Nano World Journal

https://doi.org/10.17756/nwj.2017-048

Research Article

Open Access

One Step Tissue Engineering for Cartilage Reconstruction in Severe Osteoarthritis of the Knee and Ankle: A Comprehensive Review of the Technique Resorting to Isolated BMAC or ADSCS and their Last Combination

Stefano Zanasi[1,2*] **and Carlo Ventura**[3-5]

[1]*Casa di Cura la Madonnina, San Donato Group (GSD), Milan, Italy*
[2]*Villa Erbosa Hospital, San Donato Group (GSD), University of Milan, Italy*
[3]*GUNA ATTRE (Advanced Therapies and Tissue REgeneration), Innovation Accelerator, CNR, Bologna, Italy*
[4]*Istituto Nazionale di Biostrutture e Biosistemi (INBB), Laboratory of Molecular and Cellular Biology, University of Bologna, Italy*
[5]*VID art|science/INBB, Bologna, Italy*

[*]**Correspondence to:**
Prof. Stefano Zanasi
Villa Erbosa Hospital
San Donato Group (GSD), University of Milan
Via dell'Arcoveggio 50/2, 40122 Bologna, Italy
Tel: +39-051-6315261
E-mail: zanasis.orth@alice.it

Received: May 03, 2017
Accepted: October 10, 2017
Published: October 12, 2017

Citation: Zanasi S, Ventura C. 2017. One Step Tissue Engineering for Cartilage Reconstruction in Severe Osteoarthritis of the Knee and Ankle: A Comprehensive Review of the Technique Resorting to Isolated BMAC or ADSCS and their Last Combination. *NanoWorld J* 3(3): 59-65.

Published by United Scientific Group

Abstract

An innovative clinical procedure has provided evidence that wide areas of severe cartilage defects due to osteoarthritis of the inferior limb, Kellgren stage I to III, in particular knee and ankle, can be successfully restored through transplant of human bone marrow combined with a defined human fat tissue product, like the Lipogems product. The latter is microfractured autologous human fat, containing an intact stromal vascular niche including elements of pericyte identity (Lipogems EU, http://lipogems.eu). Both are embedded within nanofabricated scaffolds with tailored-oriented architecture and fiber diameter (Chondrotissue by Biotissue, Freiburg, D http://www.biotissue.de). Such approach yields a significantly more enhanced cartilage regeneration, as compared with the rescuing effects elicited by either bone marrow or the Lipogems product alone. All these clinical outcomes are well documented by 1.5 T NMR, elastosonography and in randomized histological samples showing a higher percentage of hyaline cartilage and rare fibrous tissue compared to the outcome of the single, not combined procedures. On these bases, the combined use of autologous non-expanded tissue products made of whole bone marrow and human white adipose tissue derivatives, such as the Lipogems product, can be considered as an autologous/homologous strategy for improving the natural capacity for self-healing in damaged osteo-articular tissues.

Keywords

Regenerative medicine, Precision medicine, Cartilage reconstruction, Bone reconstruction, Osteoarthritis, Nanofabricated scaffolds, Human bone marrow, Human fat derivatives

Background

Cartilage injuries lead to joint pain and loss of function. Mature hyaline cartilage has a very low self-repair potential due to its intrinsic properties. For this reason, researchers have focused in the search of methods to reproduce the tissue characteristics of hyaline cartilage and induce complete cartilage repair [1, 2]. A new approach for the treatment of articular cartilage defects is the use of biocompatible scaffolds [3]. Scaffolds for cartilage tissue engineering have to be realized at the nanoscale level to achieve mechanical and physical properties similar to the native tissue [4].

Recently, scaffolds composed of polyglycolic acid (PGA), a hydrophilic synthetic biodegradable polymer, and hyaluronic acid (HA), have been shown to enhance tissue formation *in vitro* and also *in vivo*, when these were implanted

into rat osteochondral defects. In addition, the transplantation of PGA-HA nanofiber scaffolds embedding mesenchymal stem cells (MSCs) enhanced tissue healing at a greater extent than cell-free scaffolds, suggesting their potential as a suitable graft for articular cartilage reconstruction.

Cartilage repair represents a clinical challenge due to the inherent limited ability for selfrepair. The current treatment of cartilage defects includes intra-articular administration of HA, treatment with platelet-rich-plasma that contains bioactive proteins such as chemokines, cytokines and growth factors, and bone marrow stimulation that include subchondral drilling, abrasion, and microfracture.

Up to now, the only approved cellular-based therapy for cartilage restoration is based upon the autologous chondrocyte transplantation (ACT) after *in vitro* expansion [5, 6]. Despite the promising initial results, the limited expansion of chondrocytes *ex vivo*, and donor site morbidity limit the beneficial effects of this technique [7]. Alternative cellular therapies have focused on progenitor cell populations, e.g. bone marrow stem cells that once transplanted improved outcomes in a one-step procedure.

Since 2006, evidence has been provided for the feasibility to use a concentration technique of bone marrow cells, called BMAC, in the operating room and during the same session, focused on implanting bone marrow with significant advantages in terms of costs and time, using the so-called "one-step surgery" for cartilage repair [8-15]. This technique utilizes a disposable kit, 60 ml of bone marrow aspiration from the ipsilateral iliac crest and repeated centrifugations (5-6 times) to concentrate mesenchymal stem cells contained in the bone marrow (BM-MSCs). Using thrombin from autologous blood collection, it is possible to modify bone marrow and produce a concentrated gel that is loaded onto a scaffold (Hyaff-11) and used to reconstruct, when suitably shaped, the chondral lesion.

Unlike other multi-layered collagen or synthetic products, Hyalofast is a non-woven biodegradable HA-based scaffold, entailing a single 3D fibrous layer of Hyaff 11, a HA benzyl ester derivative. Hyalofast can be cut and adaptively placed to fit within irregular cartilage lesions, taking advantage of its non-woven and soft characteristics. Clinical data indicates that Hyalofast delivers positive results, enabling patients to regenerate hyaline-like cartilage with the goal of helping them returning to daily activities naturally.

Our results suggest that, even in severe unicompartmental osteoarthritis, BMAC-BM-MSCs resurfacing proves to act as an effective treatment for a biological coating. This provides, at least at medium-term data, remission of pain and swelling, with good functional recovery and the ability to resume previous work activity, as compared to the ACT technique, with the advantage of a single operative step and a significant cost reduction [8, 16-24].

The adipose tissue as a source for regenerative processes

Recently, the adipose tissue and adipose-derived stem cells (ADSCs) are emerging as a less invasive source for progenitors that can be differentiated into chondrocytes *in vitro* in a 3-dimensional environment, in the presence of growth factors (TGF-β superfamily), and then implanted to restore the cartilage tissue [25, 26].

Moreover, it has been observed that also uninduced (non-previously *ex vivo* committed) ADSCs were able to fully restore cartilage in ear auricle defects and in patella-femoral joints.

These promising data suggest that a minimal *ex vivo* manipulation of ADSCs, thanks to the intrinsic ability of these cells to adapt to their environment *in vivo*, could allow the development of an easy and effective clinical treatment of cartilage defects bigger than 1.5 cm in diameter, resorting to tissue engineering [26]. Adipose tissue is a connective tissue derived from embryonic mesoderm, consisting of a heterogeneous population of cells, like adipocytes, preadipocytes, smooth muscle cells, endothelial cells, mast cells, fibroblasts, and immune cells. About 10% of the adipocyte population is annually renewed. From adipose tissue manipulation it is possible to isolate the so-called stromal vascular fraction (SVF), containing, among others, MSCs. SVF is easily harvested with minimal donor site morbidity, commonly through lipoaspiration followed by *in vitro* cell isolation.

The efficiency of SVF isolation is strictly related to donor general condition, such as age and obesity. Compared to bone marrow, 1 g of adipose tissue contains about 500 times more pluripotent cells than 1 g of bone marrow aspirate. Moreover, besides showing phenotypic and transcriptional profiles similar to those of other MSCs, ADSCs present some peculiar characteristics. In particular, ADSCs express CD34 glycoprotein, stromal markers (CD13, CD29, CD44, CD63, CD73, CD90, and CD166) and endothelial cell markers (CD31, CD144, VEGFR2, and von Willebrand factor).

The benefits of adipose tissue in Regenerative Medicine are various:

- It is relatively easy to obtain it with minimally invasive harvesting;
- There is less/minimal pain at the donor site;
- There is less risk associated with autologous therapies;
- Sampling is not linked to the control of hematologic centers;
- High concentrations of regenerative cells have been found in adipose tissue;
- Cells are very abundant (unlikely to decrease with age, as bone marrow-derived cells): from 1 g of adipose tissue, about 5000 stem cells can be isolated, a remarkably higher yield, as compared to an equivalent amount of bone marrow.

The participation of MSCs in tissue regeneration has been largely investigated according to the notion that these cells can themselves differentiate into some cell types, including bone, cartilage, muscle, adipocytes, stroma, fibroblasts and endothelial cells.

The paracrine effect and the exosome nanoworld

Recent studies suggest that MSCs could participate in

tissue repair, not only by differentiating into cells of the target issue, but also by releasing several factors, contributing to restorative processes, including angiogenetic ones. The secreted trophic factors participate to tissue rescue through pro-angiogenic and anti-fibrotic mechanisms, anti-inflammatory and immunomodulatory properties, anti-apoptotic and antimicrobial characteristics. Recent studies show a direct correlation between the occurrence of MSCs and the blood vessel density in stromal vascularized tissues.

The niche is the morpho-functional unit where stem cells live and reproduce themselves. It is a particular kind of tissue within each tissue, in which a huge network of messages is fashioned through the production of the overall paracrine activity of the embedded cells, the so-called "secretome". The regenerative potency of MSCs depends mostly on their ability to afford a timely modulation in the composition of the secretome. In this new vision, the preservation of the niche is fundamental to consider MSCs as a patient-specific "molecular biology laboratory" adapting over time to the environmental cues. Components of the adipose niche released by the injured tissues are adipocytes, extracellular matrix (collagen and connective tissue), pericytes (wrapped around capillaries), pre-adipocytes (progenitor cell), microvasculature, ADSCs.

In particular, pericytes exist in adipose tissue niche wrapped around capillaries within adipocyte clusters; they facilitate communication with cellular environment and react to signals ensuing from an injury or damage. An injury-response from a pericyte transforms it into a MSC, which in turn is further activated into a regenerative MSC to produce a self-healing environment. Hence, pericytes are "Emergency Repair Cells", secreting bioactive molecules (like mini drugstores) mostly via release of nanovescicles (exosomes), with *trophic* effects which encompass anti-apoptotic, anti-scarring, angiogenic, mitotic, and anti-microbial patterning.

One of the most rapidly emerging ideas that explain paracrine mechanisms of tissue regeneration is the use of stem cell-derived micro/nano-secretory vesicles, which act as mediators of tissue regeneration following injury or disease. This is an exciting area of research, as it opens up the potential to explore non-cell-based therapy in regenerative medicine. It is now generally accepted that nano/microvesicles could aid in the transfer of genetic information between cells, as they contain proteins, messenger RNAs (mRNAs), DNAs and/or microRNAs. They also regulate the physiology and pathophysiology of cells and can be exploited for therapeutic and diagnostic purposes. Thus, nano/microvesicles could be a useful tool to treat solid organ damage, as they may act as mediators to promote anti-inflammatory, pro-angiogenic, anti-apoptotic and differentiation or mitotic factors to activate intrinsic repair and regeneration processes.

There are two main mechanisms for stem cell exosome/microvesicle-mediated regeneration of injured tissues:

Microvesicles and exosomes can be released from injured tissues and act locally on tissue-resident stem cells to prime them to release exosomes/microvesicles that naturally contain a variety of beneficial cargoes to help in repair and regeneration directly.

Microvesicles and exosomes can be released from injured tissue and act on the stem cells. These stem cells can then differentiate themselves and/or de-differentiate neighbouring cells at the injured site to replace the damaged cells.

Through the use of new, ultrasenitive low-force atomic force microscopy (AFM), and correlating the AFM data with field emission scanning electron microscopy, it has been possible to provide evidence for previously unresolvable structural and nanomechanical features of exosome nanovescicles. Addressing exosome features at the dynamic nanoscale level will no doubt contribute to unraveling the mechanistic bases through which this cellular nanoworld may act as a major conductor for fashioning and shuttling pockets of information within and among (stem) cells [27]. To this end, resolving the nanomechanical signatures embedded within exosomes will help taking a glimpse of the timely patterning that rhythmically governs the efflux and biological activity of exosome cargoes, including proangiogenic miRNAs such as miR-126 and miR-296, mRNAs for immune regulation including cytokine receptor-like factor 1 (CRLF1) and interleukin 1 receptor antagonist (IL1RN), transport proteins that assist in the transport and stability of mRNA, such as Staufen1 (Stau1) and 2 (Stau2), and factors involved in miRNA transport and processing, like Argonaute2 (Ago2).

Disclosing the complexity of exosome signaling will probably provide further understanding of some of the regenerative effects attributed to ADSCs and so far generically defined as paracrine, immunomodulatory effects. In this regard, exosome-mediated paracrine signaling and the intrinsic differentiating potential from ADSCs may timely and sequentially contribute to cartilage rescue. Shortly after transplantation, ADSCs may exert anti-inflammatory and visco supplementative effects, while at a later stage cartilage regeneration may take place.

New generation technologies in fat processing for regenerative purposes

After harvesting, adipose tissue processing has been initially performed by enzymatic digestion followed by *in vitro* isolation and expansion of a MSC-like population. This "first generation approach", despite yielding a consistent number of ADSCs, has the limitations of destroying the niche and its related signaling during the enzymatic processing. Even more important, in the effort of yielding a relevant number of cells prior to transplantation, ADSCs undergo remarkable senescence during their prolonged persistence of cells in culture. This is a major drawback, as it will negatively affect tissue regeneration as compared to the initial expectation: more stem cells, more tissue repair.

A new generation technology in the handling of adipose tissue for regenerative purposes has been provided by the Lipogems system [28, 29]. The Lipogems device and method only use mild mechanical forces, in the absence of any enzyme, additive or centrifugation. The human fat is therefore processed in a fast and cheap way through a large and then a narrow grid filter, with intermediate washing, to yield a microfractured product with an intact stromal vascular niche, embedding

elements of pericyte/ADSC identity [28]. The Lipogems product is devoid of oil, and blood residues.

Other methods and devices will appear on the scene in a near future, trying to avoid a mechanical microfragmentation of the initial lipoaspirate, but simply washing the adipose tissue while concomitantly eliminating oil, cellular debris and blood residues through an ultrafiltration process. These novel methods are holding promise for a better preservation of the ADSC nanotopography, as there is no mechanical microfracturing of fat, which is seamlessly purified of the contaminants ensuing from the lipoaspiration procedure, and soon ready for transplantation. These novel methods of preservation of vascular stroma with high concentration of mesenchymal cells and pericytes are progressively applying to wound healing, gynecology, urology, proctology, otorhinolaryngology, reconstructive plastic surgery, orthopedics and sports medicine.

The personal experience

In the personal experience of Professor Zanasi, after a bilateral mini-liposuction carried out in the inner part of the thighs, in the abdominal wall, or in the buttock, the harvested adipose tissue is initially processed through the Lipogems system. The resultant product is then loaded by capillarity onto a 3-D PGA-HA (CHONDROTISSUE) scaffold that has been cut out according to the shape of the defect we have to fill. The CHONDROTISSUE scaffold is a resorbable highly porous textile polyglicolic acid felt that guarantees initial mechanical stability, provides opportunity for stable fixation, allows optimal 3D cell distribution and therefore offers an optimal environment for MSCs. HA is a natural polymer providing an important stimulus for chondrogenic cell differentiation and the subsequent formation of a hyaline cartilage matrix, also contributing to the visco-elasticity of the cartilage while protecting against friction and impact loading. In combination with bone marrow or Lipogems or both, CHONDROTISSUE scaffold allows for optimal defect covering and filling, even though no intact surrounding cartilage is required. These features may lead to extended indications for the use of the above reported approach. In particular, it seems to be suitable for: (i) osteoarthritis (OA) where exposed kissed areas of the joint are commons; (ii) correction of abnormal biomechanical forces in the defect area (capping effect); (iii) resorption after 6 months after implantation; (iv) hyaline-like cartilage formation, as proven by hystologic analyses.

After a maximum 8-year follow-up of 74/196 OA patients we treated for cartilage reconstruction by bone marrow, and 2 yrs of preliminary experience with Lipogems isolated, we started a preliminary study based upon the combined use of bone marrow and Lipogems placed onto the same CHONDROTISSUE scaffold. The rationale behind this approach relies upon the fact that recent interrelated observations clearly demonstrate that bone marrow encompasses and it is itself a form of adipose tissue, sharing some similarity with white adipose tissue (WAT) but exhibiting peculiar features [30-33]. Within this context, it is conceivable that marrow adipose tissue (MAT) may also entail distinctive paracrine properties that may be exploited in combinatorial fashion with those typical of WAT and WAT-stem cells to synergistically enhance self-repairing mechanisms.

On these bases, the combinatorial use of autologous non-expanded tissue products made of whole bone marrow and human WAT derivatives, such as the microfractured Lipogems product, can be considered as an autologous/homologous strategy for improving the natural capacity for self-healing in damaged osteo-articular tissues.

Materials and Methods

From June/2013 to May/2015 we implanted bone marrow by arthroscopic or mini-open technique in 52 patients (29m/23f) affected by unicompartmental osteoarthritis Ahlback stage II/III for medial (11 patients) and lateral (4 patients) unicompartmental femuro-tibial OA, patella-femoral joint OA (28 patients), and 9 patients with unshouldered kissed ankle large defects. The average age was 34 yrs. (18-55 years). Most patients treated for knee OA involvement (32/43) got previous surgical procedure for meniscoplasty (19 patients), chondroplasty (31 patients), tibial tuberosity advancement (TTA) (4 patients), lateral release (13 patients). Six out of 9 ankles we treated have been developing OA, due to previous surgical procedures for tibial and/or peroneal fracture.

Average BMI was 25.6. All patients were followed for a minimum follow-up of 18 months (18-61 months). The mean size of the lesions was 5.5 cm^2 (range 4.5–26 cm^2). We recorded the ICRS as well as AOFAS score preoperatively.

Bone marrow (60 ml) was harvested from iliac crest, and then processed in the Operative theatre by a centrifuge system (Bio-Spin Magellan): concurrently in all cases, we made a miniliposuction either symmetrically bilaterally in the inner part of the thighs (6 patients), or in the abdominal wall (44 patients), or in the buttock (2 patients). We processed the harvested adipose tissue (300 ml) by the Lipogems system, obtaining a microfractured fat product ready for a subsequent loading onto a 3D PGA-HA (CHONDROTISSUE) scaffold. This scaffold consisted of a 100% synthetic and resorbable membrane (CHONDROTISSUE-) that was cut into an appropriate size according to the lesion area. The scaffold was loaded by capillarity with 2 ml bone marrow concentrate that had been previously mixed for a few minutes with the Lipogems product. The best empiric ratio for the combined embedding of the scaffold was two parts of Lipogems product and 1 part of bone marrow, as assessed throughout the best outcome in our personal experience [26, 34-37] in the reconstruction of both sides of a kissed chondral defect or a single/multiple wider loss/es of cartilage tissue (Figures 1 and 2).

After surgery, all patients have been directed to a specific rehabilitation program.

Results

We followed patients at 3, 6, 12, 18, and 24 months after surgery. The clinical evaluation was performed using the ICRS-IKDC and AOFAS protocols; the evaluation of the cartilage was performed by a 1.5 T MRI imaging and processing was performed through MOCART scoring system. The EuroQol EQ-5D was used to assess the quality of life of patients.

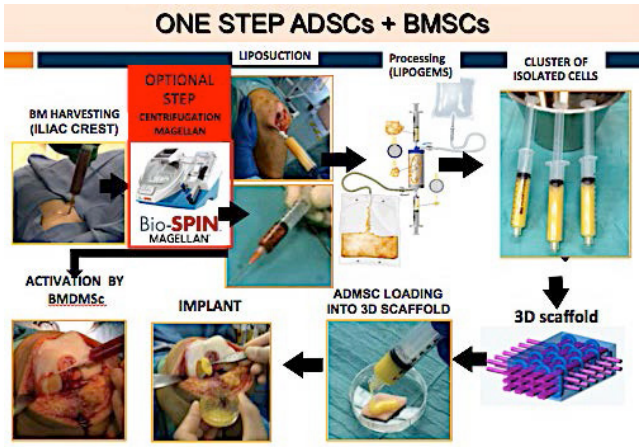

Figure 1: Tissue Engineering in OA: one step Lipogems product and bone marrow concentrate.

Figure 2: Patello-femoral kissing lesion recovered by combined embedding of Lipogems product and bone marrow concentrate within a 3D scaffold.

The ICRS-IKDC, AOFAS and CM scoring documented a statistically significant improvement in clinical objective and subjective data ($p < 0.0001$). We had no intra-operative, nor post-operative complications. The mean pre-operative ICRS-IKDC and AOFAS scores were respectively 46.4 ± 16.5 and 51 ± 11.2. The mean post-operative ICRS-IKDC score progressively improved to 82.5 ± 6.3 at 6 ms follow-up, 88.6 ± 7.9 at 12 ms, 89.9 ± 9.5 at 18 ms, and 91.4 ± 3.2 at 24 months. The mean post-operative AOFOS score further significantly improved too. The EuroQol EQ-5D index was significantly improved compared to baseline in all patients. After a mean time of almost 18 months post-surgery, 52/52 (100%) patients experienced and maintained improvement in joint function and symptoms from baseline without significant worsening (IKDC/AOFAS/CMP subjective evaluation). 49/52 (94%) patients had no pain and mobility problems at follow-up, comparable to a general reference population; 48/52 (92%) of treated joints classified as normal or nearly normal (IKDC/AOFAS objective evaluation).

According to MOCART scoring system, high-resolution 1.5 T MRI was used to analyze tissue with nine pertinent variables:

- A complete filling of the defect was found in 92.6%;
- A complete integration of the border zone to the adjacent cartilage in 94.1%;
- Homogeneous structure of the repaired tissue in 76%;
- Isointense signal intensities of the repair tissue compared to the adjacent native cartilage were seen in 95.3%;
- No adhesions and mild effusion in 56% were found.

The average value of MOCART scoring system was 81/100.

Furthermore, a limited number of complications occurred and in particular a graft failure was observed in none patients out of 52. Theoretically, due to the maintenance of the subjective and objective clinical results, none or only mild degeneration of neocartilage resurfacing tissue should occur.

From the hystological point of view, we examined 11 repair tissue biopsies at 18 to 24 ms after implantation of chondrotissue embedding both Lipogems and bone marrow. Remarkably, hematoxylin & eosin overview staining (Figure 3) showed areas with round chondrocytic cells (A: black arrow) and cells in a typical columnar arrangement in a chondron-like formation (B: white arrows).

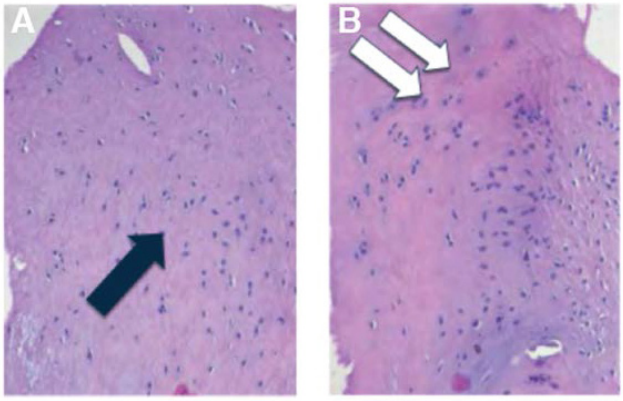

Figure 3: Patello-femoral kissing lesion recovered by implantation of CHONDROTISSUE subjected to combinatorial embedding with Lipogems product and bone marrow concentrate. Hyaline Cartilage and a repaired area are evident in the sample biopsy at 18 months follow-up. **(A)** Hematoxylin & eosin overview staining shows areas with round chondrocytic cells (black arrow). **(B)** Cells in a typical columnar arrangement in a chondron-like formation (white arrows).

On the whole, cartilage defects lead to high compression forces compared to healthy or reconstructed knee: Covering of the defect with the CHONDROTISSUE scaffold reduced the compression forces in the knee joint comparable to levels of an intact knee: Implantation of CHONDROTISSUE corrects increased compression forces in the defect that can impair the surrounding cartilage.

Conclusions

Microfractured adipose tissue combined with bone marrow for the treatment of coin, complex and contained kissing lesions (OA) of knee, ankle and shoulder, concurrently with realignment procedures, can be considered a safe and highly effective therapeutic option. Such solution provides, at least at medium-term data, normal post-operative outcomes without serious adverse events, correlated to the remission of pain and swelling, with good functional recovery within 3 months. The ability to resume previous work and sport activities demonstrates overlapping outcome versus the ACT procedure with the advantage of a single operative step, without requiring removal of a cartilage sample for cloning in a specialized Centre and replanting after about 40 days. So far, we can estimate that: total cost for open field ACI is 14,305 €; total arthroscopic ACI cost is 9696 €; total bone marrow transplantation cost is 4348 €. Average total Lipogems transplantation cost is 4320 €; Total cost for a combined Lipogems and bone marrow transplantation within CHONDROTISSUE scaffold is 4660 €. Therefore, our proposed strategy, besides leading to a remarkable improvement in the extent and timing for cartilage tissue repair, is also associated with high affordability and important cost reductions.

References

1. Hollander AP, Dickinson SC, Kafienah W. 2010. Stem cells and cartilage development: complexities of a simple tissue. *Stem Cells* 28(11): 1992-1996. https://doi.org/10.1002/stem.534
2. Mahmoudifar N, Doran PM. 2012. Chondrogenesis and cartilage tissue engineering: the longer road to technology development. *Trends Biotechnol* 30(3): 166-176. https://doi.org/10.1016/j.tibtech.2011.09.002
3. Vinatier C, Bouffi C, Merceron C, Gordeladze J, Brondello JM, et al. 2009. Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr Stem Cell Res Ther* 4(4): 318-329. https://doi.org/10.2174/157488809789649205
4. Shanmugasundaram S, Chaudhry H, Arinzeh TL. 2011. Microscale versus nanoscale scaffold architecture for mesenchymal stem cell chondrogenesis. *Tissue Eng Part A* 17(5-6): 831-840. https://doi.org/10.1089/ten.TEA.2010.0409
5. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, et al. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 331(14): 889-895. https://doi.org/10.1056/NEJM199410063311401
6. Brittberg M, Peterson L, Sjögren-Jansson E, Tallheden T, Lindahl A. 2003. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. *J Bone Joint Surg Am* 85-A(Suppl 3): 109-115.
7. Peterson L, Minas T, Brittberg M, Nilsson A, Sjögren-Jansson E, et al. 2000. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res* 374: 212-234. https://doi.org/10.1097/00003086-200005000-00020
8. Barry F, Boynton RE, Liu B, Murphy JM. 2001. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res* 268(2): 189-200. https://doi.org/10.1006/excr.2001.5278
9. Grigolo B, Lisignoli G, Desando G, Cavallo C, Marconi E, et al. 2009. Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. *Tissue Eng Part C Methods* 15(4): 647-658. https://doi.org/10.1089/ten.TEC.2008.0569
10. Haleem AM, Singergy AAE, Sabry D, Atta HM, Rashed LA, et al. 2010. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. *Cartilage* 1(4): 253-261. https://doi.org/10.1177/1947603510366027
11. Kasemkijwattana C, Hongeng S, Kesprayura S, Rungsinaporn V, Chaipinyo K, et al. 2011. Autologous bone marrow mesenchymal stem cells implantation for cartilage defects: two cases report. *J Med Assoc Thai* 94(3): 395-400.
12. Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, et al. 2007. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthr Cartil* 15(2): 226-231. https://doi.org/10.1016/j.joca.2006.08.008
13. Nejadnik H, Hui JH, Feng Choong EP, Tai B-C, Lee EH. 2010. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med* 38(6): 1110-1116. https://doi.org/10.1177/0363546509359067
14. Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, et al. 2007. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med* 1(1): 74-79. https://doi.org/10.1002/term.8
15. Wang W, Li B, Yang J, Xin L, Li Y, et al. 2010. The restoration of full-thickness cartilage defects with BMSCs and TGF-beta 1 loaded PLGA/fibrin gel constructs. *Biomaterials* 31(34): 8964-8973. https://doi.org/10.1016/j.biomaterials.2010.08.018
16. Burke J, Hunter M, Kolhe R, Isales C, Hamrick M, et al. 2016. Therapeutic potential of mesenchymal stem cell based therapy for osteoarthritis. *Clin Transl Med* 5(1): 27. https://doi.org/10.1186/s40169-016-0112-7
17. Caplan AI. 2007. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 213(2): 341-347. https://doi.org/10.1002/jcp.21200
18. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, et al. 2008. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician* 11(3): 343-353.
19. Chang YH, Liu HW, Wu KC, Ding DC. 2016. Mesenchymal stem cells and their clinical applications in osteoarthritis. *Cell Transplant* 25(5): 937-950. https://doi.org/10.3727/096368915X690288
20. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. 2011. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int J Rheum Dis* 14(2): 211-215. https://doi.org/10.1111/j.1756-185X.2011.01599.x
21. Gupta PK, Das AK, Chullikana A, Majumdar AS. 2012. Mesenchymal stem cells for cartilage repair in osteoarthritis. *Stem Cell Res Ther* 3(4): 25. https://doi.org/10.1186/scrt116
22. Koelling S, Miosge N. 2009. Stem cell therapy for cartilage regeneration in osteoarthritis. *Expert Opin Biol Ther* 9(11): 1399-1405. https://doi.org/10.1517/14712590903246370
23. Orozco L, Munar A, Soler R, Alberca M, Soler F, et al. 2013. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation* 95(12): 1535-1541. https://doi.org/10.1097/

TP.0b013e318291a2da

24. Qi Y, Feng G, Yan W. 2012. Mesenchymal stem cell-based treatment for cartilage defects in osteoarthritis. *Mol Biol Rep* 39(5): 5683-5689. https://doi.org/10.1007/s11033-011-1376-z
25. Rada T, Reis RL, Gomes ME. 2009. Adipose tissue-derived stem cells and their application in bone and cartilage tissue engineering. *Tissue Eng Part B Rev* 15(2): 113-125. https://doi.org/10.1089/ten.teb.2008.0423
26. Zanasi S. Adipose derived mesenchimal stem cells in orthopaedics. February 27th, 2015, Novi Sad, Serbia.
27. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M, et al. 2010. Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. *ACS Nano* 4(4): 1921-196. https://doi.org/10.1021/nn901824n
28. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, et al. 2013. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant* 22(11): 2063-2077. https://doi.org/10.3727/096368912X657855
29. Tremolada C, Ricordi C, Caplan AI, Ventura C. 2016. Mesenchymal stem cells in lipogems, a reverse story: from clinical practice to basic science. *Methods Mol Biol* 1416: 109-1022. https://doi.org/10.1007/978-1-4939-3584-0_6
30. Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, et al. 2014. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. *Cell Metab* 20(2): 368-375. https://doi.org/10.1016/j.cmet.2014.06.003
31. Holmes D. 2015. Stem cells: donor-derived bone marrow cells become adipocytes in recipients. *Nat Rev Endocrinol* 11(9): 506. https://doi.org/10.1038/nrendo.2015.127
32. Krings A, Rahman S, Huang S, Lu Y, Czernik PJ, et al. 2012. Bone marrow fat has brown adipose tissue characteristics, which are attenuated with aging and diabetes. *Bone* 50(2): 546-552. https://doi.org/10.1016/j.bone.2011.06.016
33. Rydén M, Uzunel M, Hård JL, Borgström E, Mold JE, et al. 2015. Transplanted bone marrow-derived cells contribute to human adipogenesis. *Cell Metab* 22(3): 408-417. https://doi.org/10.1016/j.cmet.2015.06.011
34. Zanasi S. 2016. One Step Tissue Engineering with Combined ADSCs and BMSCs in OA for Cartilage Repair: the Lipogem Approach. Presented at: Session 5-5: Stem Cell Therapy for Skin, Cartilage, Bone at the 9th World Congress of Regenerative Medicine & Stem Cell-Korea, Seoul, South Korea.
35. Zanasi S. 2016. One Step Tissue Engineering with combined ADSCs and BMSCs in OA for Cartilage Repair: the Lipogems System. Presented at: 3rd symposium On Regenerative Orthopedics, Novi Sad, Serbia.
36. Zanasi S. 2016. One Step Tissue Engineering with Combined ADSCs and BMSCs in OA for Cartilage Repair: the Lipogem Approach. Presented at: SICOT International Orthopaedics Specialized Knee Surgery-Wurzburg, Germany.
37. Zanasi S. 2016. Clinical Application of Regenerative Medicine in Orthopaedics and Sport Traumatology. Presented at: 6th EAHM Congress - Bologna 13th-14th October 2016 Sustainable Health Requires Competence and Responsibilities - Satellite EAHM Events: 13th October - Sustainable Regenerative Medicine: Research, Clinical Applications Development, Competence and Responsibilities.