Histol Histopathol (2018) 33: 639-653

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Adipose-derived stem cells in articular cartilage regeneration: current concepts and optimization strategies

Xingjian Gu[1-4], Caixin Li[4,5], Feng Yin[5] and Guanghua Yang[1-4]

[1]International Research Center for Marine Biosciences at Shanghai Ocean University, Ministry of Science and Technology, [2]National Pathogen Collection Center for Aquatic Animals, [3]Shanghai Collaborative Innovation for Aquatic Animal Genetics and Breeding, [4]Shanghai Telebio Biomedical Co., Ltd. and [5]Research Center for Translational Medicine, East Hospital, School of Medicine, Tongji University, Shanghai, P.R. China

Summary. Knee osteoarthritis (KOA) is the most common progressive joint disorder associated with disability in the world. As a chronic disease, KOA has multifactorial etiology. However, the poor self-healing ability of the articular cartilage due to its intrinsic tissue hypovascularity and hypocellularity seems to be directly incriminated in the physio-pathological mechanism of KOA. While conventional therapies result in unfavorable clinical outcomes, regenerative cell therapies have shown great promise in articular cartilage regeneration. Adipose-derived stem cells (ASCs) appear to be an ideal alternative to bone-marrow derived stem cells (BMSCs) and autologous chondrocytes, due to their lower immunogenicity, richer source and easier acquisition. Since the first case report in 2011, ASCs have demonstrated safety and efficacy for articular cartilage regeneration in several phase I/II clinical trials. However, different levels of abnormality were found in the regenerated cartilage for most of the patients. A large portion of recent publications investigated different optimization strategies to improve the therapeutic function of ASCs, including cell source selection, preconditioning and co-delivery. Herein, we give an update on the latest research progress on ASCs, with a focus on the most promising optimization strategies for ASC-based therapy.

Offprint requests to: Guanghua Yang, No. 908 Ziping Road,Building 25, Pudong New Area, Shanghai 201321, China. e-mail: ghyang119@163.com

DOI: 10.14670/HH-11-955

Key words: Adipose-derived stem cells, SVF, Cartilage, Regeneration, Osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative disease involving the whole joint, including the articular cartilage, subchondral bone, and periarticular tissue. The knee is one of the joints most commonly affected by osteoarthritis. More than one-third of people over 65 years old suffer from pain and disability caused by knee OA (Bhatia et al., 2013). Damaged articular cartilage in OA knees has poor intrinsic healing potential due to its hypovascularity and hypocellularity, which is a major problem in clinical OA treatment. Various surgical procedures have been performed to regenerate articular cartilage but have achieved limited success, including abrasion arthroplasty, subchondral drilling and microfracture (Bert, 1993; Bae et al., 2006; Sakata et al., 2013).

Recently, the cell-based regenerative therapy emerged as a promising approach to facilitate cartilage regeneration. Autologous chondrocyte transplantation (ACT) has been investigated since 1987 showing encouraging results in early studies, but its therapeutic efficacy showed no significant difference compared to microfracture in a recent randomized, controlled trial (Knutsen et al., 2004; Cole, 2008). Besides, ACT usually comes with other problems such as the dedifferentiation of chondrocytes, donor site morbidity and the two-step

surgery procedure (Dehne et al., 2010; Harris et al., 2010; Minas et al., 2014). To address these problems, mesenchymal stem cells (MSCs) have been extensively studied as an alternative cell source to chondrocytes for cartilage repair. MSCs isolated from bone marrow possess several advantages, such as immunomodulatory activity and multipotency, demonstrating outstanding safety and efficacy for cartilage repair in multiple pre-clinical and clinical studies (Johnstone and Yoo, 1999; Pittenger et al., 1999; Centeno et al., 2008, 2011). However, clinical application of bone marrow-derived mesenchymal stem cells (BMSCs) is limited by the painful and invasive surgical procedure, an extremely low cell yield and the physiological conditions of donors (Fennema et al., 2009; Alt et al., 2012; Hernigou et al., 2014).

Zuk et al. first isolated adipose tissue-derived stem cells (ASCs) from adipose tissue in 2001 (Zuk et al., 2001). During the last decade, ASCs have attracted great attention because of their abundant source and ease of accessibility as well as the comparable regenerative capability compared to BMSCs (Erickson et al., 2002; Awad et al., 2004b; Estes et al., 2008). Since the autologous SVF transplantation was approved for clinical trials in 2009 by Korea, significant progress has been made in its clinical application for cartilage regeneration. Most of the completed clinical trials delivered ASCs in the form of SVF, and reported safety and efficacy for cartilage repair (Black et al., 2007; Pak, 2011; Toghraie et al., 2012). However, current clinical data also indicated that simple SVF injection is not sufficient to fully restore the damaged cartilage back toward normal function (Koh et al., 2014, 2016; Nguyen et al., 2017), thus an optimized ASC therapy is needed to achieve better therapeutic efficacy. The work presented here gives an update on the latest information on ASCs, with a focus on the most attractive optimization strategies (A schematic representation is provided in Fig. 1).

Adipose-derived stem cells

Origin of ASCs

Since MSCs were first isolated from bone marrow cultures, BMSCs have been the most well-characterized MSC type. However, later it was found that MSCs can be isolated from other sites of the body, including umbilical cord blood, placenta, adipose tissue and elsewhere (Erices et al., 2000; Zuk et al., 2001; In 't Anker et al., 2004). In fact, current concept supports that MSCs exist within the connective tissue of virtually all organs (Meirelles et al., 2006). Since Zuk et al. first isolated a new group of multipotent cells from adipose tissue in 2001, now termed adipose-derived stem cells (ASCs), extensive studies have been performed to determine if ASCs can be an ideal substitute for BMSCs, due to the much higher stem cell yield and ease of accessibility from liposuction (Zuk et al., 2001). Adipose tissue from liposuction is first digested by collagenase, followed by centrifugation to remove the floating adipocytes, leaving the remaining cell pellets on the bottom, named stromal vascular fraction (SVF) (Lindroos et al., 2011; Pires de Carvalho et al., 2014). SVF is a heterogeneous cell population composed of ASCs (about 3%), endothelial progenitor cells, vascular mural cells, T regulatory cells, macrophages, preadipocytes and other stromal components (Riordan et al., 2009; Baer and Geiger, 2012; Rodriguez et al., 2012). Finally, ASCs are enriched by plastic adherence and subsequent expansion (Zuk et al., 2002; Mitchell et al., 2006). SVF and ASCs have both been investigated for cartilage regeneration in recent phase I/II clinical trials (Jo et al., 2014; Koh et al., 2014; Freitag et al., 2015).

Characterization of ASCs

ASCs are able to differentiate along multiple lineages into adipocytes, osteoblasts, chondrocytes, myocytes, tendon fibroblasts, neuronal-like and endothelial cells (Gimble et al., 2007; Shen et al., 2013). The surface markers of ASCs are similar to those of BMSCs with an overlapping of more than 90% (Zuk et al., 2002). There has been a consensus that ASCs are positive for the typical markers of MSCs while being

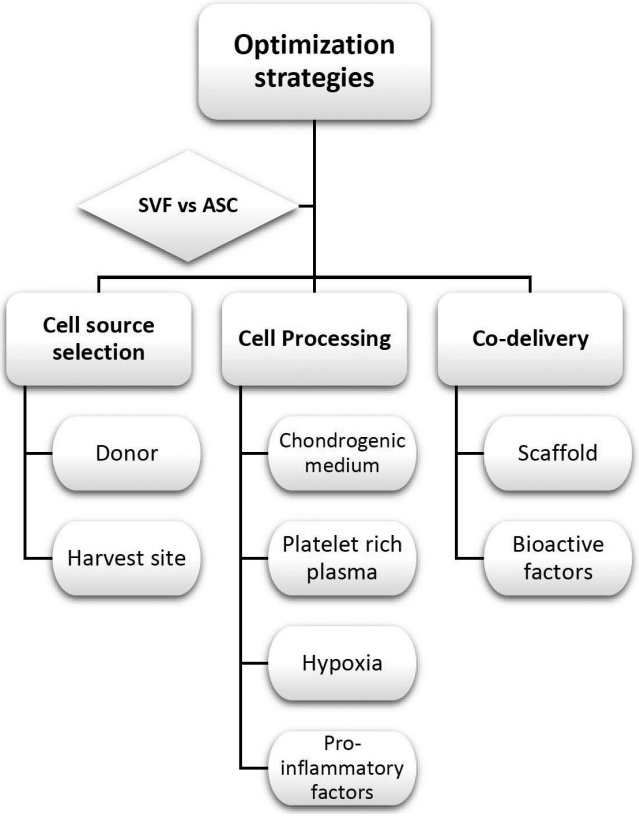

Fig. 1. Overview of the optimization strategies discussed in this work.

negative for CD31 and CD45 (Zuk, 2013). Recently in 2013, the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) proposed a set of minimal definitions for ASCs, including self-renewal ability, tri-lineage differentiation, being positive for CD90, CD73, CD105 and CD44 (>80%) while being negative for CD45 and CD31 (<2%) (Bourin et al., 2013). It was also suggested that ASCs can be distinguished from BMSCs by their positivity for CD36 and negativity for CD106 (Bourin et al., 2013). To note, multiple studies confirmed that CD34 appeared on the freshly isolated ASCs, which is typically not expressed on MSCs, but gradually disappeared during successive passages (Traktuev et al., 2008; Maumus et al., 2013).

Resembling BMSCs, ASCs secrete a variety of proteins into conditioned media, known as the trophic effect. Lately, more and more evidence suggests that paracrine effect of ASCs plays an important part during the regeneration process, exhibiting anti-apoptotic, anti-aging and anti-inflammatory abilities. For example, Platas et al. found an anti-aging effect of the conditioned medium of ASCs on OA chondrocytes, featured by down-regulation of senescence markers induced by inflammatory stress (Platas et al., 2016). A list of 68 commonly expressed proteins regardless of the specific protocols used, were thoroughly reviewed by Kapur and Katz (2013), which may serve as potential candidates of conserved secretome proteins for further research.

ASCs compared to BMSCs

The most attractive point of ASCs might be the much higher cell yield compared to BMSCs. As is known, BMSCs only constitute a very small fraction of the whole marrow nucleated cells. A recent study showed that even though the bone marrow contained 6-fold more nucleated cells than SVF, the adherent cells in SVF were 4-fold greater than bone marrow (Jang et al., 2015). Specifically, the MSC population (CD45-CD31-CD90+CD105+) was 4.28% in SVF and 0.42% in bone marrow concentration (Jang et al., 2015). It was calculated that there was a 500-fold increase in MSC yield from adipose tissue, with approximately $1\times10^{5\text{-}6}$ ASCs in 1 ml of lipoaspirate while only 50-675 BMSCs in 1 ml of bone marrow aspirate (Zuk et al., 2001; Hass et al., 2011; Li et al., 2011). Hence, the therapeutic dose of ASCs could be achieved without *in vitro* expansion, making it possible to finalize the stem cell transplantation during a one-step surgical procedure.

The proliferation potential of ASCs and BMSCs varies among species. BMSCs were reported to show higher proliferation potential than ASCs in some species, such as monkey (Izadpanah et al., 2006) and pig (Bayraktar et al., 2018). Conflicting results were reported for ASCs from mice (Ikegame et al., 2011), sheep (Ude et al., 2014), dog (Spencer et al., 2012) and human (Chen et al., 2012). Despite the species variation, the results for human cells were highly consistent (Izadpanah et al., 2006; Kern et al., 2006; Chen et al., 2012; Dmitrieva et al., 2012). Human ASCs (hASCs) demonstrated higher proliferation capacity than human MSCs (hMSCs) (Kern et al., 2006). In addition, hASCs can undergo more passages before senescence while maintaining the differentiation potential and a stable phenotype after a longer time of culture (Izadpanah et al., 2006; Dmitrieva et al., 2012). Furthermore, donor's age has less effect on the proliferation of hASCs, and this still holds true in elderly patients with osteoporosis (Chen et al., 2012), which makes it an attractive alternative to hBMSCs in clinical use.

ASCs might be better for allogenic transplantation than BMSCs. Multiple studies have demonstrated that ASCs expressed less HLA-ABC than BMSCs, indicating a lower immunogenicity (Rider et al., 2008). Furthermore, ASCs appear to have a better immunosuppressive capacity. Ivanova-Todorova et al. showed that ASCs inhibited the maturation and differentiation of human blood monocytes into DCs while enhancing the IL-10 level secreted by DCs, to a greater extent than BMSCs (Ivanova-Todorova et al., 2009). The feasibility of allogenic transplantation is particularly meaningful when the patients cannot provide enough stem cells themselves. Besides, it allows the future development of the off-the-shelf stem cell product, which is time-efficient and cost-effective.

Although several groups reported that chondrogenic potential *in vitro* of ASCs was lower than that of BMSCs (Rider et al., 2008; Diekman et al., 2010), it was also suggested that the combined use of growth factors such as transforming growth factor-β2 (TGF-β2), insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein 6 (BMP-6) could promote the chondrogenic capacity of ASCs to a comparable level to BMSCs (Hennig et al., 2007; Kim and Im, 2009). Collectively, current data indicates that ASCs could be an ideal alternative to BMSCs in cartilage regeneration.

Optimization strategies

Although ASCs have demonstrated encouraging results for cartilage regeneration in multiple animal studies and human trials, the regenerated cartilage is still inferior to the native tissue in many aspects, such as the compressive strength and the biochemical composition (Vilar et al., 2014). To achieve better clinical outcomes, ASC-based therapy needs further optimization.

SVF vs ASCs

SVF is the heterogeneous cell mix obtained after enzyme digestion of the lipoaspirates. Apart from ASCs, SVF mainly contains endothelia progenitor cells, immune cells, smooth muscle cells, pericytes and other stromal components. After isolation of the SVF, ASCs can be enriched by plastic adherence and subsequent expansion (Mitchell et al., 2006).

Currently, few studies have compared the cartilage

regeneration capacity between SVF and expanded ASCs. Jurgens et al. investigated the chondrogenic differentiation potential between SVF and culture-expanded ASCs seeded in PLA-CPL scaffold, where both of them showed similar characteristics with a slightly higher glycosaminoglycans (GAGs) deposition for SVF (Jurgens et al., 2009). Similarly, in a co-culture study with primary human chondrocytes in alginate gel, co-culture pellets of SVF-chondrocytes showed more GAGs and cartilage matrix deposition than that of ASCs-chondrocytes (Wu et al., 2016). The authors suggested that the synergic interaction between different types of cells might enhance the trophic effects of SVF (Wu et al., 2016). However, it cannot be concluded that SVF is superior to ASCs based on the weak evidence above without the evaluation of collagen type I and II content. Although no clear evidence indicates a better regenerative capacity of SVF, recent studies showed greater interest in applying SVF for clinical knee OA treatment. The main reason is that SVF transplantation is regarded as a one-step medical procedure with minimal manipulations in some countries such as Korea, whereas the expanded ASCs are generally considered as pharmaceutical products requiring rigorous clinical trials and regulatory approval (Pak et al., 2016a). In addition to the advantage in regulatory issues, SVF delivery can be done as a single surgical setting right in the operating theatre and on the same day, avoiding the time-consuming cell culture process as well as the contamination risk.

The most concern for SVF is the therapeutic efficacy. As mentioned above, current clinical data indicated that simple SVF injection is not sufficient to fully repair the damaged cartilage, especially when dealing with large cartilage lesions (Koh et al., 2014). The endothelial cells contained in SVF might induce angiogenesis, which could impede hyaline cartilage formation (Marsano et al., 2016; Staubli et al., 2017). Besides, most of the human trials using SVF required the co-delivery of platelet rich plasma (PRP) to achieve the reported therapeutic efficacy (Pak et al., 2016a), making the therapy more expensive and complicated.

Compared to SVF, culture-expanded ASCs have several advantages. First, a large dose of ASCs can be obtained after expansion. In a randomized, double-blinded and dose-escalation clinical trial conducted by Jo et al., the high dose group (1×10^8 ASCs) showed the best hyaline-like cartilage regeneration and functional recovery outcomes, while the low dose group (1×10^7 ASCs) almost did not show any improvement, indicating the importance of a larger stem cell dose (Jo et al., 2014). When the volume of SVF is not sufficient to reach the minimal therapeutic dose, an *in vitro* expansion procedure might be necessary (Jo et al., 2014; Pers et al., 2016). Apart from the dosage issue, another important point is the potential to enhance therapeutic efficacy through combination with various promising tissue engineering techniques, such as preconditioning and co-delivery, during or after the culture expansion process (Clevenger et al., 2016). Last but not least, purified and expanded ASCs may have a lower immunogenicity and higher immunosuppression property compared to the freshly isolated SVF, making it more suitable for allogenic stem cell transplantation. It is even possible to develop the off-the-shelf stem cell product, which is highly cost-effective and convenient for patients (McIntosh et al., 2006).

The main concern about expanded ASCs is the potential for malignant transformation. In vitro cultured human BMSCs were reported to acquire chromosomal aberrations eventually leading to genomic instability and tumorigenicity, so it is possible that ASCs would also undergo malignant transformation during culture expansion (Buyanovskaya et al., 2009; Tarte et al., 2010; Ben-David et al., 2011). However, so far there is no direct evidence of malignant transformation related to cultured hASCs. In a recent study investigating genomic stability of ASCs, *in vitro* expanded ASCs did not show genetic alterations and replicative senescence nor anchorage-independent growth during early passages (Neri et al., 2013). The safety of expanded ASCs has also been demonstrated on humans, without tumor development or any serious adverse events (Ra et al., 2011; Pers et al., 2016). Taken together, it is extremely important to push forward the evaluation of culture-expanded ASCs for cartilage regeneration.

Cell source selection

Different cell sources can have a great impact on the therapeutic efficacy of ASCs. Two main factors are donor's physiological condition and harvest site.

Donor

The influence of donor's age is controversial. It was shown in some studies that the proliferation of ASCs from elders was lower than that of younger subjects (Van Harmelen et al., 2004; Efimenko et al., 2011; Alt et al., 2012). The chondrogenic differentiation of ASCs was also decreased by aging in one study (Alt et al., 2012). On the contrary, some other studies found no significant correlation between these properties and aging (Chen et al., 2012; Ding et al., 2013; Abbo et al., 2017). It was suggested that the wide inconsistency could be due to the differences in donor's gender, the sources of the adipose tissue and the culture conditions (Clavijo-Alvarez et al., 2006; Ding et al., 2013). Interestingly, the results were consistent in two studies both using the low-calcium keratinocyte serum free medium (KFSM) during the cell culture, supporting the irrelevance between aging and proliferation rate of ASCs (Chen et al., 2012; Ding et al., 2013). Another concern is that ASCs might be influenced by donor's chronic diseases, since ASCs used for autologous transplantation come from these patients themselves. While in some studies obesity and diabetes showed a negative effect on the proliferation potential and clonogenic capacity of ASCs (Gu et al.,

2012), the OA condition of the knee did not show any influence on ASCs or the cellular composition in adipose tissue (Pires de Carvalho et al., 2014).

Harvest site

For ASCs from different body sites, the results showed more consistency. Pires de Carvalho et al. compared the infrapatellar fat pad (IPFP) to the periarticular subcutaneous adipose tissue (SQ) of 7 subjects and found that ASCs derived from both sites exhibited similar characteristics (Pires de Carvalho et al., 2014). Grasys et al. investigated the lipoaspirates from abdomen, thigh and knee, demonstrating no significant differences in the content of soluble factors nor the yield, proliferation and percentage of ASCs (Grasys et al., 2016). Nonetheless, the evaluation of chondrogenic differentiation and cartilage regeneration efficacy of ASCs from different body sites is rare.

With the conflicting results, hardly any suggestions can be made towards the donor selection. However, multiple studies have confirmed that harvest site does not affect the property of ASCs. Hence the ideal harvest site of ASCs seems to be the subcutaneous abdominal adipose tissue. In addition to the promising outcomes in multiple clinical trials, patients can also rest in a comfortable supine position during the surgery (Riis et al., 2015). Furthermore, most of the surgeons prefer the abdomen as the primary harvest site as well, according to a survey conducted in 2007, probably because in this case they will have no need to consider the asymmetry issue (Kaufman et al., 2007; Riis et al., 2015).

Cell processing

Differentiation of stem cells along the chondrogenic lineage is important for cartilage repair (Estes et al., 2010). It was shown that ASCs expanded in conventional growth medium without appropriate pretreatment could have a negative effect on the chondrocytes and inhibit cartilage regeneration (Lee et al., 2012). Cell proliferation and *in vivo* therapeutic function of ASCs can be greatly improved through *in vitro* cell processing. There can be a variety of cell processing techniques, herein we discuss some of the most attractive ones described in recent publications.

Chondrogenic medium (CM)

The chondrogenic medium (CM) can induce ASCs to a chondrocyte-like phenotype. The active ingredients of CM mainly include dexamethasone (Dex), ascorbic acid 2-phosphate (AA2P), transforming growth factor beta family (TGFβ) and bone morphogenetic protein family (BMP) (Estes et al., 2010). ASCs cultured in CM showed elevated levels of chondrogenic-specific genes such as Sox-9 together with increased production of collagen type II and GAG (Awad et al., 2004a; Im et al., 2006; Kolambkar et al., 2007). The positive function of growth factors, such as TGF-β1, TGF-β3 and BMP-6, was also demonstrated when incorporated in ASC-seeded scaffolds to repair cartilage defects, resulting in a significant increase of cell proliferation and chondrogenic marker expression (Sukarto et al., 2012; He and Pei, 2013; Yin et al., 2015).

However, recent studies found that different components of CM might have distinct effects on ASCs, and their functions could be largely influenced by the concentration, the patient's disease state, and the interaction between different growth factors. For example, TGF-β1 is considered to have a biphasic effect under different concentrations. It was reported that TGF-β1 inhibited endothelial cell invasion and induced ASC chondrogenesis in relatively high concentrations (5 to 10 ng/ml) while exhibiting opposite effects in low concentrations (0.1 to 1 ng/ml) (Iruela-Arispe and Sage, 1993; Pepper et al., 1993; Estes et al., 2006). Moreover, TGF-β1 showed different effects on ASCs from different donors, probably because of the differential expression of TGFβ receptors such as ALK5 in osteoarthritic cartilage (Blaney Davidson et al., 2006, 2009). Lee et al. compared different components of CM, and demonstrated their distinct effects on growth factor secretion of ASCs (Lee et al., 2013). In this study, AA2P appeared to be the most beneficial CM component. AA2P enhanced the secretion of chondrogenic factors (IGF-1, TGF-β2) and reduced the secretion of angiogenic factor (VEGF-A) and the mRNA level of chondrocyte hypertrophy factor (FGF-18), while VEGF-A was previously found to inhibit cartilage regeneration in rats (Lee et al., 2012, 2013). Their study also provided some evidence of the interaction between growth factors, which remains to be further elucidated (Lee et al., 2013). Future studies need to work out the optimal concentration and combination of growth factors in the chondrogenic medium, with the disease state of patients taken into consideration.

Platelet rich plasma (PRP)

PRP is isolated from the autologous blood by centrifugation, containing highly concentrated platelets and a hematocrit typically below 5% (Centeno et al., 2008). A rich source of growth factors is contained in the concentrated platelets via an intricate vesicular storage system, which can be immediately released by platelet activation. The growth factors in PRP mainly include basic fibroblast growth factor (FGF-2), insulin-like growth factor-1 (IFG-1), transforming growth factors (TGFs), platelet-derived growth factors (PDGFs), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and various kinds of Interleukins (IL) (Fréchette et al., 2005; Scioli et al., 2017). The potential immunogenicity and the risk of disease transmission make it unsuitable to culture ASCs with fetal bovine serum (FBS) when intended for clinical use (Bieback, 2013). While the cell functions are compromised in various serum-free solutions and the

human serum is short of source, PRP seems an ideal substitute to FBS for stem cell culture (Bieback, 2013). Hildner et al. reported that ASCs cultured in 5% platelet lysate showed better proliferation and *in vitro* chondrogenic (re)differentiation than in 10% FBS (Hildner et al., 2015). The growth rate of ASCs in 10% tPR was even much faster than in 10% FBS when using a novel rapid thrombin activation method to prepare the platelet lysate (tPR), with an at least 3-fold increase while maintaining the multipotency of ASCs (McLaughlin et al., 2016). It was suggested that the increased cell proliferation was due to the avoidance of heparin during tPR preparation, as heparin could inhibit the proliferation of ASCs (Kocaoemer et al., 2007; McLaughlin et al., 2016). Despite these encouraging results, the appropriate concentration of PRP in culture medium needs further investigation before it can eventually replace FBS.

Hypoxia

The chondrocytes in cartilage tissue reside in a hypoxia environment with only 1-6% O_2 in the deepest zone (Treuhaft and Mccarty, 1971). In vitro tests showed that the hypoxia with 1-5% oxygen tension significantly improved the proliferation and chondrogenesis of ASCs, while the morphology and surface markers did not change (Merceron et al., 2010; Portron et al., 2013; Choi et al., 2014). The analysis on growth factor expression of co-cultured ASCs and chondrocytes under hypoxia showed a significant increase of hypoxia-inducible factor-1α (HIF-1α) (Shi et al., 2016), supporting the previous finding that HIF-1α played an important role in mediating the effects of low oxygen tension (Schipani et al., 2001; Malladi et al., 2007). HIF-1α upregulates the transcriptional activity of SOX9 by binding on specific hypoxia-responsive element sequences, stimulating the extracellular matrix (ECM) synthesis of ASCs (Robins et al., 2005; Amarilio et al., 2007). Consistently, the most regulated proteins of ASC after hypoxia preconditioning were involved in the ECM synthesis and cell metabolism, confirming the ECM remodeling through HIF-1α pathway as a main mechanism of hypoxia (Riis et al., 2016). Despite the convincing *in vitro* studies, the direct evidence supporting a similar function of hypoxia *in vivo* is quite limited. It was even shown in one study that hypoxia could not exert the expected beneficial functions on *in vivo* cartilage regeneration in rabbits and humans (Portron et al., 2013). However, this could be due to various reasons, for example, the Si-HPMC hydrogel as the cell scaffold in this study might already create a hypoxia environment (Portron et al., 2013). More data from *in vivo* studies is required to confirm the function of hypoxia on cartilage regeneration, and a standard protocol of hypoxia pretreatment should be established.

Pro-inflammatory factors

Preconditioning with pro-inflammatory factors seems to be highly promising. The anti-inflammation effect of ASCs is crucial for its therapeutic function. Recent studies showed that the anti-inflammatory function of ASCs can be stimulated by various pro-inflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor (TNF), and especially the interferon-gamma (IFNγ) (Crop et al., 2010). Maumus et al. investigated IFNγ-primed ASCs on murine OA model, and found significantly enhanced anti-inflammatory and chondroprotective effects both *in vitro* and *in vivo* (Maumus et al., 2016). The authors suggested that the positive effect of IFNγ preconditioning was associated with the modulation of ASC secretome, and the effect of IFNγ-priming on the inflammatory gene profile of ASCs is shown in Fig. 2 (Maumus et al., 2016).

Besides, the therapeutic function of ASCs was

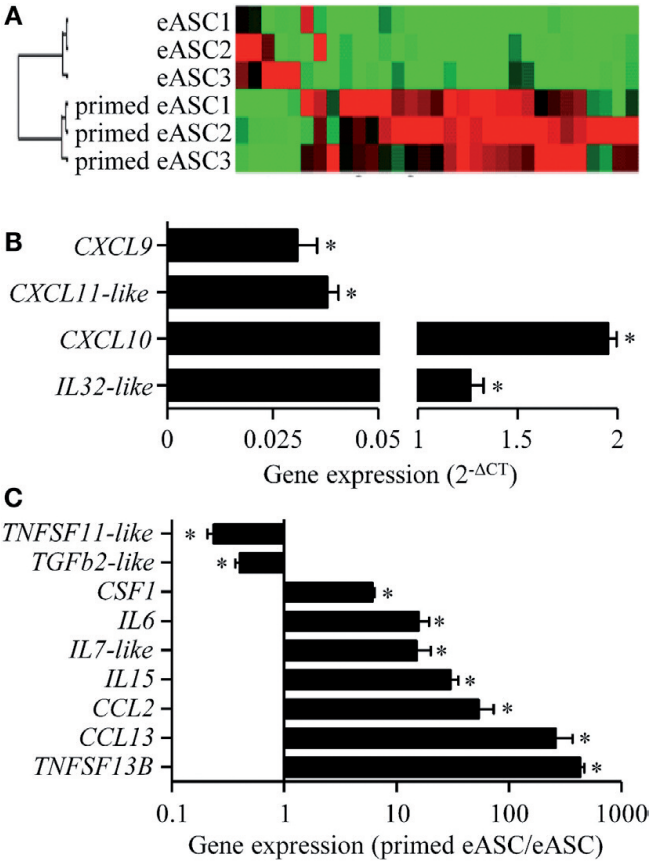

Fig. 2. Effect of IFNγ-priming on the inflammatory gene profile of eASCs. Gene array analysis of inflammatory cytokines and chemokines mRNA compared naïve and IFNγ-primed eASCs. **A.** Hierarchical clustering comparing naïve or IFNγ-primed eASCs. **B.** Induced gene expression levels in IFNγ-primed eASCs expressed as relative expression (2-ΔCT). **C.** Significantly modulated gene expression levels in IFNγ-primed eASCs. Results are represented as mean ± SEM for three independent biological replicates. Data were analyzed using the Mann-Whitney test. *p<0.05. (Adapted from Front. Immunol. 2016; 7: 392, "Utility of a Mouse Model of Osteoarthritis to Demonstrate Cartilage Protection by IFNγ-Primed Equine Mesenchymal Stem Cells" by Maumus et al., used under CC BY).

largely influenced by the inflammation status of the diseased joints, exhibiting no significant effect without severe inflammation or when they were delivered at the late stage of OA (Ter Huurne et al., 2012; Schelbergen et al., 2014). The pro-inflammatory cytokines released by the synovial macrophages during the early phase of OA might be responsible for activating the injected ASCs, which was reflected by the level of alarmins S100A8/A9 before and after the cell delivery (Schelbergen et al., 2014). And in turn the delivered ASCs could decrease these secreted pro-inflammatory factors by switching the activated-M1-like inflammatory macrophages to a M2-like phenotype (Manferdini et al., 2017). These studies indicated that in some cases pro-inflammatory factor pretreatment on ASCs might even be necessary.

Co-delivery

The delivery of ASCs alone is not sufficient to regenerate the damaged cartilage. A high rate of abnormality was found in mechanical property, chemical composition and biological function of the neo-formed cartilage, regardless of the cell dose (Jo et al., 2014; Koh et al., 2014; Pers et al., 2016). Among various co-delivery strategies, the cell-seeded scaffolds are being most widely discussed in recent publications, showing great potential in future clinical translation (summarized in Table 1).

Scaffold

Directly injected cells usually have limited cell retention and survival at the target site. The feasibility of intra-articular (IA) injected ASCs for knee joint repair was questioned, because only 15% of IA injected ASCs was detectable in the joint of experimental mice after one month, and this number further decreased to 1.5% in six months (Maumus et al., 2013). In a recent clinical trial, 76% of all patients (37 in total) showed abnormality in cartilage repair following direct ASC injection, especially those with large cartilage lesions (≥5.4 cm^2) (Koh et al., 2014). It was suggested that an appropriate cell scaffold should be developed for treating patients with large cartilage defects, since ASCs seeded in scaffolds may have better viability, retention and aggregation (Koh et al., 2014). The same group evaluated fibrin glue as a scaffold for ASC implantation. The results showed a significant difference in International Cartilage Repair Society (ICRS) grades between the scaffold group and non-scaffold group, where 12 of the 17 lesions (58%) and 9 of the 39 lesions (23%) achieved a grade I or II in each group, respectively (Kim et al., 2015). Their preliminary clinical data provides direct evidence that a well-designed bioactive scaffold can really improve the therapeutic function of ASCs for cartilage regeneration. However, the *in vivo* evaluation of ASC-seeded scaffolds for cartilage regeneration remains scarce. The beneficial effects of scaffolds on *in vitro* proliferation, adhesion, migration and chondrogenic differentiation of ASCs were also reported in other studies with convincing data, although in these studies the *in vivo* characterizations of ASC-seeded scaffolds did not set the control group of ASCs alone (Zhang et al., 2013; Li et al., 2015; Scioli et al., 2017; Gwon et al., 2017). Thus, the beneficial role of scaffolds on ASCs needs further investigation, particularly in large-scale randomized and double-blinded human trials.

There is a considerable amount of recent papers discussing the influence of scaffolds on ASCs. However,

Table 1. ASC-seeded scaffolds for cartilage regeneration reviewed in this work.

Material	Model	Conclusion	Reference
CDM scaffold	Rabbit	ASC-ECM scaffold regenerates hyaline cartilage, comparable to native cartilage	Kang et al., 2014
Chitosan hydrogel + RGD/growth factors	N/A	Young's modulus affects ASC viability and retention; RGD peptide improves ASC viability; growth factor-loaded MPs enhance ASC chondrogenesis	Sukarto et al., 2012
Genipin-crosslinked CDM scaffold	N/A	Chemical crosslinking by genipin prevents scaffold contraction while preserving the chondrogenic potential of CDM	Cheng et al., 2013
Poly(L-glutamic acid)-chitosan scaffold	Rabbit	PLGA/CHI scaffold repairs full-thickness cartilage defects, comparable to native cartilage	Zhang et al., 2013
CDM-chitosan hydrogel	N/A	Col II enhances ASC condensation and chondrogenesis through increased cell–matrix adhesion, mediated by integrin α10	Choi et al., 2014
CDM-PCL scaffold	N/A	Multi-layer electrospun constructs enhance cell infiltration; inclusion of CDM stimulates chondrogenesis	Garrigues et al., 2014
CDM scaffold	N/A	Scaffolds with larger pore size have greater ASC migration, proliferation, and chondrogenic differentiation	Almeida et al., 2015
TGF-β1-conjugated chitosan hydrogel	Rat	Covalently conjugated TGF-β1 via SMCC linker significantly reduces burst release	Choi et al., 2015
Poly(L-glutamic acid)-chitosan scaffold	Rabbit	Non-fouling scaffold drives ASCs into multicellular spheroids, which facilitates hyaline-like cartilage Regeneration	Zhang et al., 2015
Fibrin glue	Human	Scaffolds improve the cartilage regeneration capacity of ASCs for large cartilage lesions	Kim et al., 2015
Type I collagen scaffold + PRP/insulin	N/A	3D culture and PRP/insulin treatment enhance ASC chondrogenesis	Scioli et al., 2017
Heparin-HA hydrogel	N/A	Hydrogel degradation is necessary for 3D cellular activities; Hep-HA superior to Hep-PEG and PEG-HA	Gwon et al., 2017

most of them focused their attention on natural scaffolds instead of synthetic scaffolds. One reason is that some novel scaffolds fabricated by natural polymers exhibited impressive bioactivity. For example, multiple studies described the superiority of CDM and collagen scaffolds for cartilage tissue engineering. The natural polymeric molecules contained in these scaffolds, especially collagen type II (Col II), were able to stimulate chondrogenic differentiation of ASCs (Cheng et al., 2013; Portron et al., 2013; Choi et al., 2014; Garrigues et al., 2014; Kang et al., 2014; Almeida et al., 2015; Scioli et al., 2017). ASCs underwent morphological and ultrastructure changes when seeded in 3D collagen scaffold (Scioli et al., 2017). It was suggested that collagen type II promotes chondrogenic differentiation of ASCs by evoking a round cell shape through beta1 integrin-mediated Rho A/Rock signaling pathway (Lu et al., 2010). Consistently, it was found that Col II enhanced condensation and chondrogenesis of ASCs through increased cell–matrix adhesion, and this was mainly mediated by integrin $\alpha 10\beta 1$-Col II interaction (Choi et al., 2014). Notably, the implantation of scaffolds containing allogenic or xenogeneic materials, such as collagen, could induce the host immune response (Hassanbhai et al., 2017). However, very little attention is given to this issue (Badylak and Gilbert, 2008; Keane and Badylak, 2015). The influence of the potential immune response to patients and cartilage regeneration process induced by biological scaffold materials needs careful examination before clinical application.

Tailoring the physical parameters of scaffolds can significantly influence the cellular behavior of ASCs. The effect of Young's modulus showed a non-linear pattern. The viability and retention of ASCs were enhanced in the chitosan gel when the Young's modulus was between 225 and 380 kpa (Sukarto et al., 2012). In accordance, ASCs showed decreased cell attachment and ECM formation on the genipin-crosslinked CDM scaffold when the crosslinking degree increased from 50% to 89% (Cheng et al., 2013). Teong et al. demonstrated that modulating the stiffness of methacrylated hyaluronan (MeHA) hydrogel could enhance the chondrogenesis of ASCs, and the hydrogel with 140% degree of methacrylation (8 kPa) exhibited the highest rates of GAG and collagen type II synthesis (Teong et al., 2018). The pore size of porous scaffolds also affects the cartilage repair ability of ASCs. It was shown that the migration, proliferation, and chondrogenic differentiation of ASCs were enhanced in scaffolds with the larger pore size (Im et al., 2012; Almeida et al., 2015). And the enhanced performance of ASCs was confirmed when regenerating cartilage in rabbits (Im et al., 2012). However, there is limited data from *in vivo* studies that can help to understand its inherent molecular mechanism.

The importance of cell-binding affinity and biodegradability were also highlighted recently. It was demonstrated that stem cell spreading, proliferation, adhesion and migration was much better in heparin-HA hydrogel compared to heparin-PEG and HA-PEG hydrogel, due to the combination of the cell-binding affinity from heparin and the biodegradability from HA (Gwon et al., 2017). In addition, HA was also able to initiate and enhance ASCs chondrogenesis through increased cell-matrix adhesion via HA-CD44 interaction (Wu et al., 2010, 2013). Consistently, grafting RGD-contained peptides onto N-methacrylate glycol chitosan (MGC) gel increased the *in vitro* viability and retention of encapsulated ASCs (Sukarto et al., 2012). However, of our particular interest, Zhang et al. claimed that the excessive cell adhesion and spreading on scaffolds might actually have negative influence on ASC chondrogenesis, generating fibrous tissue in neo-cartilage, therefore limiting the success of current scaffold technology (Zhang et al., 2015). By designing an anti-adhesive poly(L-glutamic acid)/chitosan (PLGA/CS) scaffold and another cell-adhesive PLGA/CS scaffold through a combination of air-drying and freeze-drying procedures, they demonstrated that ASCs in anti-adhesive scaffold exhibited high-level GAG and collagen type II but low-level collagen type I deposition both *in vitro* and *in vivo*, similar to normal cartilage (shown in Fig. 3), as opposed to the cell-adhesive group (Zhang et al., 2015). It was suggested that ASCs formed multicellular spheroids through spontaneous cellular aggregation on the anti-adhesive scaffold, mimicking the "condensation" step during embryonic limb development which promoted chondrogenesis (Zhang et al., 2015). This study provided a unique strategy to design new scaffolds which might better mimic the *in vivo* biological environment.

In summary, with the safety of ASC-seeded scaffolds already demonstrated in multiple pre-clinical animal models and small-scale humans trials (Kim et al., 2015), the next step is to confirm their safety and efficacy in large-scale human trials. And the ASC-seeded scaffolds should be compared with the delivery of ASCs alone. In addition, the material, structure and physicochemical parameters of scaffolds require further optimization based on the latest findings to provide better regenerative capacity for ASCs.

Bioactive factors

Co-delivery of growth factors or PRP is another effective way to enhance the proliferation and chondrogenesis of ASCs (Li et al., 2015; Yin et al., 2015; Scioli et al., 2017). In fact, the co-delivery of PRP as a source of growth factors has already been applied to most of the recent clinical trials using SVF (Pak et al., 2016a,b). Nevertheless, the delivery of growth factors in these studies was still in an uncontrolled manner. Some growth factors might not exert the expected function when delivered in an inappropriate concentration, for example, the TGF-$\beta 1$. The homogenous and sustained drug release could be achieved by covalent conjugation

of growth factors onto the scaffolds. Choi et al. significantly reduced the burst release by covalently linking TGF-β1 to the chitosan hydrogel via SMCC linker (Choi et al., 2015). Another choice is encapsulating growth factors in drug carrier systems, such as microspheres (Sukarto et al., 2012; Yin et al., 2015; Deepthi and Jayakumar, 2016). Certain natural materials could be used to fabricate the microspheres, such as chondroitin sulphate and ECM, and these natural-derived molecules might enhance the bioactivity of the co-delivered scaffolds, especially the synthetic scaffolds (Gibson et al., 2014; Deepthi and Jayakumar, 2016). However, before moving into human trials, the safety of micro- or nano-phase drug carriers still needs careful investigation for any potential side-effects.

Future perspective

Various pre-operative optimization strategies have been investigated recently, including cell source selection, preconditioning and co-delivery. Despite the encouraging results already achieved, few of these methods has really moved into the clinical stage. One reason is that the underlying mechanisms behind these methods are not fully understood. Besides, the healing process of treated cartilage and the biological process ASCs undergo following delivery needs further elucidation. If these basic questions were clearly answered, the optimized therapies could achieve a more consistent result in pre-clinical studies, thereby accelerating their pace into clinical stage.

Except for the pre-operative optimization, some post-operative strategies can also be considered. Some pilot studies demonstrated that special mechanical stimuli can induce ASC chondrogenesis, for example, low-intensity ultrasound (Shafaei et al., 2013). In the future, effective physical therapies could be designed to further improve the therapeutic function of ASCs post-operatively, as a supplement to the surgical treatments.

To conduct large-scale clinical trials and eventually enter the market, more efforts should be made on the stem cell quality control. Recently, some pilot studies have already been conducted. For liposuction, the commercialized Bodyjet® water-jet-assisted liposuction and ultrasound-assisted liposuction (UAL) have shown no influence on the cell yield, viability and

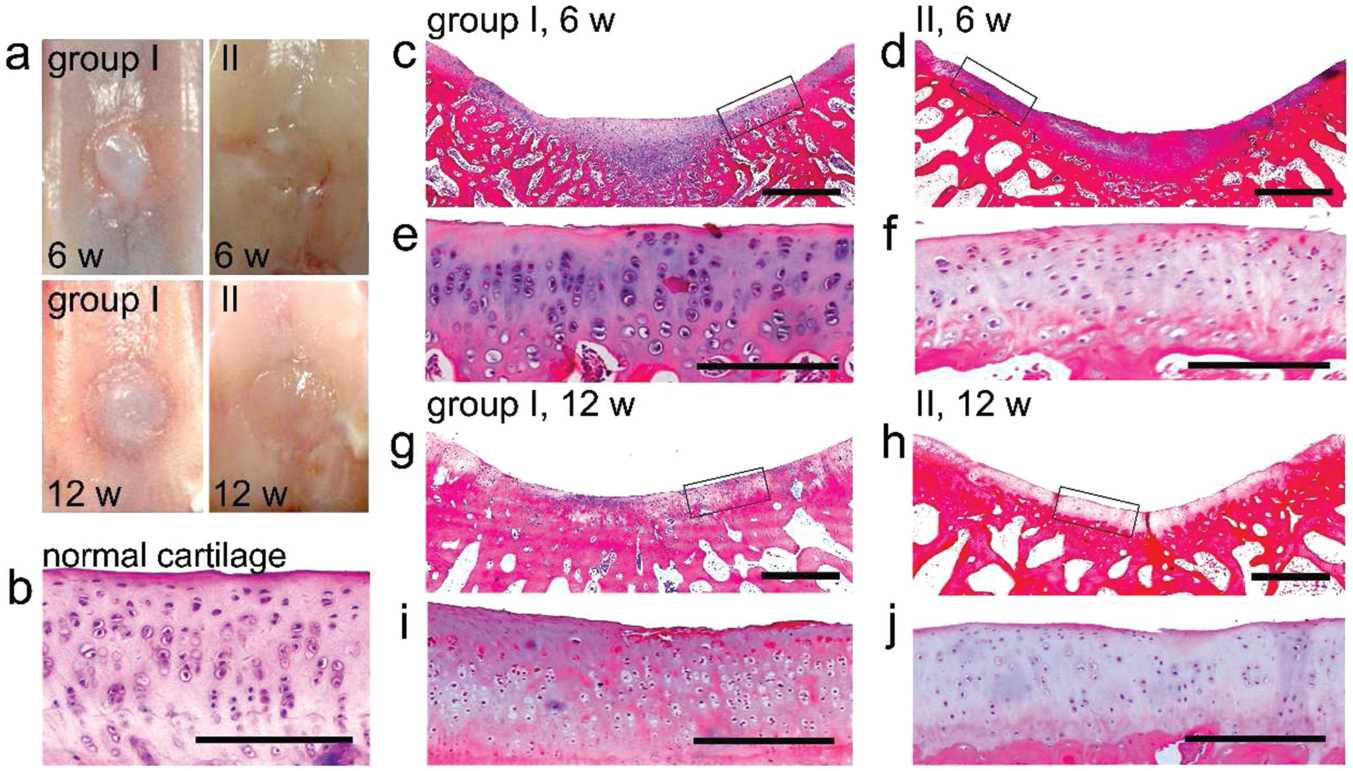

Fig. 3. Morphological evaluation of neo-cartilage in critical-size rabbit cartilage defects. Defect repaired with the ASC spheroids/scaffold A construct was set as group I. Defect repaired with adherent ASCs/scaffold B construct was set as group II. a, Gross appearance of the neo-cartilage at 6 w and 12 w. b, H&E staining of normal cartilage. H&E staining of group I **(c)**, group II **(d)** at 6 w, and group I **(g)**, group II **(h)** at 12 w. **e, f, i and j** were higher-magnification images selected from the regenerated areas outlined by the rectangles in **c, d, g and h**, respectively. H&E staining showed that cells in the regenerated tissue of group I possessed larger volume and more obvious cartilage lacuna structure than those in group II. And the arrangement features of cells in group I were more obvious. (Adapted from Biomaterials 2015, 71: 24-34, with permission from Elsevier). Scale bars: c, d, g, h, 1000 μm; b, e, f, i, g, 250 μm.

differentiation potential of ASCs, demonstrating good compatibility for ASC production (Bony et al., 2015; Duscher et al., 2016). For SVF isolation, fully-automatic systems are attractive due to the minimal manipulation which minimizes the contamination risk and variability between batches. Incellator® (Tissue Genesis™) and Celution® (Cytori) are two kinds of commercialized systems that automatically isolate SVF from adipose tissue, with comparable or even better efficiency compared to the manual procedure (Riis et al., 2015).

Short-term hypothermic preservation, which is necessary for quality control inspection and cell product transportation, remains an unsolved problem because of the cell injury and death upon rewarming. Hajmousa et al. proposed a modified 6-chromanol SUL-109 as a novel single molecule cell preservation additive agent in the culture medium to protect ASCs from hypothermic damage while maintaining the differentiation capacity, showing great potential for future application (Hajmousa et al., 2017).

Conventional static monolayer culture techniques in the laboratory are not suitable in industry due to low efficiency, risk of contamination and difficulties in quality control. Thus, a well-designed novel culture system needs to be developed. Yu et al. fabricated a novel non-chemically crosslinked decellularized adipose tissue (DAT) porous microcarrier for dynamic culture of ASCs (Yu et al., 2017). After over one month of continuous culture, ASCs that expanded on the DAT microcarriers maintained their immunophenotype and multilineage differentiation capacity while exhibiting stronger chondrogenesis than baseline control, showing great promise in ASC mass culture (Yu et al., 2017).

Conclusion

Adipose-derived stem cells are an ideal alternative to BMSCs, due to the similar regenerative capacity but more abundant source and easier accessibility. To date, most of the completed clinical trials delivered ASCs in the form of SVF and reported safety and efficacy for cartilage regeneration, exhibiting great potential for clinical knee osteoarthritis treatment. However, simple SVF injection is not sufficient to fully restore the damaged cartilage back toward normal function, thus an optimized ASC therapy is needed for an enhanced therapeutic function. Based on current technology, culture-expanded ASCs are more promising than crude SVF in future application. Among various optimization strategies investigated recently, preconditioning and co-delivery showed the most encouraging results in preliminary studies, but more convincing data from well-designed pre-clinical and clinical trials are needed before practical use. Due to the bright future of expanded ASCs, techniques involving isolation, expansion and storage of ASC products also need to be explored to ensure the good quality of stem cell products and the smooth industrial translation.

Acknowledgements. The authors would like to thank for the financial support from Shanghai Telebio Biomedical Co., Ltd.

Disclosures. The authors indicate no potential conflicts of interest.

References

Abbo O., Taurand M., Monsarrat P., Raymond I., Arnaud E., De Barros S., Auriol F., Galinier P., Casteilla L. and Planat-Benard V. (2017). Comparison between pediatric and adult adipose mesenchymal stromal cells. Cytotherapy 19, 395-407.

Almeida H.V., Cunniffe G.M., Vinardell T., Buckley C.T., O'Brien F.J. and Kelly D.J. (2015). Coupling freshly isolated CD44(+) infrapatellar fat pad-derived stromal cells with a TGF-β3 eluting cartilage ECM-derived scaffold as a single-stage strategy for promoting chondrogenesis. Adv. Healthc. Mater. 4, 1043-1053.

Alt E.U., Senst C., Murthy S.N., Slakey D.P., Dupin C.L., Chaffin A.E., Kadowitz P.J. and Izadpanah R. (2012). Aging alters tissue resident mesenchymal stem cell properties. Stem Cell Res. 8, 215-225.

Amarilio R., Viukov S.V., Sharir A., Eshkaroren I., Johnson R.S. and Zelzer E. (2007). HIF1α regulation of Sox9 is necessary to maintain differentiation of hypoxic prechondrogenic cells during early skeletogenesis. Development 134, 3917-3928.

Awad H.A., Halvorsen Y.D., Gimble J.M. and Guilak F. (2004a). Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells. Tissue Eng. 9, 1301-1312.

Awad H.A., Wickham M.Q., Leddy H.A., Gimble J.M. and Guilak F. (2004b). Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. Biomaterials 25, 3211-3222.

Badylak S.F. and Gilbert T.W. (2008). Immune response to biologic scaffold materials. Semin. Immunol. 20, 109-116.

Bae D.K., Yoon K.H. and Song S.J. (2006). Cartilage healing after microfracture in osteoarthritic knees. Arthroscopy 22, 367-374.

Baer P.C. and Geiger H. (2012). Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. Stem Cells Int. 2012, 812693.

Bayraktar S., Jungbluth P., Deenen R., Grassmann J., Schneppendahl J., Eschbach D., Scholz A., Windolf J., Suschek C.V. and Grotheer V. (2018). Molecular- and microarray-based analysis of diversity among resting and osteogenically induced porcine mesenchymal stromal cells of several tissue origin. J. Tissue Eng. Regen. Med. 12, 114-128.

Ben-David U., Mayshar Y. and Benvenisty N. (2011). Large-scale analysis reveals acquisition of lineage-specific chromosomal aberrations in human adult stem cells. Cell Stem Cell 9, 97-102.

Bert J.M. (1993). Role of abrasion arthroplasty and debridement in the management of osteoarthritis of the knee. Rheum. Dis. Clin. North Am. 19, 725-739.

Bhatia D., Bejarano T. and Novo M. (2013). Current interventions in the management of knee osteoarthritis. J. Pharm. Bioallied Sci. 5, 30-38.

Bieback K. (2013). Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transfus. Med. Hemother. 40, 326-335.

Black L.L., Gaynor J., Gahring D., Adams C., Aron D., Harman S.,

Gingerich D.A. and Harman R. (2007). Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. Vet. Ther. 8, 272-284.

Blaney Davidson E.N., Vitters E.L., van der Kraan P.M. and van den Berg W.B. (2006). Expression of transforming growth factor-beta (TGFbeta) and the TGFbeta signalling molecule SMAD-2P in spontaneous and instability-induced osteoarthritis: role in cartilage degradation, chondrogenesis and osteophyte formation. Ann. Rheum. Dis. 65, 1414-1421.

Blaney Davidson E.N., Remst D.F., Vitters E.L., van Beuningen H.M., Blom A.B., Goumans M.J., van den Berg W.B. and van der Kraan P.M. (2009). Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. J. Immunol. 182, 7937-7945.

Bony C., Cren M., Domergue S., Toupet K., Jorgensen C. and Noël D. (2015). Adipose mesenchymal stem cells isolated after manual or water-jet-assisted liposuction display similar properties. Front. Immunol. 6, 655.

Bourin P., Bunnell B.A., Casteilla L., Dominici M., Katz A.J., March K.L., Redl H., Rubin J.P., Yoshimura K. and Gimble J.M. (2013). Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Ccience (IFATS) and the International Society for Celluar Therapy (ISCT). Cytotherapy 15, 641-648.

Buyanovskaya O.A., Kuleshov N.P., Nikitina V.A., Voronina E.S., Katosova L.D. and Bochkov N.P. (2009). Spontaneous aneuploidy and clone formation in adipose tissue stem cells during different periods of culturing. Bull. Exp. Biol. Med. 148, 109-112.

Centeno C.J., Busse D., Kisiday J., Keohan C., Freeman M. and Karli D. (2008). Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician 11, 343-353.

Centeno C.J., Schultz J.R., Cheever M., Freeman M., Faulkner S., Robinson B. and Hanson R. (2011). Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr. Stem Cell Res. Ther. 6, 368-378.

Chen H.T., Lee M.J., Chen C.H., Chuang S.C., Chang L.F., Ho M.L., Hung S.H., Fu Y.C., Wang Y.H., Wang H.I., Wang G.J., Kang L. and Chang J.K. (2012). Proliferation and differentiation potential of human adipose-derived mesenchymal stem cells isolated from elderly patients with osteoporotic fractures. J. Cell. Mol. Med. 16, 582-593.

Cheng N.C., Estes B.T., Young T.H. and Guilak F. (2013). Genipin-crosslinked cartilage-derived matrix as a scaffold for human adipose-derived stem cell chondrogenesis. Tissue Eng. Part A 19, 484-496.

Choi B., Kim S., Lin B., Wu B.M. and Lee M. (2014). Cartilaginous extracellular matrix-modified chitosan hydrogels for cartilage tissue engineering. ACS Appl. Mater. Interfaces. 6, 20110-20121.

Choi B., Kim S., Fan J., Kowalski T., Petrigliano F., Evseenko D. and Lee M. (2015). Covalently conjugated transforming growth factor-β1 in modular chitosan hydrogels for the effective treatment of articular cartilage defects. Biomater. Sci. 3, 742-752.

Choi J.R., Pingguan-Murphy B., Wan Abas W.A., Noor Azmi M.A., Omar S.Z., Chua K.H. and Wan Safwani W.K. (2014). Impact of low oxygen tension on stemness, proliferation and differentiation potential of human adipose-derived stem cells. Biochem. Biophys. Res. Commun. 448, 218-224.

Clavijo-Alvarez J.A., Rubin J.P., Bennett J., Nguyen V.T., Dudas J., Underwood C. and Marra K.G. (2006). A novel perfluoroelastomer seeded with adipose-derived stem cells for soft-tissue repair. Plast. Reconstr. Surg. 118, 1132-1142.

Clevenger T.N., Luna G., Fisher S.K. and Clegg D.O. (2016). Strategies for bioengineered scaffolds that support adipose stem cells in regenerative therapies. Regen. Med. 11, 589-599.

Cole B.J. (2008). A randomized trial comparing autologous chondrocyte implantation with microfracture. J. Bone Joint Surg. Am. 90, 1165-1166.

Crop M.J., Baan C.C., Korevaar S.S., Ijzermans J.N., Pescatori M., Stubbs A.P., van Ijcken W.F., Dahlke M.H., Eggenhofer E., Weimar W. and Hoogduijn M.J. (2010). Inflammatory conditions affect gene expression and function of human adipose tissue-derived mesenchymal stem cells. Clin. Exp. Immunol. 162, 474-486.

Deepthi S. and Jayakumar R. (2016). Prolonged release of TGF-β from polyelectrolyte nanoparticle loaded macroporous chitin-poly(caprolactone) scaffold for chondrogenesis. Int. J. Biol. Macromol. 93, 1402-1409.

Dehne T., Schenk R., Perka C., Morawietz L., Pruss A., Sittinger M., Kaps C. and Ringe J. (2010). Gene expression profiling of primary human articular chondrocytes in high-density micromasses reveals patterns of recovery, maintenance, re- and dedifferentiation. Gene 462, 8-17.

Diekman B.O., Rowland C.R., Lennon D.P., Caplan A.I. and Guilak F. (2010). Chondrogenesis of adult stem cells from adipose tissue and bone marrow: Induction by growth factors and cartilage-derived matrix. Tissue Eng. Part A 16, 523-533.

Ding D.C., Chou H.L., Hung W.T., Liu H.W. and Chu T.Y. (2013). Human adipose-derived stem cells cultured in keratinocyte serum free medium: Donor's age does not affect the proliferation and differentiation capacities. J. Biomed. Sci. 20, 59.

Dmitrieva R.I., Minullina I.R., Bilibina A.A., Tarasova O.V., Anisimov S.V. and Zaritskey A.Y. (2012). Bone marrow- and subcutaneous adipose tissue-derived mesenchymal stem cells: Differences and similarities. Cell Cycle 11, 377-383.

Duscher D., Atashroo D., Maan Z.N., Luan A., Brett E.A., Barrera J., Khong S.M., Zielins E.R., Whittam A.J., Hu M.S., Walmsley G.G., Pollhammer M.S., Schmidt M., Schilling A.F., Machens H.G., Huemer G.M., Wan D.C., Longaker M.T. and Gurtner G.C. (2016). Ultrasound-assisted liposuction does not compromise the regenerative potential of adipose-derived stem cells. Stem Cells Transl. Med. 5, 248-257.

Efimenko A., Starostina E., Kalinina N. and Stolzing A. (2011). Angiogenic properties of aged adipose derived mesenchymal stem cells after hypoxic conditioning. J. Transl. Med. 9, 10.

Erices A., Conget P. and Minguell J.J. (2000). Mesenchymal progenitor cells in human umbilical cord blood. Br. J. Haematol. 109, 235-242.

Erickson G.R., Gimble J.M., Franklin D.M., Rice H.E., Awad H. and Guilak F. (2002). Chondrogenic potential of adipose tissue-derived stromal cells *in vitro* and *in vivo*. Biochem. Biophys. Res. Commun. 290, 763-769.

Estes B.T., Wu A.W. and Guilak F. (2006). Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. Arthritis Rheum. 54, 1222-1232.

Estes B.T., Diekman B.O. and Guilak F. (2008). Monolayer cell

expansion conditions affect the chondrogenic potential of adipose-derived stem cells. Biotechnol. Bioeng. 99, 986-995.

Estes B.T., Diekman B.O., Gimble J.M. and Guilak F. (2010). Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. Nat. Protoc. 5, 1294-1311.

Fennema E.M., Renard A.J., Leusink A., van Blitterswijk C.A. and de Boer J. (2009). The effect of bone marrow aspiration strategy on the yield and quality of human mesenchymal stem cells. Acta Orthop. 80, 618-621.

Fréchette J.P., Martineau I. and Gagnon G. (2005). Platelet-rich plasmas: growth factor content and roles in wound healing. J. Dent. Res. 84, 434-439.

Freitag J., Ford J., Bates D., Boyd R., Hahne A., Wang Y., Cicuttini F., Huguenin L., Norsworthy C. and Shah K. (2015). Adipose derived mesenchymal stem cell therapy in the treatment of isolated knee chondral lesions: design of a randomised controlled pilot study comparing arthroscopic microfracture versus arthroscopic microfracture combined with postoperative mesenchymal stem cell injections. BMJ Open 5, e009332.

Garrigues N.W., Little D., Sanchez-Adams J., Ruch D.S. and Guilak F. (2014). Electrospun cartilage-derived matrix scaffolds for cartilage tissue engineering. J. Biomed. Mater. Res. A 102, 3998-4008.

Gibson M., Beachley V., Coburn J., Bandinelli P.A., Mao H.Q. and Elisseeff J. (2014). Tissue extracellular matrix nanoparticle presentation in electrospun nanofibers. Biomed. Res. Int. 2014, 469120.

Gimble J.M., Katz A.J. and Bunnell B.A. (2007). Adipose-derived stem cells for regenerative medicine. Circ. Res. 100, 1249-1260.

Grasys J., Kim B.S. and Pallua N. (2016). Content of soluble factors and characteristics of stromal vascular fraction cells in lipoaspirates from different subcutaneous adipose tissue depots. Aesthet. Surg. J. 36, 831-841.

Gu J.H., Lee J.S., Kim D.W., Yoon E.S. and Dhong E.S. (2012). Neovascular potential of adipose-derived stromal cells (ASCs) from diabetic patients. Wound Repair Regen. 20, 243-252.

Gwon K., Kim E. and Tae G. (2017). Heparin-hyaluronic acid hydrogel in support of cellular activities of 3D encapsulated adipose derived stem cells. Acta Biomater. 49, 284-295.

Hajmousa G., Vogelaar P., Brouwer L.A., van der Graaf A.C., Henning R.H. and Krenning G. (2017). The 6-chromanol derivate SUL-109 enables prolonged hypothermic storage of adipose tissue-derived stem cells. Biomaterials 119, 43-52.

Harris J.D., Siston R.A., Pan X. and Flanigan D.C. (2010). Autologous chondrocyte implantation: a systematic review. J. Bone Joint Surg. Am. 92, 2220-2233.

Hass R., Kasper C., Böhm S. and Jacobs R. (2011). Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun. Signal. 9, 12.

Hassanbhai A.M., Lau C.S., Wen F., Jayaraman P., Goh B.T., Yu N. and Teoh S.H. (2017). In vivo immune responses of cross-linked electrospun tilapia collagen membrane. Tissue Eng. Part A 23, 1110-1119

He F. and Pei M. (2013). Extracellular matrix enhances differentiation of adipose stem cells from infrapatellar fat pad toward chondrogenesis. J. Tissue Eng. Regen. Med. 7, 73-84.

Hennig T., Lorenz H., Thiel A., Goetzke K., Dickhut A., Geiger F. and Richter W. (2007). Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J. Cell. Physiol. 211, 682-691.

Hernigou P., Desroches A., Queinnec S., Flouzat Lachaniette C.H., Poignard A., Allain J., Chevallier N. and Rouard H. (2014). Morbidity of graft harvesting versus bone marrow aspiration in cell regenerative therapy. Int. Orthop. 38, 1855-1860.

Hildner F., Eder M.J., Hofer K., Aberl J., Redl H., van Griensven M., Gabriel C. and Peterbauer-Scherb A. (2015). Human platelet lysate successfully promotes proliferation and subsequent chondrogenic differentiation of adipose-derived stem cells: a comparison with articular chondrocytes. J. Tissue Eng. Regen. Med. 9, 808-818.

Ikegame Y., Yamashita K., Hayashi S., Mizuno H., Tawada M., You F., Yamada K., Tanaka Y., Egashira Y., Nakashima S., Yoshimura S., and Iwama T. (2011). Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 13, 675-685.

Im G.I., Jung N.H. and Tae S.K. (2006). Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. Tissue Eng. 12, 527-536.

Im G.I., Ko J.Y. and Lee J.H. (2012). Chondrogenesis of adipose stem cells in a porous polymer scaffold: Influence of the pore size. Cell Transplant. 21, 2397-2405.

In 't Anker P.S., Scherjon S.A., Kleijburg-van der Keur C., de Groot-Swings G.M., Claas F.H., Fibbe W.E. and Kanhai H.H. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 22, 1338-1345.

Iruela-Arispe M.L. and Sage E.H. (1993). Endothelial cells exhibiting angiogenesis *in vitro* proliferate in response to TGF-beta 1. J. Cell. Biochem. 52, 414-430.

Ivanova-Todorova E., Bochev I., Mourdjeva M., Dimitrov R., Bukarev D., Kyurkchiev S., Tivchev P., Altunkova I. and Kyurkchiev D.S. (2009). Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunol. Lett. 126, 37-42.

Izadpanah R., Trygg C., Patel B., Kriedt C., Dufour J., Gimble J.M. and Bunnell B.A. (2006). Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. J. Cell. Biochem. 99, 1285-1297.

Jang Y., Koh Y.G., Choi Y.J., Kim S.H., Yoon D.S., Lee M. and Lee J.W. (2015). Characterization of adipose tissue-derived stromal vascular fraction for clinical application to cartilage regeneration. In Vitro Cell. Dev. Biol. Anim. 51, 142-150.

Jo C.H., Lee Y.G., Shin W.H., Kim H., Chai J.W., Jeong E.C., Kim J.E., Shim H., Shin J.S., Shin I.S., Ra J.C., Oh S. and Yoon K.S. (2014). Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem Cells 32, 1254-1266.

Johnstone B. and Yoo J.U. (1999). Autologous mesenchymal progenitor cells in articular cartilage repair. Clin. Orthop. Relat. Res. 367, S156-162.

Jurgens W.J., van Dijk A., Doulabi B.Z., Niessen F.B., Ritt M.J., van Milligen F.J. and Helder M.N. (2009). Freshly isolated stromal cells from the infrapatellar fat pad are suitable for a one-step surgical procedure to regenerate cartilage tissue. Cytotherapy 11, 1052-1064.

Kang H., Peng J., Lu S., Liu S., Zhang L., Huang J., Sui X., Zhao B., Wang A., Xu W., Luo Z. and Guo Q. (2014). In vivo cartilage repair using adipose-derived stem cell-loaded decellularized cartilage ECM scaffolds. J. Tissue Eng. Regen. Med. 8, 442-453.

Kapur S.K. and Katz A.J. (2013). Review of the adipose derived stem cell secretome. Biochimie 95, 2222-2228.

Kaufman M.R., Bradley J.P., Dickinson B., Heller J.B., Wasson K., O'Hara C., Huang C., Gabbay J., Ghadjar K. and Miller T.A. (2007). Autologous fat transfer national consensus survey: trends in techniques for harvest, preparation, and application, and perception of short- and long-term results. Plast. Reconstr. Surg. 119, 323-331.

Keane T.J. and Badylak S.F. (2015). The host response to allogeneic and xenogeneic biological scaffold materials. J. Tissue Eng. Regen. Med. 9, 504-511.

Kern S., Eichler H., Stoeve J., Kluter H. and Bieback K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24, 1294-1301.

Kim H.J. and Im G.I. (2009). Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: greater doses of growth factor are necessary. J. Orthop. Res. 27, 612-619.

Kim Y.S., Choi Y.J., Suh D.S., Heo D.B., Kim Y.I., Ryu J.S. and Koh Y.G. (2015). Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? Am. J. Sports Med. 43, 176-185.

Knutsen G., Engebretsen L., Ludvigsen T.C., Drogset J.O., Grontvedt T., Solheim E., Strand T., Roberts S., Isaksen V. and Johansen O. (2004). Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J. Bone Joint Surg. Am. 86-A, 455-464.

Kocaoemer A., Kern S., Kluter H. and Bieback K. (2007). Human AB serum and thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for the expansion of mesenchymal stem cells from adipose tissue. Stem Cells 25, 1270-1278.

Koh Y.G., Choi Y.J., Kwon O.R. and Kim Y.S. (2014). Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. Am. J. Sports Med. 42, 1628-1637.

Koh Y.G., Kwon O.R., Kim Y.S., Choi Y.J. and Tak D.H. (2016). Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: 2-year follow-up of a prospective randomized trial. Arthroscopy 32, 97-109.

Kolambkar Y.M., Peister A., Soker S., Atala A. and Guldberg R.E. (2007). Chondrogenic differentiation of amniotic fluid-derived stem cells. J. Mol. Histol. 38, 405-413.

Lee C.S., Burnsed O.A., Raghuram V., Kalisvaart J., Boyan B.D. and Schwartz Z. (2012). Adipose stem cells can secrete angiogenic factors that inhibit hyaline cartilage regeneration. Stem Cell Res. Ther. 3, 35.

Lee C.S., Watkins E., Burnsed O.A., Schwartz Z. and Boyan B.D. (2013). Tailoring adipose stem cell trophic factor production with differentiation medium components to regenerate chondral defects. Tissue Eng. Part A 19, 1451-1464.

Li G., Fu N., Xie J., Fu Y., Deng S., Cun X., Wei X., Peng Q., Cai X. and Lin Y. (2015). Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) based electrospun 3D scaffolds for delivery of autogeneic chondrocytes and adipose-derived stem cells: evaluation of cartilage defects in rabbit. J. Biomed. Nanotechnol. 11, 105-116.

Li J., Wong W.H., Chan S., Chim J.C., Cheung K.M., Lee T.L., Au W.Y., Ha S.Y., Lie A.K., Lau Y.L., Liang R.H. and Chan G.C. (2011). Factors affecting mesenchymal stromal cells yield from bone marrow aspiration. Chin. J. Cancer Res. 23, 43-48.

Lindroos B., Suuronen R. and Miettinen S. (2011). The potential of adipose stem cells in regenerative medicine. Stem Cell Rev. 7, 269-291.

Lu Z., Doulabi B.Z., Huang C., Bank R.A. and Helder M.N. (2010). Collagen type II enhances chondrogenesis in adipose tissue-derived stem cells by affecting cell shape. Tissue Eng. Part A 16, 81-90.

Malladi P., Xu Y., Chiou M., Giaccia A.J. and Longaker M.T. (2007). Hypoxia inducible factor-1alpha deficiency affects chondrogenesis of adipose-derived adult stromal cells. Tissue Eng. 13, 1159-1171.

Manferdini C., Paolella F., Gabusi E., Gambari L., Piacentini A., Filardo G., Fleury-Cappellesso S., Barbero A., Murphy M. and Lisignoli G. (2017). Adipose stromal cells mediated switching of the pro-inflammatory profile of M1-like macrophages is facilitated by PGE2: *in vitro* evaluation. Osteoarthritis Cartilage 25, 1161-1171.

Marsano A., Medeiros da Cunha C.M., Ghanaati S., Gueven S., Centola M., Tsaryk R., Barbeck M., Stuedle C., Barbero A., Helmrich U., Schaeren S., Kirkpatrick J.C., Banfi A. and Martin I. (2016). Spontaneous *in vivo* chondrogenesis of bone marrow-derived mesenchymal progenitor cells by blocking vascular endothelial growth factor signaling. Stem Cells Transl. Med. 5, 1730-1738.

Maumus M., Manferdini C., Toupet K., Peyrafitte J.A., Ferreira R., Facchini A., Gabusi E., Bourin P., Jorgensen C., Lisignoli G. and Noël D. (2013). Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis. Stem Cell Res. 11, 834-844.

Maumus M., Roussignol G., Toupet K., Penarier G., Bentz I., Teixeira S., Oustric D., Jung M., Lepage O., Steinberg R., Jorgensen C. and Noel D. (2016). Utility of a mouse model of osteoarthritis to demonstrate cartilage protection by IFNγ-primed equine mesenchymal stem cells. Front. Immunol. 7, 392.

McIntosh K., Zvonic S., Garrett S., Mitchell J.B., Floyd Z.E., Hammill L., Kloster A., Di Halvorsen Y., Ting J.P., Storms R.W., Goh B., Kilroy G., Wu X. and Gimble J.M. (2006). The immunogenicity of human adipose-derived cells: temporal changes *in vitro*. Stem Cells 24, 1246-1253.

McLaughlin M., Gagnet P., Cunningham E., Yeager R., D'Amico M., Guski K., Scarpone M. and Kuebler D. (2016). Allogeneic platelet releasate preparations derived via a novel rapid thrombin activation process promote rapid growth and increased BMP-2 and BMP-4 expression in human adipose-derived stem cells. Stem Cells Int. 2016, 7183734.

Meirelles L.D.S., Chagastelles P.C. and Nardi N.B. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J. Cell Sci. 119, 2204-2213.

Merceron C., Vinatier C., Portron S., Masson M., Amiaud J., Guigand L., Cherel Y., Weiss P. and Guicheux J. (2010). Differential effects of hypoxia on osteochondrogenic potential of human adipose-derived stem cells. Am. J. Physiol. Cell Physiol. 298, C355-364.

Minas T., Von Keudell A., Bryant T. and Gomoll A.H. (2014). The John Insall Award: A minimum 10-year outcome study of autologous chondrocyte implantation. Clin. Orthop. Relat. Res. 472, 41-51.

Mitchell J.B., McIntosh K., Zvonic S., Garrett S., Floyd Z.E., Kloster A., Di Halvorsen Y., Storms R.W., Goh B., Kilroy G., Wu X. and Gimble J.M. (2006). Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 24, 376-385.

Neri S., Bourin P., Peyrafitte J.A., Cattini L., Facchini A. and Mariani E. (2013). Human adipose stromal cells (ASC) for the regeneration of injured cartilage display genetic stability after *in vitro* culture expansion. PLoS One 8, e77895.

Nguyen P.D., Tran T.D., Nguyen H.T., Vu H.T., Le P.T., Phan N.L., Vu N.B., Phan N.K. and Van Pham P. (2017). Comparative clinical observation of arthroscopic microfracture in the presence and absence of a stromal vascular fraction injection for osteoarthritis. Stem Cells Transl. Med. 6, 187-195.

Pak J. (2011). Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. J. Med. Case Rep. 5, 296.

Pak J., Lee J.H., Kartolo W.A. and Lee S.H. (2016a). Cartilage regeneration in human with adipose tissue-derived stem cells: current status in clinical implications. Biomed. Res. Int. 2016, 4702674.

Pak J., Lee J.H., Park K.S., Jeong B.C. and Lee S.H. (2016b). Regeneration of cartilage in human knee osteoarthritis with autologous adipose tissue-derived stem cells and autologous extracellular matrix. Biores. Open Access 5, 192-200.

Pepper M.S., Vassalli J.D., Orci L. and Montesano R. (1993). Biphasic effect of transforming growth factor-beta 1 on *in vitro* angiogenesis. Exp. Cell Res. 204, 356-363.

Pers Y.M., Rackwitz L., Ferreira R., Pullig O., Delfour C., Barry F., Sensebe L., Casteilla L., Fleury S., Bourin P., Noël D., Canovas F., Cyteval C., Lisignoli G., Schrauth J., Haddad D., Domergue S., Noeth U., Jorgensen C. and on behalf of the ADIPOA Consortium (2016). Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. Stem Cells Transl. Med. 5, 847-856.

Pires de Carvalho P., Hamel K.M., Duarte R., King A.G., Haque M., Dietrich M.A., Wu X., Shah F., Burk D., Reis R.L., Rood J., Zhang P., Lopez M., Gimble J.M. and Dasa V. (2014). Comparison of infrapatellar and subcutaneous adipose tissue stromal vascular fraction and stromal/stem cells in osteoarthritic subjects. J. Tissue Eng. Regen. Med. 8, 757-762.

Pittenger M.F., Mackay A.M., Beck S.C., Jaiswal R.K., Douglas R., Mosca J.D., Moorman M.A., Simonetti D.W., Craig S. and Marshak D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284, 143-147.

Platas J., Guillén M.I., Pérez Del Caz M.D., Gomar F., Castejón M.A., Mirabet V. and Alcaraz M.J. (2016). Paracrine effects of human adipose-derived mesenchymal stem cells in inflammatory stress-induced senescence features of osteoarthritic chondrocytes. Aging (Albany NY) 8, 1703-1717.

Portron S., Merceron C., Gauthier O., Lesoeur J., Sourice S., Masson M., Fellah B.H., Geffroy O., Lallemand E., Weiss P., Guicheux J. and Vinatier C. (2013). Effects of *in vitro* low oxygen tension preconditioning of adipose stromal cells on their *in vivo* chondrogenic potential: Application in cartilage tissue repair. PLoS One 8, e62368.

Ra J.C., Shin I.S., Kim S.H., Kang S.K., Kang B.C., Lee H.Y., Kim Y.J., Jo J.Y., Yoon E.J., Choi H.J. and Kwon E. (2011). Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev. 20, 1297-1308.

Rider D.A., Dombrowski C., Sawyer A.A., Ng G.H., Leong D., Hutmacher D.W., Nurcombe V. and Cool S.M. (2008). Autocrine fibroblast growth factor 2 increases the multipotentiality of human adipose-derived mesenchymal stem cells. Stem Cells 26, 1598-1608.

Riis S., Zachar V., Boucher S., Vemuri M.C., Pennisi C.P. and Fink T. (2015). Critical steps in the isolation and expansion of adipose-derived stem cells for translational therapy. Expert Rev. Mol. Med. 17, e11.

Riis S., Stensballe A., Emmersen J., Pennisi C.P., Birkelund S., Zachar V. and Fink T. (2016). Mass spectrometry analysis of adipose-derived stem cells reveals a significant effect of hypoxia on pathways regulating extracellular matrix. Stem Cell Res. Ther. 7, 52.

Riordan N.H., Ichim T.E., Min W.P., Wang H., Solano F., Lara F., Alfaro M., Rodriguez J.P., Harman R.J., Patel A.N., Murphy M.P., Lee R.R. and Minev B. (2009). Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J. Transl. Med. 7, 29.

Robins J.C., Akeno N., Mukherjee A., Dalal R.R., Aronow B.J., Koopman P. and Clemens T.L. (2005). Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. Bone 37, 313-322.

Rodriguez J.P., Murphy M.P., Hong S., Madrigal M., March K.L., Minev B., Harman R.J., Chen C.S., Timmons R.B., Marleau A.M. and Riordan N.H. (2012). Autologous stromal vascular fraction therapy for rheumatoid arthritis: rationale and clinical safety. Int. Arch. Med. 5, 5.

Sakata K., Furumatsu T., Abe N., Miyazawa S., Sakoma Y. and Ozaki T. (2013). Histological analysis of failed cartilage repair after marrow stimulation for the treatment of large cartilage defect in medial compartmental osteoarthritis of the knee. Acta Med. Okayama 67, 65-74.

Schelbergen R.F., van Dalen S., ter Huurne M., Roth J., Vogl T., Noël D., Jorgensen C., van den Berg W.B., van de Loo F.A., Blom A.B. and van Lent P.L. (2014). Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels. Osteoarthritis Cartilage 22, 1158-1166.

Schipani E., Ryan H.E., Didrickson S., Kobayashi T., Knight M. and Johnson R.S. (2001). Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev. 15, 2865-2876.

Scioli M.G., Bielli A., Gentile P., Cervelli V. and Orlandi A. (2017). Combined treatment with platelet-rich plasma and insulin favours chondrogenic and osteogenic differentiation of human adipose-derived stem cells in three-dimensional collagen scaffolds. J. Tissue Eng. Regen. Med. 11, 2398-2410.

Shafaei H., Esfandiari E., Esmaeili A., Razavi S., Hashemibeni B., Nasr Esfahani M.H., Shiran M.B., Isfahani S.H. and Mardani M. (2013). Optimizing a novel method for low intensity ultrasound in chondrogenesis induction. Adv. Biomed. Res. 2, 79.

Shen H., Gelberman R.H., Silva M.J., Sakiyama-Elbert S.E. and Thomopoulos S. (2013). BMP12 induces tenogenic differentiation of adipose-derived stromal cells. PLoS One 8, e77613.

Shi S., Xie J., Zhong J., Lin S., Zhang T., Sun K., Fu N., Shao X. and Lin Y. (2016). Effects of low oxygen tension on gene profile of soluble growth factors in co-cultured adipose-derived stromal cells and chondrocytes. Cell Prolif. 49, 341-351.

Spencer N.D., Chun R., Vidal M.A., Gimble J.M. and Lopez M.J. (2012). In vitro expansion and differentiation of fresh and revitalized adult canine bone marrow-derived and adipose tissue-derived stromal cells. Vet. J. 191, 231-239.

Staubli S.M., Cerino G., Gonzalez De Torre I., Alonso M., Oertli D., Eckstein F., Glatz K., Rodriguez Cabello J.C. and Marsano A. (2017). Control of angiogenesis and host response by modulating the cell adhesion properties of an elastin-like recombinamer-based hydrogel. Biomaterials 135, 30-41.

Sukarto A., Yu C., Flynn L.E. and Amsden B.G. (2012). Co-delivery of adipose-derived stem cells and growth factor-loaded microspheres in RGD-grafted N-methacrylate glycol chitosan gels for focal chondral repair. Biomacromolecules 13, 2490-2502.

Tarte K., Gaillard J., Lataillade J.J., Fouillard L., Becker M., Mossafa H., Tchirkov A., Rouard H., Henry C., Splingard M., Dulong J., Monnier D., Gourmelon P., Gorin N.C., Sensebé L. and on behalf of Société Française de Greffe de Moelle et Thérapie Cellulaire (2010). Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. Blood 115, 1549-1553.

Teong B., Wu S.C., Chang C.M., Chen J.W., Chen H.T., Chen C.H., Chang J.K. and Ho M.L. (2018). The stiffness of a crosslinked hyaluronan hydrogel affects its chondro-induction activity on hadscs. J. Biomed. Mater. Res. B Appl. Biomater. 106, 808-816.

Ter Huurne M., Schelbergen R., Blattes R., Blom A., De Munter W., Grevers L.C., Jeanson J., Noël D., Casteilla L., Jorgensen C., van den Berg W. and van Lent P.L. (2012). Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. Arthritis Rheum. 64, 3604-3613.

Toghraie F., Razmkhah M., Gholipour M.A., Faghih Z., Chenari N., Torabi Nezhad S., Nazhvani Dehghani S. and Ghaderi A. (2012). Scaffold-free adipose-derived stem cells (ASCs) improve experimentally induced osteoarthritis in rabbits. Arch. Iran. Med. 15, 495-499.

Traktuev D.O., Merfeld-Clauss S., Li J., Kolonin M., Arap W., Pasqualini R., Johnstone B.H. and March K.L. (2008). A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ. Res. 102, 77-85.

Treuhaft P.S. and Mccarty D.J. (1971). Synovial fluid pH, lactate, oxygen and carbon dioxide partial pressure in various joint diseases. Arthritis Rheum. 14, 475-484.

Ude C.C., Sulaiman S.B., Min-Hwei N., Hui-Cheng C., Ahmad J., Yahaya N.M., Saim A.B. and Idrus R.B. (2014). Cartilage regeneration by chondrogenic induced adult stem cells in osteoarthritic sheep model. PLoS One 9, e98770.

Van Harmelen V., Röhrig K. and Hauner H. (2004). Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. Metabolism 53, 632-637.

Vilar J.M., Batista M., Morales M., Santana A., Cuervo B., Rubio M., Cugat R., Sopena J. and Carrillo J.M. (2014). Assessment of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. BMC Vet. Res. 10, 143.

Wu L., Prins H.J., Leijten J., Helder M.N., Evseenko D., Moroni L., van Blitterswijk C.A., Lin Y. and Karperien M. (2016). Chondrocytes cocultured with stromal vascular fraction of adipose tissue present more intense chondrogenic characteristics than with adipose stem cells. Tissue Eng. Part A 22, 336-348.

Wu S.C., Chang J.K., Wang C.K., Wang G.J. and Ho M.L. (2010). Enhancement of chondrogenesis of human adipose derived stem cells in a hyaluronan-enriched microenvironment. Biomaterials 31, 631-640.

Wu S.C., Chen C.H., Chang J.K., Fu Y.C., Wang C.K., Eswaramoorthy R., Lin Y.S., Wang Y.H., Lin S.Y., Wang G.J. and Ho M.L. (2013). Hyaluronan initiates chondrogenesis mainly via CD44 in human adipose-derived stem cells. J. Appl. Physiol. (1985) 114, 1610-1618.

Yin F., Cai J., Zen W., Wei Y., Zhou W., Yuan F., Singh S.R. and Wei Y. (2015). Cartilage regeneration of adipose-derived stem cells in the TGF-β1-immobilized PLGA-gelatin scaffold. Stem Cell Rev. 11, 453-459.

Yu C., Kornmuller A., Brown C., Hoare T. and Flynn L.E. (2017). Decellularized adipose tissue microcarriers as a dynamic culture platform for human adipose-derived stem/stromal cell expansion. Biomaterials 120, 66-80.

Zhang K., Zhang Y., Yan S., Gong L., Wang J., Chen X., Cui L. and Yin J. (2013). Repair of an articular cartilage defect using adipose-derived stem cells loaded on a polyelectrolyte complex scaffold based on poly(l-glutamic acid) and chitosan. Acta Biomater. 9, 7276-7288.

Zhang K., Yan S., Li G., Cui L. and Yin J. (2015). In-situ birth of MSCs multicellular spheroids in poly(l-glutamic acid)/chitosan scaffold for hyaline-like cartilage regeneration. Biomaterials 71, 24-34.

Zuk P. (2013). Adipose-derived stem cells in tissue regeneration: a review. ISRN Stem Cells 2013.

Zuk P.A., Zhu M., Mizuno H., Huang J., Futrell J.W., Katz A.J., Benhaim P., Lorenz H.P. and Hedrick M.H. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 7, 211-228.

Zuk P.A., Zhu M., Ashjian P., Ugarte D.A.D., Huang J.I., Mizuno H., Alfonso Z.C., Fraser J.K., Benhaim P. and Hedrick M.H. (2002). Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13, 4279-4295.

Accepted December 15, 2017