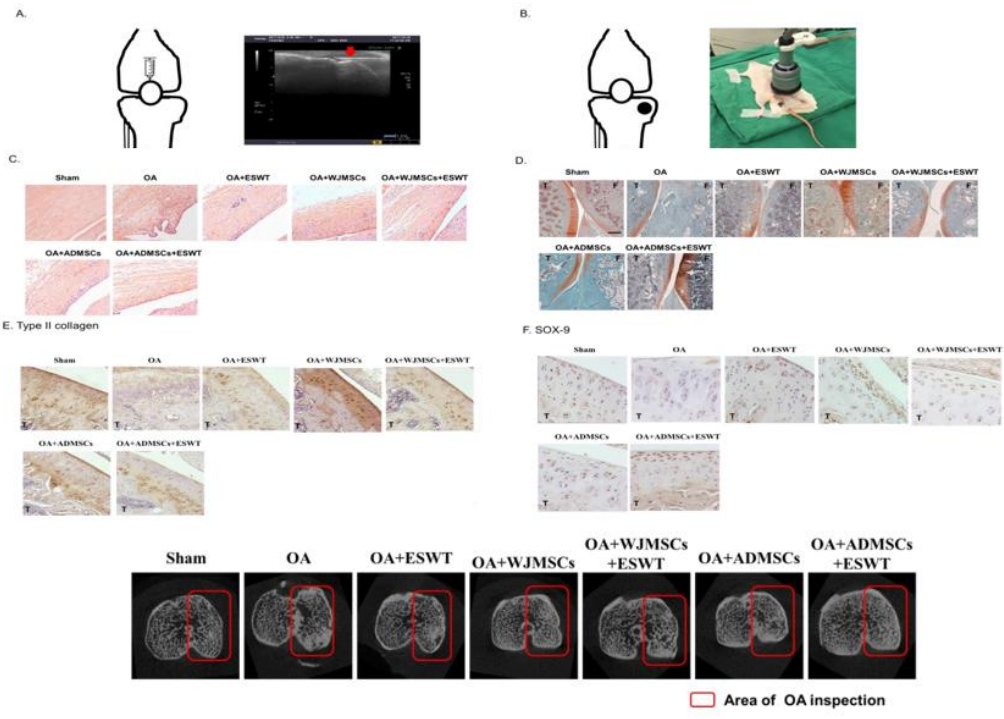

Figure 4. OA for rat model

Figure 4 A shows the location of injection of solution in the rats. During the period of injection, shockwave was used simultaneously. Researchers made different combination and compared them, the OA+ means the rats had disease. It was proved that mesenchymal stem cells really reduce the OA, and got a relatively considerable result.

Similarly, Rheumatoid arthritis (RA) is also a chronic autoimmune inflammatory joint disease which may lead to both bone and cartilage defect. Collagen-induced arthritis (CIA), which occurs in mice, are similar to human RA(Marinova-Mutafchieva et al.,1997). Studies also demonstrated that MSC transplantation could treat mice CIA (Augello et al.,2007), which mesenchymal stem cells can also be used to treat Rheumatoid arthritis.

4. Future Promotion

In the case of the seven-year-old girl, it took approximately 2 hours to process the autologous adipose tissue, which was very time-consuming and complex, including reparation, expansion and reimplantation into the receptor, even when the urgent time came, there was no time to prepare for the surgery. Some studies have shown that MSCs may be immune privileged cells (Di Nicola et al.,2002). Therefore, allogeneic MSCs can be isolated from one or more donors, providing an unlimited source for easy expansion and cryopreservation.

Meanwhile, doctors chose to extract Cancellous bone and transplanted into skull

for the seven year old girl, but what about the bone is not available? Therefore, short of graft for large bone defect to fix the location of mesenchymal stem cells is another problem.

It is undeniable that MSCs transplantation is an interdisciplinary surgery, which means it needs both Surgeons and biologists. Surgeons for the success of surgery, biologists for the culture of stem cells. Therefore, it is difficult to coordinate these two parts in clinic. For this reason, it requires a professional team which means it difficult to popularize to basic primary hospitals. May be in the future, some packages will be available, just like blood bags, they can use these packages which have prepared in the storage to induce the patients' cells to stem cells quickly. The process is supposed to be easy, so that it can popularize to more hospitals.

Besides, the proliferation and differentiation ability of mesenchymal stem cells decreases with age increasing(Hasegawa et al.,2017), therefore, in vitro amplification and allogeneic transplantation should be developed in the future.

Meanwhile, a large number of animal models have been used successfully for the treatment in some studies, but there is missing link of clinical test and long-term follow-up pathology.

Although there are many problems to be taken into consideration, admittedly, mesenchymal stem cells really utilized in multiple application. In conclusion, the continuous progress of scientific development will provide broad application prospects for the development of mesenchymal stem cells.

5. References

[1] Meirelles, L., & Nardi, N. B. (2003). Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. British journal of haematology, 123(4), 702–711. https://doi.org/10.1046/j.1365-2141.2003.04669.x

[2] Ilizarov G. A. (1989). The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clinical orthopaedics and related research, (239), 263–285.

[3] Bianco, P., Cao, X., Frenette, P. S., Mao, J. J., Robey, P. G., Simmons, P. J., & Wang, C. Y. (2013). The meaning, the sense and the significance: Translating the science of mesenchymal stem cells into medicine. Nature Medicine, 19(1), 35-42. https://doi.org/10.1038/nm.3028

[4] Phinney, D.G. (2012), Functional heterogeneity of mesenchymal stem cells: Implications for cell therapy. J. Cell. Biochem., 113: 2806-2812. https://doi.org/10.1002/jcb.24166

[5] Zuk, P. A., Zhu, M., Ashjian, P., De Ugarte, D. A., Huang, J. I., Mizuno, H., Alfonso, Z. C., Fraser, J. K., Benhaim, P., & Hedrick, M. H. (2002). Human adipose tissue is a source of multipotent stem cells. Molecular biology of the cell, 13(12), 4279–4295. https://doi.org/10.1091/mbc.e02-02-0105

[6] Mizuno, H., Zuk, P. A., Zhu, M., Lorenz, H. P., Benhaim, P., & Hedrick, M. H. (2002). Myogenic differentiation by human processed lipoaspirate cells. Plastic and reconstructive surgery, 109(1), 199–211.
https://doi.org/10.1097/00006534-200201000-00030

[7] Lendeckel, S., Jödicke, A., Christophis, P., Heidinger, K., Wolff, J., Fraser, J. K., Hedrick, M. H., Berthold, L., & Howaldt, H. P. (2004). Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery, 32(6), 370–373. https://doi.org/10.1016/j.jcms.2004.06.002

[8] Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P.,

Lorenz, H. P., & Hedrick, M. H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue engineering, 7(2), 211–228. https://doi.org/10.1089/107632701300062859

[9] Mesimäki, K., Lindroos, B., Törnwall, J., Mauno, J., Lindqvist, C., Kontio, R., Miettinen, S., & Suuronen, R. (2009). Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. International journal of oral and maxillofacial surgery, 38(3), 201–209. https://doi.org/10.1016/j.ijom.2009.01.001

[10] Solchaga,L. A., Penick,K., Porter, J.D., Goldberg, V. M., Caplan, A.I., & Welter, J. F.(2005). FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. Journal of Cellular Physiology, 203(2),398–409.

[11] Auletta, J. J., Zale, E. A., Welter, J. F., & Solchaga, L. A. (2011). Fibroblast Growth Factor-2 Enhances Expansion of Human Bone Marrow-Derived Mesenchymal Stromal Cells without Diminishing Their Immunosuppressive Potential. Stem cells international, 2011, 235176. https://doi.org/10.4061/2011/235176

[12] Felka, T., Schäfer, R., De Zwart, P., & Aicher, W. K. (2010). Animal serum-free expansion and differentiation of human mesenchymal stromal cells. Cytotherapy, 12(2), 143–153. https://doi.org/10.3109/14653240903470647

[13] Cournil-Henrionnet, C., Huselstein, C., Wang, Y., Galois, L., Mainard, D., Decot, V., Netter, P., Stoltz, J. F., Muller, S., Gillet, P., & Watrin-Pinzano, A. (2008). Phenotypic analysis of cell surface markers and gene expression of human mesenchymal stem cells and chondrocytes during monolayer expansion. Biorheology, 45(3-4), 513–526.

[14] Gardner, O. F., Archer, C. W., Alini, M., & Stoddart, M. J. (2013). Chondrogenesis of mesenchymal stem cells for cartilage tissue engineering. Histology and histopathology, 28(1), 23–42. https://doi.org/10.14670/HH-28.23

[15] Adesida, A.B., Mulet-Sierra, A. & Jomha, N.M.(2012).Hypoxia mediated isolation and expansion enhances the chondrogenic capacity of bone marrow

mesenchymal stromal cells. Stem Cell Res Ther 3(2),9.https://doi.org/10.1186/scrt100

[16]Thabet, A. M., Paley, D., Kocaoglu, M., Eralp, L., Herzenberg, J. E., & Ergin, O. N. (2008). Periosteal grafting for congenital pseudarthrosis of the tibia: a preliminary report. Clinical orthopaedics and related research, 466(12), 2981–2994. https://doi.org/10.1007/s11999-008-0556-1

[17]Shah, H., Rousset, M., & Canavese, F. (2012). Congenital pseudarthrosis of the tibia: Management and complications. Indian journal of orthopaedics, 46(6), 616–626. https://doi.org/10.4103/0019-5413.104184

[18]Agashe, M. V., Song, S. H., Refai, M. A., Park, K. W., & Song, H. R. (2012). Congenital pseudarthrosis of the tibia treated with a combination of Ilizarov's technique and intramedullary rodding. Acta orthopaedica, 83(5), 515–522. https://doi.org/10.3109/17453674.2012.736170

[19] Gouron, R.,Deroussen, F.,Segarra, M., Ursu, C., Plancq, M.-C, & Collet, L.-M. (2011). Early resection of congenital pseudarthrosis of the tibia and successful reconstruction using the Masquelet technique. The Journal of bone and joint surgery, British volume, 93(4), 552-554.

[20]Granchi, D., Devescovi, V., Baglìo, S. R., Leonardi, E., Donzelli, O., Magnani, M., Stilli, S., Giunti, A., & Baldini, N. (2010). Biological basis for the use of autologous bone marrow stromal cells in the treatment of congenital pseudarthrosis of the tibia. Bone, 46(3), 780–788. https://doi.org/10.1016/j.bone.2009.10.044

[21]Tikkanen, J. , Leskelä,, H. V. , Lehtonen, S. T. , Vähäsarja, V. , Melkko, J. , & L. Ahvenjärvi, L., et al. (2010). Attempt to treat congenital pseudarthrosis of the tibia with mesenchymal stromal cell transplantation. Cytotherapy, 12(5), 593-604.

[22]Granchi, D., Devescovi, V. , Baglio, S. R. , Magnani, M. , Donzelli, O. , & Baldini, N.. (2012). A regenerative approach for bone repair in congenital pseudarthrosis of the tibia associated or not associated with type 1 neurofibromatosis: correlation between laboratory findings and clinical outcome. Cytotherapy, 14(3), 306-314.

[23]Giuliani, N., Lisignoli, G., Magnani, M., Racano, C., Bolzoni, M., Dalla Palma, B., Spolzino, A., Manferdini, C., Abati, C., Toscani, D., Facchini, A., & Aversa, F. (2013). New insights into osteogenic and chondrogenic differentiation of human bone marrow mesenchymal stem cells and their potential clinical applications for bone regeneration in pediatric orthopaedics. Stem cells international, 2013, 312501. https://doi.org/10.1155/2013/312501

[24]Peng, Y., Huang, D., Li, J., Liu, S., Qing, X., & Shao, Z. (2020). Genipin-crosslinked decellularized annulus fibrosus hydrogels induces tissue-specific differentiation of bone mesenchymal stem cells and intervertebral disc regeneration. Journal of tissue engineering and regenerative medicine, 14(3), 497–509. https://doi.org/10.1002/term.3014

[25]Luoma, K., Riihimaki, H., Luukkonen, R., Raininko, R., Viikari-Juntura, E., &Lamminen, A. (2000). Low back pain in relation to lumbar disc degeneration. Spine (Phila Pa 1976), 25, 487–492.
https://doi.org/10.1097/00007632-200002150-00016

[26]Buser, Z., Chung, A. S., Abedi, A., & Wang, J. C. (2019). The future of disc surgery and regeneration. International Orthopaedics, 43, 995–1002.
https://doi.org/10.1007/s00264-018-4254-7

[27]Battie, M. C., Lazary, A., Fairbank, J., Eisenstein, S., Heywood, C., BraydaBruno, M.,& McCall, I. (2014). Disc degeneration-related clinical phenotypes. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society, 23(3), S305–S314.
https://doi.org/10.1007/s00586-013-2903-5

[28]Fjeld, O. R., Grovle, L., Helgeland, J., Smastuen, M. C., Solberg, T. K.,Zwart, J.-A., & Grotle, M. (2019). Complications, reoperations, readmissions, and length of hospital stay in 34 639 surgical cases of lumbar disc herniation. The Bone & Joint Journal, 101-B, 470–477.
https://doi.org/10.1302/0301-620X.101B4.BJJ-2018-1184.R1

[29]Bejleri, D., & Davis, M. E. (2019). Decellularized extracellular matrix materials for cardiac repair and regeneration. Advanced Healthcare Materials, 8, e1801217. https://doi.org/10.1002/adhm.201801217

[30]Xu, H., Xu, B., Yang, Q., Li, X., Ma, X., Xia, Q., & Zhang, Y. (2014). Comparison of decellularization protocols for preparing a decellularized porcine annulus fibrosus scaffold. PLoS ONE, 9, e86723. https://doi.org/10.1371/journal.pone.0086723

[31]Younger, E. M., & Chapman, M. W. (1989). Morbidity at bone graft donor sites. Journal of orthopaedic trauma, 3(3), 192–195. https://doi.org/10.1097/00005131-198909000-00002

[32]Fu, T. S., Chang, Y. H., Wong, C. B., Wang, I. C., Tsai, T. T., Lai, P. L., Chen, L. H., & Chen, W. J. (2015). Mesenchymal stem cells expressing baculovirus-engineered BMP-2 and VEGF enhance posterolateral spine fusion in a rabbit model. The spine journal : official journal of the North American Spine Society, 15(9), 2036–2044. https://doi.org/10.1016/j.spinee.2014.11.002

[33]Bruder, S. P., Jaiswal, N., Ricalton, N. S., Mosca, J. D., Kraus, K. H., & Kadiyala, S. (1998). Mesenchymal stem cells in osteobiology and applied bone regeneration. Clinical orthopaedics and related research, (355 Suppl), S247–S256. https://doi.org/10.1097/00003086-199810001-00025

[34]Solchaga, L. A. , Johnstone, B. , Yoo, J. U. , Goldberg, V. M. , & Caplan, A. I. . (1999). High variability in rabbit bone marrow-derived mesenchymal cell preparations. Cell Transplantation, 8(5), 511-519.

[35]Hanada, K., Dennis, J. E., & Caplan, A. I. (1997). Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 12(10), 1606–1614. https://doi.org/10.1359/jbmr.1997.12.10.1606

[36]Noshi, T., Yoshikawa, T., Ikeuchi, M., Dohi, Y., Ohgushi, H., Horiuchi, K., Sugimura, M., Ichijima, K., & Yonemasu, K. (2000). Enhancement of the in vivo

osteogenic potential of marrow/hydroxyapatite composites by bovine bone morphogenetic protein. Journal of biomedical materials research, 52(4), 621–630. https://doi.org/10.1002/1097-4636(20001215)52:4<621::aid-jbm6>3.0.co;2-a

[37]Noshi, T., Yoshikawa, T., Dohi, Y., Ikeuchi, M., Horiuchi, K., Ichijima, K., Sugimura, M., Yonemasu, K. & Ohgushi, H. (2001), Recombinant Human Bone Morphogenetic Protein-2 Potentiates the In Vivo Osteogenic Ability of Marrow/Hydroxyapatite Composites. Artificial Organs, 25: 201-208. https://doi.org/10.1046/j.1525-1594.2001.025003201.x

[38]Ho, Y. C. , Lee, H. P. , Hwang, S. M. , Lo, W. H. , Chen, H. C. , & Chung, C. K. , et al. (2006). Baculovirus transduction of human mesenchymal stem cell-derived progenitor cells: variation of transgene expression with cellular differentiation states. Gene Therapy, 13(20), 1471-9.

[39]Buckwalter, J. A., Mankin, H. J., & Grodzinsky, A. J. (2005). Articular cartilage and osteoarthritis. Instructional course lectures, 54, 465–480.

[40]Kalaszczynska, I., & Ferdyn, K. (2015). Wharton's jelly derived mesenchymal stem cells: future of regenerative medicine? Recent findings and clinical significance. BioMed research international, 2015, 430847. https://doi.org/10.1155/2015/430847

[41]Pak, J., Lee, J. H., & Lee, S. H. (2014). Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells. BioMed research international, 2014, 436029. https://doi.org/10.1155/2014/436029

[42]Hsu, C. C., Cheng, J. H., Wang, C. J., Ko, J. Y., Hsu, S. L., & Hsu, T. C. (2020). Shockwave Therapy Combined with Autologous Adipose-Derived Mesenchymal Stem Cells Is Better than with Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells on Knee Osteoarthritis. International journal of molecular sciences, 21(4), 1217. https://doi.org/10.3390/ijms21041217

[43]Marinova-Mutafchieva, L., Williams, R. O., Mason, L. J., Mauri, C., Feldmann, M., & Maini, R. N. (1997). Dynamics of proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis (CIA). Clinical and experimental immunology, 107(3), 507–512. https://doi.org/10.1046/j.1365-2249.1997.2901181.x

[44] Augello, A., Tasso, R., Negrini, S. M., Cancedda, R., & Pennesi, G. (2007). Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis and rheumatism, 56(4), 1175–1186. https://doi.org/10.1002/art.22511

[45] Di Nicola, M., Carlo-Stella, C., Magni, M., Milanesi, M., Longoni, P. D., Matteucci, P., Grisanti, S., & Gianni, A. M. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood, 99(10), 3838–3843. https://doi.org/10.1182/blood.v99.10.3838

[46] Hasegawa, A., Yonezawa, T., Taniguchi, N., Otabe, K., Akasaki, Y., Matsukawa, T., Saito, M., Neo, M., Marmorstein, L. Y., & Lotz, M. K. (2017). Role of Fibulin 3 in Aging-Related Joint Changes and Osteoarthritis Pathogenesis in Human and Mouse Knee Cartilage. Arthritis & rheumatology (Hoboken, N.J.), 69(3), 576–585. https://doi.org/10.1002/art.39963