Journal of Cell Science & Therapy

Trzeciak, et al., J Cell Sci Ther 2014, 5:4
DOI: 10.4172/2157-7013.1000172

Review Article Open Access

Induced Pluripotent and Mesenchymal Stem Cells as a Promising Tool for Articular Cartilage Regeneration

Tomasz Trzeciak, Ewelina Augustyniak, Magdalena Richter, Jacek Kaczmarczyk and Wiktoria Suchorska

Department of Orthopaedics and Traumatology, Poznan University of Medical Sciences, Poland

***Corresponding author:** Tomasz Trzeciak, Department of Orthopaedics and Traumatology, Poznan University of Medical Sciences, 28 czerwca Street No 135/147-61 545 Poznan, Poland, Tel: +48 61 8310 359; Fax: +48 61 8310 163; E-mail: doktortrzeciak@gmail.com

Rec date: Jul 24, 2014; **Acc date:** Aug 25, 2014; **Pub date:** Aug 27, 2014

Abstract

The application of stem cells in regenerative medicine has recently become a rapidly growing field, holding promise for combating a number of orthopedic disorders including osteodegenerative ones (osteoporosis and osteoarthritis). Although the differentiation of stem cells into chondrocytes is now intensively investigated on a laboratory scale, implementing the laboratory protocols in clinical practice requires a scale-up culture. In order to apply this technique many aspects of stem cell bioprocessing such as optimal culture conditions for anchorage-dependent or anchorage-independent cells and the type of culture must be taken into account. The presence of microcarriers and/or scaffolds for adherent cells is essential, since they provide a three-dimensional microenvironment indispensable for cell growth. For treatment of osteoarthritis, induced pluripotent stem cells and mesenchymal stem cells seem to be the best choice. Although, the scale-up culture using stem cells has been intensively investigated on a laboratory scale, the scale-up culture for clinical application still requires further technical improvements.In this review stem cell bioprocessing including the use of biomaterials, bioreactors, and factors affecting this process, as well as scale-up culture of induced Pluripotent and mesenchymal stem cells were presented and discussed.

Keywords: Stem cell bioprocessing; Induced pluripotent stem cells; Mesenchymal stem cells; Osteoarthritis; Bioreactor; Scale-up culture; Good Manufacturing Practice

Introduction

Isolated partial-thickness articular cartilage defects show very limited capacity for self-repair due to the lack of blood vessels, nerves and lymphatic vessels. However, cartilage lesions extending to subchondral bone show the potential to heal due to formation of blood clots from subchondral bone vessels and the release of bone marrow-derived stem cells. Surface erosion following focal cartilage defects may lead over the time to osteoarthritis (OA), which is characterized by cartilage structure breakdown and subchondral bone remodeling [1]. Both OA and focal injuries result in joint malfunction and pain that significantly impair the quality of life. Options available for treating the symptomatic cartilage defects range from the conservative to the most advanced cell-based therapies. Conservative methods include pharmacological treatment and physical therapy, and aim at reducing the symptoms. However, there is no evidence that these methods improve the joint structure and function [2]. Currently accessible surgical methods of treating chondral and osteochondral defects, based on cell and tissue grafting (autologous chondrocyte implantation (ACI), mosaicplasty, and osteochondral autograft transplantation (OATS)) have several limitations such as (i) donor site morbidity, (ii) limited availability of tissue and cells, (iii) graft de-differentiation, and (iv) cell apoptosis [3]. Moreover, marrow stimulation techniques, such as microfractures, are suitable only for small focal lesions and result in the formation of hyaline-like cartilage, fibrous tissue or bone [4]. Tissue-engineered grafts, generated from the patients' own cells and seeded on an appropriate degradable biomaterial, seem to be a promising tool in cartilage lesion repair and ultimately OA treatment. Cell-based methods allow one to: (i) culture the tissue from a small number of cells, (ii) match the specific size and shape of the tissue, (iii) reinforce the medium with biochemical factors to enhance graft integration in the site of the lesion [5].

First introduced in the early eighties of the last century, ACI has been used so far with a combination of periosteal flap or collagen membranes, applied as a cover for implanted cells [6]. Although the usefulness of this technique in treating larger cartilage lesions (sized 2 to 10 cm2) has been shown in numerous clinical studies, ACI still has some limitations, such as:
(i) inadequate number of cells available for harvesting from the donor's non-weight-bearing cartilage surface, (ii) inability to preserve chondrogenic potential of the cell and (iii) failure to maintain cell differentiation and tissue formation after the implantation [7]. To overcome these limitations, the stem cell-based approach seems to be a promising alternative. Over the past several years both mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs) have been checked for their therapeutic potential in numerous tissue engineering studies, including bone and cartilage. MSCs are multipotent stem cells primarily isolated from bone marrow. These cells differentiate into several cell lineages including osteogenic and chondrogenic [8,9]. More recently described induced pluripotent stem cells (iPSCs) have the potential to differentiate into somatic cells, including chondrocytes [10-12].

An important point in stem cell-based tissue engineering is developing and maintaining appropriate cell culture system, which would mimic the *in vivo* cell microenvironment. Implementation of stem cell-based techniques requires the ability to produce a large number of cells of high purity with well-defined properties. Over the past 10 years bioreactor systems have been used to obtain uniform cell culture conditions [13]. This review presents recent advances in stem

cell bioprocessing using bioreactors and summarizes data from the literature concerning the parameters of culture conditions that ensure efficient stem cell expansion and differentiation into chondrocytes.

Stem Cell Bioprocessing

Bioreactors in mammalian cell culture

The principal challenge in an advanced tissue culture technique is provision of bioreactors used for controlling environmental conditions in cell culture. There are three main types of bioreactor systems used for: (i) cells in suspension culture, (ii) anchorage-dependent cells and (iii) micro-carrier systems [14]. For cells cultured in suspension, the stirred tank bioreactor, rotary cell culture system (RCCS) and wave bioreactor are recommended. Adherent cells can be easily cultured in a microcarrier-based bioreactor, microencapsulation-based bioreactor, fiber membrane bioreactor or as cells immobilized on 3D scaffolds (fixed bed, fluidized bed, fibrous bed) [15]. Bioreactors are predominantly used for cell expansion, differentiation or for both processes carried on simultaneously. The two-dimensional (2D) culture of pluripotent stem cells has been successfully used on a laboratory scale. However, introduction of the third dimension proved fundamental for large-scale culture [16]. The 3D culture conditions are similar to those present in a developing tissue. Furthermore, specific cellular behavior is barely noticed in conventional monolayer culture [17]. It is worth mentioning that in bone marrow hMSCs represent only 0.001 to 0.01% of the nucleated cells. Thus, the isolation of an adequate number of hMSC cells for regeneration of injured tissues is troublesome. Moreover, these cells have a brief lifespan. In addition, the 2D monolayer MSC culture is expensive, labor intensive, time consuming and results in an insufficient number of cells. Consequently, the development and improvement of large-scale, long-term 3D culture is critical for its clinical application [18]. It has also been reported that in contrast to cells cultured in monolayer, the 3D cell culture shows better proliferative activity, and given appropriate signals the cells are more prone to alter their shape and function [19].

Biomaterials used in tissue and organ engineering

Types of biomaterials used in tissue and organ engineering include naturally derived, like collagen, alginate and hyaluronic acid (HA), and synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA) as well as poly(-lactic-co-glycolic) acid (PLGA).

Collagen derives from ECM and constitutes natural adhesive ligand promoting cell attachment. Moreover, the U.S. Food and Drug Administration (FDA) approved collagen products for medical applications. HA is a versatile, linear polysaccharide that naturally occurs in cartilage. Alginate is an anionic polysaccharide with crosslinking properties. Synthetic polyesters such as PGA, PLA and PLGA are biodegradable, biocompatible and are also approved by FDA for clinical applications [20].

In bioprocessing many microstructures are used: microcarriers, 3D cell aggregates, advanced scaffolds obtained from natural, non-animal polymers such as sponges, and gels made of synthetic materials. Microcarriers are porous or nonporous structures 170 to 6000μm in diameter. In order to improve cell attachment and growth, they can be coated with ECM proteins [21]. Seeding cells onto scaffolds at high densities enhances tissue formation and cartilage matrix production in the 3D structures [22]. Nevertheless, the efficient and uniform distribution of cells at high densities, even on small scaffolds, is a challenge [23].

Factors affecting bioprocessing

As far as bioprocessing is concerned, the following issues should be considered: (i) maintaining a uniform cell concentration within the scaffold during cell seeding (ii) controlling aseptic parameters and (iii) availability of automated processing steps [24]. Successful bioprocessing depends upon several important factors. The expansion and differentiation of stem cells is difficult due to complicated kinetics of culture. Cells tend to develop unstable subpopulations, due to parameters, such as oxygen tension, growth factor concentration and cell-to-cell interactions. So far, however, there are no established stem cells culture protocols [25].

Cell growth is determined by the dissolved oxygen concentration (dO_2) and pH. In the scale-up cell culture continuous and stable oxygen supply plays a crucial role because its concentration significantly decreases with increasing biomass. The changing values of these two parameters indicate the beginning of apoptosis [26]. Although almost all mammalian cell cultures are conducted at pH 7.4, in 20% oxygen, 5% CO_2, and at 37˚C, the conditions of expansion and subsequent differentiation of stem cells must be individually selected, because distinct stem cell cultures require different optimal parameters [27]. Suspension culture technology ensures obtaining a relatively homogenous environment, which allows online monitoring and control of the two parameters as well as the concentration of nutrients and cytokines [28]. However, the prolonged culture at atmospheric oxygen concentration may lead to an oxidative stress. The MSCs reveal more efficient expansion at 2% O_2, whereas the human embryonic stem cells (hESCs) prefer to grow under 20% or even 30% O_2 [29,30]. With respect to the chondrogenic differentiation, this process is favored by hypoxic conditions (2 to 5% O_2) [31].

Temperature is another significant parameter. Stem cells are typically cultured at 37˚C [32]. Nevertheless, it was reported that MSCs could be more effectively cultured at 32˚C. Moreover, the growth of MSCs at lower temperatures reduces the oxidative stress and affects stem cell self-renewal throughout the regulation of p21 and p53 levels [33].

Osmolarity is a function of the osmotic pressure of the medium and influences stem cell functioning. The extracellular osmolarity of healthy articular cartilage fluctuates between 350 and 480 mOsm. For comparison, the osmolarity of standard culture media is similar to its levels in plasma (280 mOsm). The Nfat5 gene that regulates the response of cells to osmolarity changes, most likely takes part in chondrogenic differentiation through the influence on a key chondrogenic transcription factor Sox9 [34]. The hydrodynamic shear stress is proportional to impeller diameter, geometry and location, as well as to the frequency of agitation (rpm) during cell culture in the bioreactor. The shear stress is also correlated with the presence of probes and other vessel internals due to disruption of radial flow patterns [30]. In spite of those disadvantages, agitation is very important, since it allows for interaction between the cultured cells with the components of culture medium and keeps the aggregates or microcarriers in suspension [35]. Study by Liovic et al. [36] showed that shear in bioreactor culture influences hMSCs differentiation due to induction of the mitogen activated protein kinase (MAPK) signaling, mechano-transduction (mechanical forces) as well as the wnt signaling pathway.

Another aspect of culture is cells' tendency to grow in agglomerates. Unfortunately, necrotic areas may be created in centers of the structure due to the limited nutrient and oxygen transport [37]. The transport of dissolved oxygen in a bioreactor takes place in three main regions: (i) bulk fluid phase of the bioreactor, called global mass transfer (ii) internal mass transfer, that appears from bulk to the surface of the aggregates and (iii) external mass transfer, which occurs among the aggregated cells [24]. In contrast to single cells, the aggregates or the cells seeded on the microcarriers are affected at lower agitation frequency, because of inverse relationship between Kolmogorov eddy size and agitation intensity [30].

Nutrients are indispensable for the appropriate metabolism of stem cells. The composition of the basal medium is more complex for pluripotent- than for mesenchymal stem cells. Therefore it is important to provide suitable ingredients at proper concentrations. Glucose and glutamine play the most important role in cell nutrition [38]. The demand for glucose and oxygen varies and depends on the phase of stem cell growth. The expansion of stem cells depends on glycolysis, whereas the process of differentiation of stem cells relies on oxidative phosphorylation. During the reprogramming stage the metabolic shift from oxidative phosphorylation to glycolysis is observed [39]. On one hand, it is essential to ensure an adequate supply of the nutrients for cells, but on the other hand it is crucial to keep the concentration of metabolic waste products below the toxic level. It has been proven that as growth-inhibiting metabolites, ammonia and lactate constitute a serious danger. Ammonia appears as a product of the oxidative deamination of mainly glutamate and/or derives from the deamination of glutamine, whereas lactate excretion is related to anaerobic glycolysis during the early stages of culture [40].

Essential for chondrogenic differentiation are growth factor- and/or cytokine- supplemented media. The members of TGF-β superfamily are the most important growth factors in directing chondrogenesis of stem cells [41]. Numerous different media containing Bone Morphogenetic Proteins (BMPs), TGF-β1 and/or TGF-β3 were used successfully in stem cell culture [42-44]. However, for bioreactor-based chondrocyte expansion and chondrogenic differentiation, prolonged culturing in TGF-β3 supplemented medium creates a problem, since at 37˚C, TGF-β3 bioactivity rapidly decreases in both serum-free and serum-containing media [45].

Scale-up pluripotent stem cell culture

The principal step in the differentiation of embryonic stem cells (ESCs) is the embryoid bodies (EBs) formation. On a laboratory scale, the EBs are obtained in hanging drop- or static suspension culture, encapsulation of the ESC culture, entrapment of the ESC culture or with the help of low adherence, 96 well plates [46]. On a larger scale, they are formed in spinner flasks, rotating cell culture system or rotary orbital culture [47]. Another procedure includes mouse ESC expansion as aggregates of EBs during long-term culture in suspension bioreactors [48]. The blastocyst-stage human embryos express E-cadherin – an adhesion molecule, which mediates mutual attachment between EBs. The EB encapsulation in size-specified agarose capsules allows the control of cell-to-cell interactions in scalable culture [49]. Human embryoid bodies (hEBs) can be effectively created from hESC within the 3D porous alginate scaffolds in a rotating bioreactor system. Alginate scaffolds resemble ECM and ensure efficient cell seeding. Their porosity can be controlled during the production process [50]. However, the EB agglomeration might inhibit cell growth and differentiation; but a hydrogel encapsulation approach eliminates this impediment and facilitates direct differentiation of ESCs in stirred suspension culture [51]. Recent reports indicate that mouse embryonic stem cells (mESCs) can be effectively expanded in both suspension and in fibrous bed bioreactor (FBB), and their ability to form EBs is maintained. However, the culture in FBB ensures better cell growth, with less frequent passaging, and less medium and labor is required [52]. Another promising result is the successful transfer of single cell-inoculated suspension culture of hESCs and hiPSCs to a fully controlled, stirred bioreactor. This procedure includes the usage of a fully defined serum-free medium and a Rho-associated coiled-coil kinase (ROCK) inhibitor (RI) resulting in a long-term expansion of hESCs and hiPSCs, independent of any extracellular matrices or scaffolds [53]. Shafa and colleagues [54] reported promising results of reprogramming mouse embryonic fibroblasts (MEFs) in stirred suspension bioreactors. They proved that this process could be successfully conducted on a larger scale while maintaining high expression of pluripotency markers of reprogrammed cells.

It is important to evaluate the translationabilty of scalable or expanded cells. It was reported that hESC-derived chondrocytes, cultured in HA-based hydrogel, maintain long-term ability to repair of critical-sized osteochondral defects in rat, with no evidence of tumorigenicity [55]. Alfred and co-workers [56] developed serum-free protocols for the production of murine stem cell-derived osteoblasts and chondrocytes for large-scale using CultiSpher S microcarriers in stirred suspension bioreactors. CultiSpher S is a biodegradable microcarrier that provides suitable environment for stem cell expansion and differentiation. Moreover, in this study the stem cell derived-cells did not reveal the tumorigenic risk in a mouse fracture model. In the other study, the usefulness of murine iPSCs in cartilage-repair therapies was demonstrated [57]. After initial chondrogenic differentiation, iPSCs were treated with type II collagen-driven green fluorescent protein (GFP) and the GFP positive cells were seeded onto 1% agarose, delivered to the defect and chondrogenic differentiation was successfully performed for 21 days.

Craft et al. [58] demonstrated that the manipulation of appropriate signal pathways of mouse ESCs, allows obtaining both hypertrophic and non-hypertrophic chondrocyte populations. In this experiment chondrocyte populations were able to form cartilage-like tissue in vitro and support cartilage tissue phenotype within niche of immunodeficient recipients *in vivo*. *In vitro* cartilage tissue engineering models help to investigate the oncogenic risk and identify abnormal human iPSCs lines without taking advantage of animal transplantation experiments [59]. The translationability of pluripotent stem cells is still an emerging field, therefore further investigations are necessary. The reprogramming is currently carried out throughout genetic modifications, including the use of viral vectors. Therefore, iPSC lines are not in clinical use, because of their tumorigenic potential and the ethical issues involved.

Scale-up mesenchymal stem cell culture

For clinical applications, efficient and high-yield methods of scale-up culture for adherent MSCs have been developed. Chen et al. [60] performed a 6-day experiment with the use of Myelocult medium containing a combination of supplementary factors and Augst et al. [61] in a three-week experiment in a rotating bioreactor demonstrated the ability of hMSCs to undergo chondrogenic differentiation. The cells were cultured on silk scaffolds characterized by high biocompatibility, slow degradation and the potential to generate structure of desired porosity and mechanical properties. Zhang et al.

[62] demonstrated that culture of MSCs in a Biaxial Rotating (BXR) bioreactor provides satisfying results. The human fetal mesenchymal stem cells (hfMSCs) seeded onto macroporous polycaprolactone/tri-calcium phosphate (PCL-TCP) scaffolds in the BXR bioreactor, showed better cellular proliferation and osteogenic induction compared with other bioreactors such as spinner flask bioreactor, perfusion bioreactor or rotating wall vessel bioreactor [62]. The MSC proliferation profile was evaluated in a microcarrier-based stirred bioreactor. It has been shown that Cultispher-S is the most efficient microcarrier for the MSC expansion. The enhanced cell proliferation or chondrogenic differentiation can also be achieved by manipulation of actin organization within the cells. Cytopore-2 promotes chondrogenesis throughout the disorganization of actin forms [63]. Another example is the xenogeneic-free microcarrier–based bone marrow MSCs culture [64]. The controlled stirred-tank bioreactor-base culture was conducted for 7 days. In this experiment the cell growth under different Airsat and various modes of operation reached practically the same level. Schirmaier and colleagues [65] achieved maximum living cell densities in stirred single-use bioreactors under low-serum conditions on both benchtop and pilot scales. Moreover, the maximum cell densities were reached 4 to 7 days earlier than those reported by other research groups [65].

Over the last decade the therapeutic use of MSCs in injuries and osteodegenerative disorders was intensively investigated. Different types of MSCs including bone marrow-derived MSCs, adipose-derived MSCs, umbilical cord blood MSCs and peripheral blood MSCs have been studied. MSCs were injected into the injured knee of the rat in order to verify their regenerative properties and it was found that the GFP positive MSCs contributed to regeneration of the intraarticular cartilage injuries [66]. Valonen et al. [67] obtained mechanically functional cartilage grafts from adult mesenchymal stem cells based on 3D-woven poly(ε-caprolactone) (PCL) scaffold. It was shown that production of tissue engineered cartilage constructs in the oscillating bioreactor was faster in comparison with the static dishes, while the features characteristic for articular cartilage, such as an expression of collagen II, was maintained. Polycaprolactone- tricalcium phosphate (PCL-TP) as a composite scaffold was also investigated in animal models [68,69]. In the investigation of cartilaginous repair, an *ex vivo* model of cartilage defect was also examined. For this purpose, the addition of chondrogenic cytokines and transfection of growth factors genes to MSCs was studied. It is worth mentioning that cartilage regeneration in osteochondral and chondral defects with the use of MSCs transfected with the TGF-β-gene was more effective in contrast to non-transfected MSCs. This phenomenon might be caused by diffusion of TGF-β3 molecules form the transfected cells to the medium [70]. Swine bone marrow-derived mesenchymal stem cells were successfully differentiated into chondrocytes, and then were seeded onto a three dimensional PGA-derived scaffold to construct cartilage. This two-step procedure resulted in the formation of the mature cartilage that was implanted subcutaneously into nude mice. The use of this procedure facilitates stable chondrogenesis *in vivo* [71]. The investigations of Thorpe et al. [72] revealed the importance of oxygen tension in cartilaginous tissue engineering of MSCs seeded onto hydrogels. The control of the depth of developing constructs and oxygen level was achieved by application of dynamic compression and confinement of constructs, the principal parameter being the depth of the constructs. The latest achievement in the translational field is the creation of the functional human cartilage from MSCs. It was achieved by imitation of the mesenchymal condensation occurring during chondrogenesis. The creation of condensed mesenchymal cell bodies and their fusion into homogenous aggregates gives rise to well-differentiated cartilage. Its functionality was confirmed in a cartilage defect model [73].

Currently Orth et al. [74] collected and reviewed literature (17 publications) concerning the use of stem cell-based therapy of knee injuries. Patients were subjected to surgery, or in some cases joint injection, and mostly BMSCs or PBMSCs, were implanted. The number of cases ranged from 1 to 70 and the time of observation from several months to 6 years. In almost all cases clinical improvement was observed and either hyaline-like of fibrocartilaginous cartilage was obtained. In treatment of OA, mostly BMCs or ASCs were used. Patients overcome knee injection and in one of the cases surgical procedure. The results were described in 9 publications and the number of patients ranged from 1 to 25 and the time of observation ranged from 3 to 24 months. Similarly to the treatment of injuries, OA therapy using stem cells result in clinical improvement. In one of the publication hyaline-like tissue was observed. This clearly indicates that in the nearest future treatment of articular knee injuries, but also OA therapy by surgical means, will be dominated by the use of stem cells.

Mesenchymal stem cells obtained from dental pulp (dental pulp stems cells, DPSCs) seem to be an interesting alternative, since their multipotency is comparable with the differentiation abilities of bone marrow mesenchymal stem cells. Moreover, DPSCs demonstrate better proliferation rate and availability represented by greater cell number [75]. Nevertheless, there are only few clinical trials investigating human tissue regeneration [76].

Good Manufacturing Practice (GMP)

European law requires cell-based medical components to be produced in accordance with the Good Manufacturing Practice (GMP) [77]. More precisely, products in compliance with GMP should include the highest standards of sterility, quality control, and documentation, following a standard operating procedure (SOP), at each phase of production from cell isolation to freezing and storage [78,79]. The human pluripotent stem cell-derived products must be developed under well-defined conditions, ensuring the maintenance of pluripotency and/or differentiation of the original stem cells [80].

Another significant point is establishing the animal-free culture system. The use of animal-derived research-grade products might constitute a risk of infecting the cells with animal pathogens or might cause rejection the product after transplantation. Especially, the nonhuman sialic acid Neu5Gc molecules secreted by feeder cells may contribute to the rejection [81]. Human bone marrow- and adipose-derived MSCs were effectively expanded in serum-free and xeno-free culture medium (SFM-XF), and their capacity to differentiate into adipogenic, chondrogenic or osteogenic lineages was preserved [82]. Animal-free hESC pluripotency can be maintained by their encapsulation in calcium alginate hydrogels and their growth in basic medium is retained [83]. Ohmine et al. [84] investigated the generation of iPSCs from mobilized hematopoietic progenitor cells (HPCs) and immobilized peripheral blood mononuclear cells (PBMCs) under the GMP-compliant process. The protocol established by this research group offers a promising procedure for future clinical application. It has also been reported, that the derivation of hiPSCs from adult dermal fibroblasts according to the demands of GMP can be attained. For this purpose, chemically defined, feeder was used and the exogenous DNA-free protocol was established [85].

The products used for medical purposes must meet two basic conditions: (i) derivation of cells and cell products under GMP instructions and (ii) manipulation of cells or cell products according to GMP requirements [86]. Obtaining iPSCs and iPSC-derived products according to GMP standards will be possible in the near future, but this requires further investigations.

Conclusions

Implementation of the laboratory results into a manufacturing process is one of the most challenging steps in a large-scale production of cell-based therapeutics. Stem cell bioprocessing is a very promising field of research; therefore, it is rapidly expanding. Unfortunately, achievements on the laboratory scale do not guarantee implementation of identical protocols on a larger scale, to say nothing of their application in clinical medicine. Stem cell cultures on the benchtop and on a larger scale are not easily comparable, because the culture conditions and stem cell behavior are dissimilar. Moreover, cell-based medical products must be created in accordance with GMP at each stage of production. This requires very restrictive standards and compliance with high quality. The main problem is that application of bioprocesses on a clinical scale, according to the GMP standards, considerably raises the costs.

The expansion and differentiation of stem cells in an integrated bioreactor seems to be very tempting approach. In particular, the production of mature chondrocytes or chondroprogenitor cells from stem cells on a large scale would reveal unlimited treatment options in cell-based therapy of musculoskeletal diseases. The iPSCs, because of their self-renewal and pluripotency, are believed to be the best candidates for chondrocyte production on a clinical scale. However, many issues must still be taken into consideration, such as: the safety of reprogrammed cells, their efficient expansion and the effective chondrogenic differentiation. All of these steps should be scalable, and should meet GMP demands, but will require further efforts, which no doubt will advance research in this field.

References

1. Heir S, Nerhus TK, Røtterud JH, Løken S, Ekeland A, et al. (2010) Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis: a comparison of knee injury and osteoarthritis outcome score in 4 patient categories scheduled for knee surgery. Am J Sports Med 38: 231-237.
2. Browne JE, Branch TP (2000) Surgical alternatives for treatment of articular cartilage lesions. J Am Acad Orthop Surg 8: 180-189.
3. Cole BJ, Pascual-Garrido C, Grumet RC (2009) Surgical Management of Articular Cartilage Defects in the Knee. J Bone Joint Surg Am 91: 1778-1790.
4. Nehrer S, Spector M, Minas T (1999) Histologic analysis of tissue after failed cartilage repair procedures. Clin Orthop Relat Res 365: 149-162.
5. Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noël D (2009) Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. Trends Biotechnol 27: 307-314.
6. Bartlett W, Skinner JA, Gooding CR, Carrington RW, Flanagan AM, et al. (2005) Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br 87: 640-645.
7. Jones DG, Peterson L (2006) Autologous Chondrocyte Implantation. J Bone Joint Surg Am 88: 2501-2520.
8. Cooke ME, Allon AA, Cheng T, Kuo AC, Kim HT, et al. (2011) Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. Osteoarthritis Cartilage 19: 1210-1218.
9. Diao Y, Ma Q, Cui F, Zhong Y (2009) Human umbilical cord mesenchymal stem cells: osteogenesis in vivo as seed cells for bone tissue engineering. J Biomed Mater Res A 91: 123-131.
10. Diekman BO, Christoforou N, Willard VP, Sun H, Sanchez-Adams J, et al. (2012) Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells.Proc Nat Acad Sci 109: 19172-19177.
11. Li F, Niyibizi C (2012) Cells derived from murine induced pluripotent stem cells (iPSC) by treatment with members of TGF-beta family give rise to osteoblasts differentiation and form bone in vivo. BMC Cell Biol 13: 35.
12. Qu C, Puttonen KA, Lindeberg H, Ruponen M, Hovatta O, et al. (2013) Chondrogenic differentiation of human pluripotent stem cells in chondrocyte co-culture. Int J Biochem Cell Biol 45: 1802-1812.
13. Liu M, Liu N, Zang R, Li Y, Yang ST (2013) Engineering stem cell niches in bioreactors. World J Stem Cells 5: 124-135.
14. Naing MW, Williams DJ (2011) Three-dimensional culture and bioreactors for cellular therapies. Cytotherapy 13: 391-399.
15. Liu N, Zang R, Yang ST, Li Y (2014) Stem cell engineering in bioreactors for large-scale bioprocessing. Engineering in Life Sciences 14: 4–15.
16. Storm MP, Orchard CB, Bone HK, Chaudhuri JB, Welham MJ (2010) Three-dimensional culture systems for the expansion of pluripotent embryonic stem cells. Biotechnol Bioeng 107: 683-695.
17. Hwang NS, Kim MS, Sampattavanich S, Baek JH, Zhang Z, et al. (2006) Effects of three-dimensional culture and growth factors on the chondrogenic differentiation of murine embryonic stem cells. Stem Cells 24: 284–91.
18. Cao Y, Li D, Shang C, Yang ST, Wang J, et al. (2010) Three-dimensional culture of human mesenchymal stem cells in a polyethylene terephthalate matrix. Biomed Mater 5: 065013.
19. Handschel J, Naujoks C, Depprich R, Lammers L, Kübler N, et al. (2011) Embryonic stem cells in scaffold-free three-dimensional cell culture: osteogenic differentiation and bone generation. Head Face Med 7: 12.
20. Murphy SV, Atala A (2013) Organ engineering--combining stem cells, biomaterials, and bioreactors to produce bioengineered organs for transplantation. BioEssays? News and Reviews in Molecular, Cellular and Developmental Biology 35: 163–72.
21. Hambor JE (2012) Bioreactor Design and Bioprocess Controls for Industrialized Cell Processing. BioProcess International 10.
22. Wendt D, Jakob M, Martin I (2005) Bioreactor-based engineering of osteochondral grafts: from model systems to tissue manufacturing. J Biosci Bioeng 100: 489-494.
23. Martin I, Wendt D, Heberer M (2004) The role of bioreactors in tissue engineering. Trends Biotechnol 22: 80-86.
24. Salehi-Nik N, Amoabediny G, Pouran B, Tabesh H, Shokrgozar MA, et al. (2013) Engineering parameters in bioreactor's design: a critical aspect in tissue engineering. Biomed Res Int 2013: 762132.
25. Rodrigues CA, Fernandes TG, Diogo MM, da Silva CL, Cabral JM (2011) Stem cell cultivation in bioreactors. Biotechnol Adv 29: 815-829.
26. Naciri M, Kuystermans D, Al-Rubeai M (2008) Monitoring pH and dissolved oxygen in mammalian cell culture using optical sensors. Cytotechnology 57: 245-250.
27. Placzek MR, Chung IM, Macedo HM, Ismail S, Mortera Blanco T, et al. (2009) Stem cell bioprocessing: fundamentals and principles. J R Soc Interface 6: 209-232.
28. Baptista RP, Fluri DA, Zandstra PW (2013) High density continuous production of murine pluripotent cells in an acoustic perfused bioreactor at different oxygen concentrations. Biotechnology and Bioengineering 110: 648–55.
29. Serra M, Brito C, Sousa MF, Jensen J, Tostões R, et al. (2010) Improving expansion of pluripotent human embryonic stem cells in perfused bioreactors through oxygen control. Journal of Biotechnology 148: 208–215.

30. King JA, Miller WM (2007) Bioreactor development for stem cell expansion and controlled differentiation. Curr Opin Chem Biol 11: 394-398.
31. Toh WS, Lee EH, Cao T (2011) Potential of human embryonic stem cells in cartilage tissue engineering and regenerative medicine. Stem Cell Rev 7: 544-559.
32. Kowalczyk M, Waldron K, Kresnowati P, Danquah MK (2011) Process challenges relating to hematopoietic stem cell cultivation in bioreactors. J Ind Microbiol Biotechnol 38: 761-767.
33. Stolzing A, Scutt A (2006) Effect of reduced culture temperature on antioxidant defences of mesenchymal stem cells. Free Radic Biol Med 41: 326-338.
34. Caron MM, van der Windt AE, Emans PJ, van Rhijn LW, Jahr H, et al. (2013) Osmolarity determines the in vitro chondrogenic differentiation capacity of progenitor cells via nuclear factor of activated T-cells 5. Bone 53: 94–102.
35. Tandon N, Marolt D, Cimetta E, Vunjak-Novakovic G (2013) Bioreactor engineering of stem cell environments. Biotechnol Adv 31: 1020-1031.
36. Liovic P, Šutalo ID, Stewart R, Glattauer V, Meagher L (2012) Fluid Flow and Stresses On Microcarriers In Spinner Flask Bioreactors. Ninth International Conference on CFD in the Minerals and Process Industries CSIRO.
37. Sen A, Kallos MS, Behie LA (2002) Passaging protocols for mammalian neural stem cells in suspension bioreactors. Biotechnol Prog 18: 337-345.
38. Handorf AM, Li WJ (2014) Induction of mesenchymal stem cell chondrogenesis through sequential administration of growth factors within specific temporal windows. Journal of Cellular Physiology 229: 162–171.
39. Sart S, Agathos SN, Li Y (2014) Process engineering of stem cell metabolism for large scale expansion and differentiation in bioreactors. Biochemical Engineering Journal 84: 74–82.
40. Lavric V, OfiÅ£eru ID, Woinaroschy A (2005) A sensitivity analysis of the fed-batch animal-cell bioreactor with respect to some control parameters. Biotechnol Appl Biochem 41: 29-35.
41. Keller B, Yang T, Chen Y, Munivez E, Bertin T, et al. (2011) Interaction of TGFÎ² and BMP signaling pathways during chondrogenesis. PLoS One 6: e16421.
42. Abdallah BM, Harkness L, Mahmood A, Kassem M (2011) Direct Differentiation of Human Embryonic Stem Cells toward Osteoblasts and Chondrocytes through an Intermediate Mesenchyme Progenitor Lineage, Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis.
43. Gong G, Ferrari D, Dealy CN, Kosher RA (2010) Direct and progressive differentiation of human embryonic stem cells into the chondrogenic lineage. J Cell Physiol 224: 664-671.
44. Shen B, Wei A, Whittaker S, Williams LA, Tao H, et al. (2010) The role of BMP-7 in chondrogenic and osteogenic differentiation of human bone marrow multipotent mesenchymal stromal cells in vitro. J Cell Biochem 109: 406-416.
45. Vonwil D, Wendt D, Strobel S, Wallny HJ, Gygax D, et al. (2008) Assessment of the stability of TGFß3 bioactivity for potential bioreactor applications. Biochemical Engineering Journal 39: 586–589.
46. Gerecht-Nir S, Itskovitz-Eldor J (2004) Human embryonic stem cells: a potential source for cellular therapy. American Journal of Transplantation? Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons 4 Suppl 6: 51–57.
47. Rungarunlert S, Techakumphu M, Pirity MK, Dinnyes A (2009) Embryoid body formation from embryonic and induced pluripotent stem cells: Benefits of bioreactors. World J Stem Cells 1: 11-21.
48. zur Nieden NI, Cormier JT, Rancourt DE, Kallos MS (2007) Embryonic stem cells remain highly pluripotent following long term expansion as aggregates in suspension bioreactors. J Biotechnol 129: 421-432.
49. Dang SM, Gerecht-Nir S, Chen J, Itskovitz-Eldor J, Zandstra PW (2004) Controlled, scalable embryonic stem cell differentiation culture. Stem Cells 22: 275-282.
50. Gerecht-Nir S, Cohen S, Ziskind A, Itskovitz-Eldor J (2004) Three-dimensional porous alginate scaffolds provide a conducive environment for generation of well-vascularized embryoid bodies from human embryonic stem cells. Biotechnology and Bioengineering 88: 313–320.
51. Bauwens C, Yin T, Dang S, Peerani R, Zandstra PW (2005) Development of a perfusion fed bioreactor for embryonic stem cell-derived cardiomyocyte generation: oxygen-mediated enhancement of cardiomyocyte output. Biotechnology and Bioengineering 90: 452–461.
52. Liu N, Li Y, Yang ST (2014) Expansion of embryonic stem cells in suspension and fibrous bed bioreactors. J Biotechnol 178: 54-64.
53. Olmer R, Lange A, Selzer S, Kasper C, Haverich A, et al. (2012) Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Tissue Eng Part C Methods 18: 772-784.
54. Shafa M, Sjonnesen K, Yamashita A, Liu S, Michalak M, et al. (2012) Expansion and long-term maintenance of induced pluripotent stem cells in stirred suspension bioreactors. J Tissue Eng Regen Med 6: 462-472.
55. Toh WS, Lee EH, Guo XM, Chan JK, Yeow CH, et al. (2010) Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. Biomaterials 31: 6968-6980.
56. Alfred R, Taiani JT, Krawetz RJ, Yamashita A, Rancourt DE, et al. (2011) Large-scale production of murine embryonic stem cell-derived osteoblasts and chondrocytes on microcarriers in serum-free media. Biomaterials 32: 6006-6016.
57. Diekman BO, Christoforou N, Willard VP, Sun H, Sanchez-Adams J, et al. (2012) Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. Proc Natl Acad Sci U S A 109: 19172-19177.
58. Craft AM, Ahmed N, Rockel JS, Baht GS, Alman BA, et al. (2013) Specification of chondrocytes and cartilage tissues from embryonic stem cells. Development 140: 2597-2610.
59. Yamashita T, Abe K (2014) Direct reprogrammed neuronal cells as a novel resource for cell transplantation therapy. Cell Transplant 23: 435-439.
60. Chen X, Xu H, Wan C, McCaigue M, Li G (2006) Bioreactor expansion of human adult bone marrow-derived mesenchymal stem cells. Stem Cells 24: 2052-2059.
61. Augst A, Marolt D, Freed LE, Vepari C, Meinel L, et al. (2008) Effects of chondrogenic and osteogenic regulatory factors on composite constructs grown using human mesenchymal stem cells, silk scaffolds and bioreactors. Journal of the Royal Society, Interface / the Royal Society 5: 929–939.
62. Zhang ZY, Teoh SH, Teo EY, Khoon Chong MS, Shin CW, et al. (2010) A comparison of bioreactors for culture of fetal mesenchymal stem cells for bone tissue engineering. Biomaterials 31: 8684-8695.
63. Sart S, Errachid A, Schneider YJ, Agathos SN (2011) Controlled expansion and differentiation of mesenchymal stem cells in a microcarrier based stirred bioreactor. BMC Proceedings 5: 55.
64. Dos Santos F, Campbell A, Fernandes-Platzgummer A, Andrade PZ, Gimble JM, et al. (2014) A xenogeneic-free bioreactor system for the clinical-scale expansion of human mesenchymal stem/stromal cells. Biotechnol Bioeng 111: 1116-1127.
65. Schirmaier C, Jossen V, Kaiser SC, Jüngerkes F, Brill S, et al. (2014) Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions. Engineering in Life Sciences 14: 292–303.
66. Agung M, Ochi M, Yanada S, Adachi N, Izuta Y, Yamasaki T, Toda K (2006) Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. Knee Surg Traumatol Arthrosc 14:1307-1314.
67. Valonen PK, Moutos FT, Kusanagi A, Moretti MG, Diekman BO, et al. (2010) In vitro generation of mechanically functional cartilage grafts

based on adult human stem cells and 3D-woven poly(e-caprolactone) scaffolds. Biomaterials 31: 2193-2200.

68. Swieszkowski W, Tuan BH, Kurzydlowski KJ, Hutmacher DW (2007) Repair and regeneration of osteochondral defects in the articular joints. Biomol Eng 24: 489-495.
69. Khojasteh A, Behnia H, Hosseini FS, Dehghan MM, Abbasnia P, et al. (2013) The effect of PCL-TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: a preliminary report. J Biomed Mater Res B Appl Biomater 101: 848-854.
70. Iwai R, Fujiwara M, Wakitani S, Takagi M (2011) Ex vivo cartilage defect model for the evaluation of cartilage regeneration using mesenchymal stem cells. J Biosci Bioeng 111: 357-364.
71. Xue K, Qi L, Zhou G, Liu K (2013) A two-step method of constructing mature cartilage using bone marrow-derived mesenchymal stem cells. Cells Tissues Organs 197: 484-495.
72. Thorpe SD, Nagel T, Carroll SF, Kelly DJ (2013) Modulating Gradients in Regulatory Signals within Mesenchymal Stem Cell Seeded Hydrogels: A Novel Strategy to Engineer Zonal Articular Cartilage. PLoS One 16: e60764.
73. Bhumiratana S, Eton RE, Oungoulian SR, Wan LQ, Ateshian GA, et al. (2014) Large, stratified, and mechanically functional human cartilage grown in vitro by mesenchymal condensation. Proc Natl Acad Sci U S A 111: 6940-6945.
74. Orth P, Rey-Rico A, Venkatesan JK, Madry H, Cucchiarini M (2014) Current perspectives in stem cell research for knee cartilage repair. Stem Cells Cloning 7: 1-17.
75. La Noce M, Paino F, Spina A, Naddeo P, Montella R, et al. (2014) Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy. J Dent 42: 761-768.
76. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, et al. (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 18: 75-83.
77. Aktas M, Buchheiser A, Houben A, Reimann V, Radke T, et al. (2010) Good manufacturing practice-grade production of unrestricted somatic stem cell from fresh cord blood. Cytotherapy 12: 338-348.
78. Unger C, Skottman H, Blomberg P, Dilber MS, Hovatta O (2008) Good manufacturing practice and clinical-grade human embryonic stem cell lines. Hum Mol Genet 17: R48-53.
79. Pham PV, Vu NB, Pham VM, Truong NH, Pham TL, et al. (2014) Good manufacturing practice-compliant isolation and culture of human umbilical cord blood-derived mesenchymal stem cells. J Transl Med 12: 56.
80. Prathalingam N, Ferguson L, Young L, Lietz G, Oldershaw R, et al. (2012) Production and validation of a good manufacturing practice grade human fibroblast line for supporting human embryonic stem cell derivation and culture. Stem Cell Res Ther 3: 12.
81. Tannenbaum SE, Turetsky TT, Singer O, Aizenman E, Kirshberg S, et al. (2012) Derivation of xeno-free and GMP-grade human embryonic stem cells--platforms for future clinical applications. PLoS One 7: e35325.
82. Chase LG, Yang S, Zachar V, Yang Z, Lakshmipathy U, et al. (2012) Development and characterization of a clinically compliant xeno-free culture medium in good manufacturing practice for human multipotent mesenchymal stem cells. Stem Cells Transl Med 1: 750-758.
83. Siti-Ismail N, Bishop AE, Polak JM, Mantalaris A (2008) The benefit of human embryonic stem cell encapsulation for prolonged feeder-free maintenance. Biomaterials 29: 3946-3952.
84. Ohmine S, Dietz AB, Deeds MC, Hartjes KA, Miller DR, et al. (2011) Induced pluripotent stem cells from GMP-grade hematopoietic progenitor cells and mononuclear myeloid cells. Stem Cell Research & Therapy 2: 46.
85. Durruthy-Durruthy J, Briggs SF, Awe J, Ramathal CY, Karumbayaram S, et al. (2014) Rapid and efficient conversion of integration-free human induced pluripotent stem cells to GMP-grade culture conditions. PLoS One 9: e94231.
86. Bosse R, Kulmburg P, von Kalle C, Engelhardt M, Dwenger A, et al. (2000) Production of stem-cell transplants according to good manufacturing practice. Ann Hematol 79: 469-476.