OPINION

Regenerative medicine in rheumatic disease—progress in tissue engineering

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Abstract | Joint destruction occurs in both osteoarthritis and rheumatoid arthritis. Even in the era of biologic agents, this destruction can be delayed but not averted. As cartilage has limited ability to self-regenerate, joint arthroplasty is required. Here, we outline current tissue engineering procedures (including autologous chondrocyte implantation and *in situ* mesenchymal stem cell recruitment) that are routinely applied for the regenerative treatment of injured or early osteoarthritic cartilage. Potential future regenerative therapies, including administration of multipotent or pluripotent stem cells, are also discussed. In the future, cell-free, material-based (for cartilage lesions) or cell-free, factor-based (for osteoarthritic cartilage) therapies to facilitate the recruitment of repair cells and improve cartilage metabolism are likely to become more important. Moreover, delivery of anti-inflammatory factors or immunomodulatory cells could be a regenerative treatment option for rheumatoid arthritis. Tissue engineering faces a crucial phase to translate products into clinical routine and the regulatory framework for cell-based products in particular is an important issue.

Introduction

Injury of chondral or osteochondral tissue often leads to osteoarthritis, owing to the limited ability to self-regenerate. As a result of the personal and social burden of joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA), tissue engineering or regenerative medicine has developed as a means to regenerate cartilage and bone. The basic tools and approaches for joint tissue engineering are illustrated in Figure 1. Owing to the complex bone physiology and structure and, therefore, challenging repair process, only joint cartilage tissue engineering approaches have been translated into clinical routine. In this Perspectives, we discuss current and prospective tissue engineering approaches for joint repair in patients with rheumatic disease. Here, we focus on the clinical aspects of cell administration, *in situ* recruitment of mesenchymal stem cells (MSCs) and factor-based or MSC-based anti-inflammatory therapies. Moreover, we discuss the scientific and regulatory challenges in translating these approaches to the clinic.

Cell-based clinical products

Today, routinely applied cell-based cartilage tissue engineering products are based on autologous chondrocytes. These cells are isolated from healthy tissue from nonload-bearing cartilage, proliferated and then administered as a suspension or combined with matrices. During autologous chondrocyte implantation (ACI)—originally described in 1989[1]—chondrocytes are injected as a suspension into cartilage lesions covered by a periosteal flap or collagen sheet to avoid cell leakage (Figure 2). In matrix-assisted ACI (MACI), grafts of autologous chondrocytes and scaffolds are glued or sutured in cartilage lesions (Figure 2).[2] Different MACI products based on scaffold materials such as bioresorbable poly(DL-lactide acid-co-glycolic acid) polymers,[3] esterified hyaluronic acid polymers[4] and collagen I/III membranes[5] are currently available on the market.

In both techniques, chondrocytes secrete extracellular cartilage matrix. During MACI,[6] the use of a scaffold enables homogenous distribution of chondrocytes and cartilage formation, initial mechanical stability, simplified surgical handling and, depending on the type of scaffold and approach, arthroscopic surgery. In ACI, arthrotomy sometimes leads to adverse effects such as postoperative pain, scar formation, limited mobility and fibroarthrosis.[6] Furthermore, a perifocal solid cartilage shoulder is necessary to fix the periosteal flap or collagen sheet, which limits ACI to the regenerative treatment of focal cartilage lesions. By contrast, if grafts can be directly fixed onto subchondral bone, MACI enables stable fixation of the transplant in diffuse defects. Thus, in the future, MACI could be used for joint repair in patients with advanced arthritis.

Thousands of patients with chondral or osteochondral defects have been treated with ACI and MACI. In one study, 10–20 year long-term follow-up data of 224 patients with a cartilage defect were evaluated.[7] On the basis of results from patient survey and outcome scores, the authors concluded that ACI has emerged as an effective and durable technique for the regenerative treatment of large full-thickness cartilage and osteochondral lesions of the knee joint. However, in the literature, the outcome of ACI compared with other treatments is controversially discussed. For example, in one clinical study, 2 year and 5 year data have shown no statistically significant advantage of ACI over microfracture[8]—the standard technique used to stimulate cartilage self-regeneration based on blood clot formation. By contrast, in a similar clinical study, ACI was superior to microfracture when treating symptomatic cartilage defects of the knee.[9] In line with these observations, the clinical outcome of MACI also varies between different studies. In general, promising midterm (4–5 year) clinical results have been reported, which are independent of the scaffold material.[3–5,10] Although encouraging, in some instances the results for MACI were equivalent, but not superior, to ACI[11,12] and microfracture.[11] Importantly, ACI and MACI do not result in true native tissue, but in pain relief and formation of
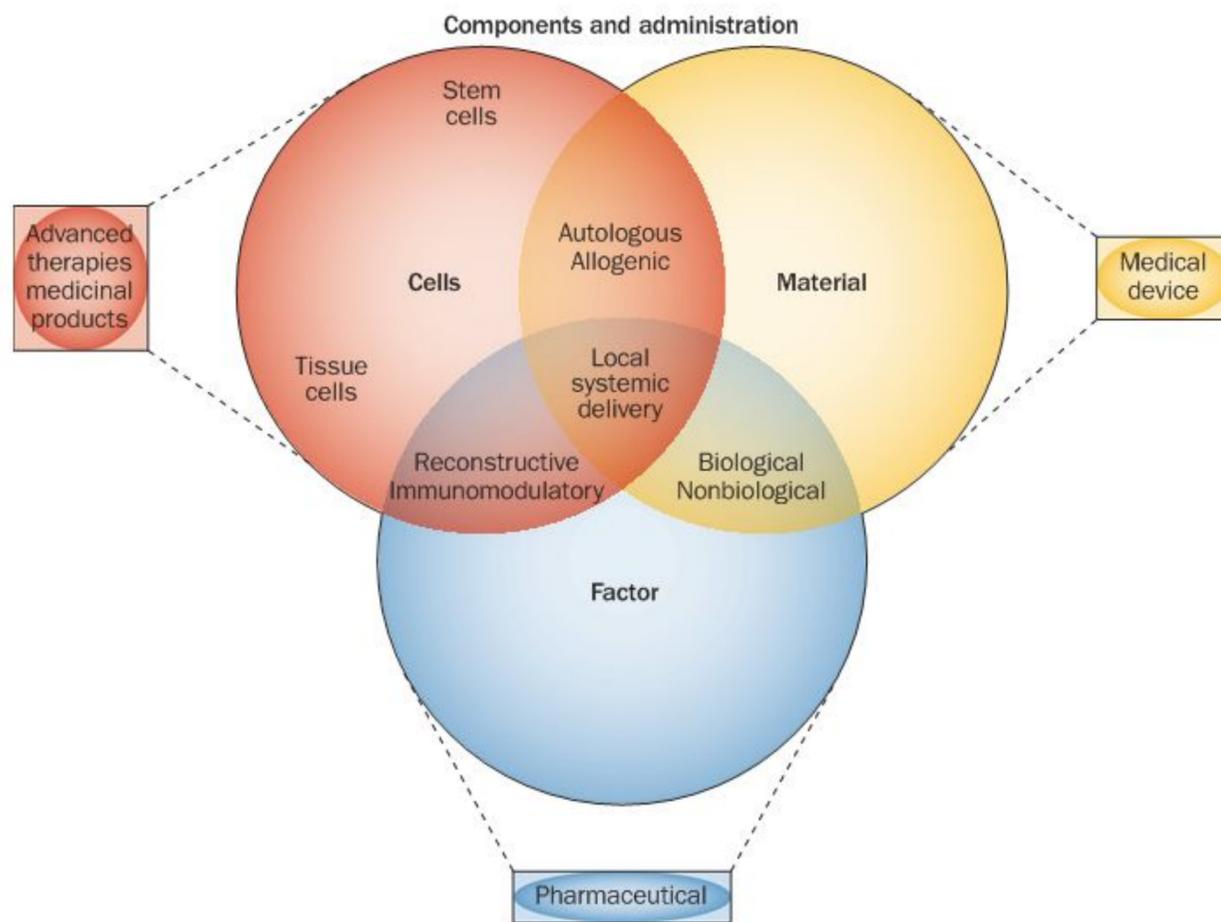
Figure 1 | Components, mode of administration and regulatory categories of regenerative therapies. Depending on the main components—cells, factors or materials—regenerative treatments are categorized differently, as a pharmaceutical, advanced therapies medicinal product or medical device. For the use of cells, distinction is needed between autologous or allogeneic cell sources and between stem or tissue cells. Cell delivery into the patient can be administered either locally or systemically. In addition, various different combinations of cells, factors and biomaterials are suggested in the literature.

fibrocartilage and/or articular cartilage and, therefore, delay OA and its adverse effects.

The feasibility of MACI in patients with advanced OA is an important area under investigation. Chondrocytes from patients with end-stage OA have similar growth and matrix-forming abilities as healthy donor cells.[13] Using MACI protocols, after seeding osteoarthritic chondrocytes on esterified hyaluronic acid polymers *in vitro*, articular cartilage has formed that is comparable to cartilage produced by donor chondrocytes from healthy donors. Moreover, the outcome of MACI in otherwise healthy individuals with cartilage lesions or patients with early OA with such lesions is comparable.[3,14,15] These evaluations were, however, based on short-term (1–3 years)[3,14] and mid-term data (4 years)[15] of only about 100 patients in total. Long-term studies of these techniques in patients with advanced OA are lacking.

Alternative cell sources
Today, solely autologous chondrocytes are routinely applied for the regenerative treatment of joint cartilage. However, the process of cartilage biopsy, chondrocyte culture and implantation is stressful for the patient and expensive. Furthermore, expansion of

chondrocytes in monolayer culture results in dedifferentiation to fibroblast-like cells with decreased cartilage formation ability.[13] Challenging 3D cultures reduce dedifferentiation and support redifferentiation, but do not overcome this problem, which could explain why ACI or MACI sometimes generate fibrocartilage. Therefore, the administration of stem cells is often discussed as an advanced treatment option.

Bone marrow MSCs
Bone marrow MSCs (also termed multipotent mesenchymal stromal cells) are easy to isolate and expand and differentiate into cartilage and bone, and thus can be used for the regenerative treatment of chondral and osteochondral lesions.[16,17] Moreover, these cells can migrate to diseased organs and secrete factors (such as immunosuppressive mediators for T cells), therefore enabling the use of allogeneic donor bone marrow MSCs (available in cell banks) to serve as a kind of factor-release system. Thus, they provide a regenerative environment, referred to as trophic activity, which stimulates regeneration by tissue intrinsic repair cells or stem cells.

Already in 2003, the therapeutic benefit of intra-articular injection of bone marrow

MSCs was proved in concept using a preclinical large-animal model of OA in goats.[18] There, OA was induced unilaterally in the knee joint by complete excision of the medial meniscus and resection of the anterior cruciate ligament, and then treated by intra-articular injection of MSCs suspended in hyaluronan.[18] This approach resulted in the beginning of regeneration of the medial meniscus and marked reduction of cartilage destruction, osteophyte remodelling and subchondral sclerosis during OA. In a 2011 phase I study to examine if MSC transplantation could reverse the progress of OA, MSCs were locally administered in the knee joints of four patients with OA.[19] After 1-year follow-up, pain relief and enhanced mobility with no adverse effects were observed.[19] In a further study, the efficacy of MSC-based ACI was compared with standard ACI in 72 matched patients with cartilage lesions.[20] The study authors concluded that MSC injection is as effective as ACI, but is less expensive and minimizes donor morbidity.[20]

Besides MSC injection approaches, the development of MSC-based MACI approaches is currently underway. In three case studies, MSCs were combined with collagen scaffolds and implanted in healthy individuals with full-thickness cartilage defects,[21,22] or patients with OA who also have such defects, with positive results.[23] Even if MSC-based MACI resulted in an enhanced formation of articular cartilage, these studies are preliminary and, from a clinical point of view, are far from established chondrocyte-based MACI.

Furthermore, although the MSC data from patients with OA are conflicting, we now know that MSCs derived from patients with end-stage OA can be extensively cultured and after stimulation with transforming growth factor β (TGF-β) form articular cartilage comparable to cartilage from healthy donor MSCs, using standard high-density pellet culture and MSC-based MACI approaches.[24,25] Nevertheless, most probably, allogeneic cells derived from healthy donors will be used in the future. Despite these promising data, only about six clinical studies on MSC-based cartilage regeneration are published, and in the ClinicalTrials.gov database roughly 20 trials (currently recruiting or completed) with focus on cartilage and/or OA are listed. In summary, although the administration of autologous chondrocytes is becoming increasingly important in cartilage regeneration, the application of MSCs is still

under investigation. On the basis of existing clinical data, it is too early to draw a final conclusion if, and for which diagnosis, administration of MSCs is preferable.

Other MSC sources

MSCs with similar properties to those derived from bone marrow are available from adipose tissue, corticospongeous bone, muscle, periosteum, synovium and umbilical cord, although naming the most appropriate MSC type is not yet possible.[26] Bone marrow MSCs are the best characterized and aspiration is a standardized and safe technique, but can be painful. Adipose tissue is easily accessible and MSCs derived from this tissue have a proliferation and differentiation capacity similar to bone marrow MSCs. Synovial MSCs are also comparable to bone marrow MSCs but, according to *in vitro* and animal data, have the most pronounced chondrogenic potential.[27] Strikingly, *in vitro* chondrogenic differentiated MSCs from synovium failed to form ectopic stable cartilage *in vivo*,[28] which is one reason to explain the slow development of clinical products based on administration of MSCs.

Pluripotent stem cells

In the past few years, embryonic stem cells and induced pluripotent stem cells (iPS) have been differentiated into cartilage and bone, and discussed as progenitors for joint tissue cells.[29,30] Interestingly for autologous tissue engineering, in 2011, MSCs, chondrocytes and osteoblasts have been generated from iPS derived from synovial fibroblasts of patients with advanced OA.[29] These results could pave the way for joint repair based on pluripotent autologous cells. However, topics including the use of viral vectors for iPS generation, lack of protocols for optimal 3D differentiation of cells, teratoma formation and regulatory affairs have to be solved before clinical application. Moreover, as for all new cells, the question is whether their possible advantages justify the labour and cost-intensive launch of a new clinical product. With these disadvantages in mind, we think that pluripotent cells are probably not a realistic clinical option for the foreseeable future.

Cell-free clinical products
Passive regenerative treatment

Biopsy is stressful and cell culture is expensive. Moreover, *in vitro* incubation of cell grafts is less efficient than *in vivo* incubation. Finally, cell-free material-based products are classified as medical devices and
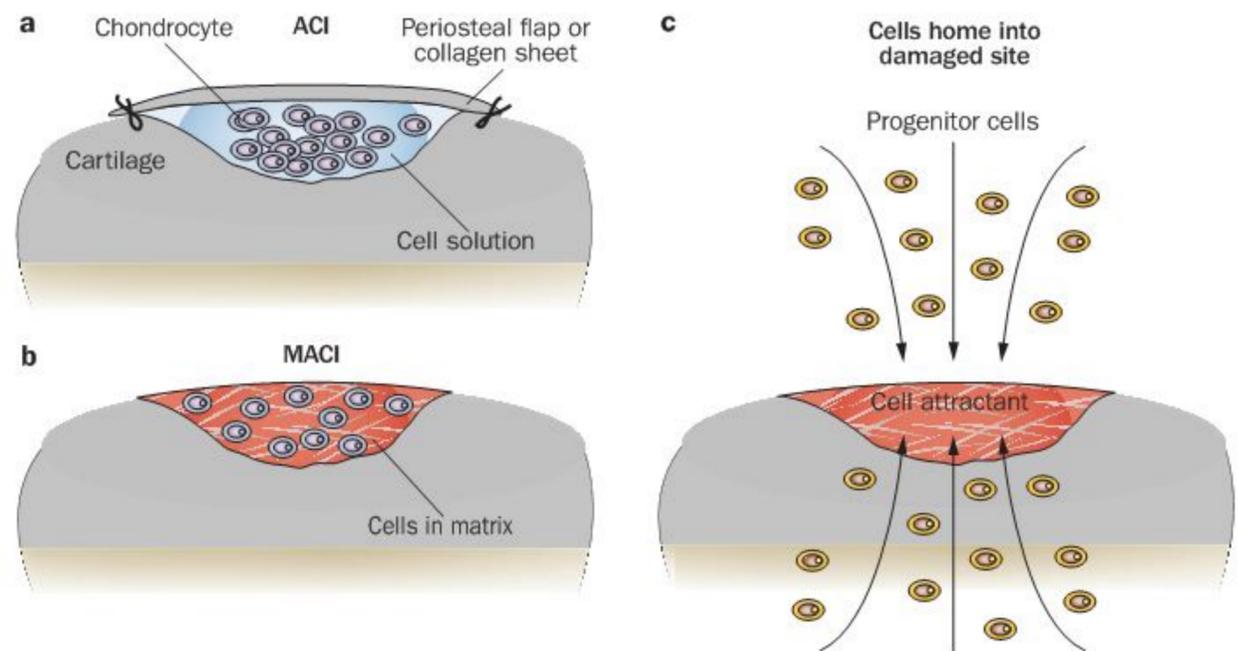

Figure 2 | Major principles for the regenerative treatment of cartilage lesions. **a** | During ACI, chondrocytes are harvested from a small cartilage biopsy sample, cultured and implanted as a cell suspension into a cartilage defect. To prevent a leakage of cells, the defect is covered by a periosteal flap or collagen sheet. **b** | During MACI, isolated and culture-expanded chondrocytes are seeded into matrices. Besides other advantages, such as homogeneous cell distribution, initial mechanical stability and simplified surgical handling, over ACI, such grafts can be fixed onto subchondral bone, which, therefore, enables a stable fixation in diffuse defects. Consequently, in the future, MACI could be used for joint repair during advanced arthritis. **c** | In newly emerging *in situ* tissue engineering strategies, cell-free implants consisting of matrices and/or local factor delivery systems for progenitor cell recruitment and differentiation are applied. This process can, for example, promote the recruitment of mesenchymal stem cells from bone marrow or synovium into a defect-filling chondroinductive matrix where they form new cartilage. Microfracture can also be applied to create small channels between the defect and subchondral bone, which, therefore, improves the level of contact between the defect and bone marrow. Abbreviations: ACI, autologous chondrocyte implantation; MACI, matrix-assisted autologous chondrocyte implantation.

regulatory approval is, therefore, easier than for cell-based products (Figure 1). Consequently, for so-called 'passive' regenerative treatment of focal joint cartilage lesions, cell-free material-based approaches are of increasing clinical interest.

One such passive technique, autologous matrix-induced chondrogenesis (AMIC), combines microfracture with the implantation of a collagen I/III membrane (Chondro-Gide® matrix, Geistlich Surgery, Wolhusen, Switzerland).[31] In a one-step approach, after arthroscopic evaluation, mini arthrotomy is performed. Here, the defect is exposed and cleaned before microfracture is performed. After microfracture, blood and bone marrow containing MSCs flow in and form a blood clot with all the cartilage-forming elements. Subsequently, the collagen I/III membrane is added, glued or sutured and the wound is closed. The matrix improves clot stability and initial mechanical stability for the new cartilage tissue. According to clinical data, AMIC is safe and leads to clinical improvement of symptomatic full-thickness chondral defects and to a regenerative defect filling.[32] The value of AMIC relative to ACI, MACI and microfracture is not currently clearly

defined. As with ACI and MACI, AMIC achieves pain relief, restores functionality of the joint and slows down cartilage destruction.[32] Chondrotissue® (BioTissue, Zurich, Switzerland) is a similar commercially available product used in combination with microfracture.[33,34] This cell-free implant consists of a polyglycolic acid polymer combined with autologous serum or autologous platelet-rich plasma (PRP) and hyaluronic acid.[34,35] Both autologous serum and PRP support the recruitment of MSCs from bone marrow, and hyaluronic acid aids the development of recruited MSCs to new cartilage. In one study, 1-year follow-up data of 52 patients with a full-thickness knee cartilage defect were evaluated.[35] At that time, the knee injury and OA score (KOOS) was markedly enhanced. Moreover, histological evaluation of biopsies available from five patients showed a homogeneous hyaline-like cartilage repair tissue.[35]

Active *in situ* approaches

In passive cartilage regeneration therapies such as AMIC, MSCs (but also other cells) flow into the carrier matrices. In newly emerging so-called 'active' cell-free, factor-based approaches aiming to regenerate

osteoarthritic joint tissues, a controlled local delivery of factors stimulates the selective recruitment and enrichment of tissue-specific cells or stem cells and induces enhanced cartilage formation. Moreover, it triggers cartilage responses and, in this way, recovery of chondrocyte remodelling. We speculate that active approaches are promising, with major value for patients with chronic cartilage degeneration.

In this context, it has been reported that articular cartilage contains cells with MSC-like character.[36] This finding means that the tissues relevant to the joint—cartilage, bone, synovium—and the surrounding bone marrow harbour MSCs, and provides the basis for *in situ* tissue engineering. One such possible strategy is to combine matrices with chemotactic molecules and factors that induce joint tissue regeneration and to inject or implant these cell-free grafts. This results in active *in situ* recruitment of MSCs to the sites of degenerated tissues, thus enabling their use in factor-guided joint repair (Figure 2).[25] As it contains MSC-recruiting autologous serum or PRP and chondrogenesis-supporting hyaluronic acid, Chondrotissue® can be considered to have 'active' properties.[33]

As a prerequisite for *in situ* therapies, knowledge of MSC migration or recruitment factors and the underlying mechanisms has dramatically increased. Chemokines,[37] PRP,[38] bone morphogenetic proteins,[39] synovial fluid[40] and serum from whole blood[41] have a dose-dependent migratory effect on MSCs derived from different tissues. For arthritis, that synovial fluid of the joint of healthy donors and patients with OA both similarly recruit MSCs in Boyden chamber chemotaxis assays, whereas synovial fluid of donors with RA have markedly reduced recruitment activity, is of special interest.[40] This finding implies that, in 'active' cell-free, factor-based approaches in patients with OA and RA, most likely different chemotactic stimuli will have to be used.

In addition, factors that stimulate chondrogenesis or improve cartilage metabolism, such as matrix molecules (hyaluronan), members of the TGF-β superfamily (TGF-β1 and TGF-β3 and bone morphogenetic proteins BMP2, BMP6, BMP7 and BMP11), members of the fibroblast growth factor family, insulin-like growth factor I and platelet-derived growth factor have been described.[42] Most frequently, TGF-β1 was used to induce and control chondrogenesis; it limits hypertrophy and prevents vascularization and development to bone.[17,42]

Furthermore, a phase II trial to show safety and efficacy of an implant of chondrocytes secreting TGF-β1 in patients with advanced OA is ongoing.[43] Also promising, is the use of PRP, conditioned serum and bone marrow concentrates as methods for combined growth factor delivery.

For local factor delivery, controlled-release systems have been developed. In some studies, factors are incorporated in, or bonded on, matrices for simultaneous cell growth and drug delivery;[44] in other studies they are released out of particles.[42,45] For instance, TGF-β3 encapsulation in nanoparticles was used as a controlled delivery system to improve maintenance of the bioavailability of this factor, and in rabbits resulted in development of cartilage superior to control cultures treated with empty nanoparticles.[46]

Antagonizing inflammation

The effectiveness of tissue engineering approaches will be limited by inflammatory conditions in the joint. In diseases such as RA, formation of cartilage will be impaired and new cartilage degraded over time. In the past few years, the therapeutic importance of controlled delivery of anti-rheumatic drugs has been reported.[47] Most of the available therapies do not have tissue specificity and, therefore, to reach sufficient drug concentrations, high drug doses are systemically delivered, which causes high costs and adverse systemic and extra-articular effects. Local drug delivery systems are a promising approach to overcome this problem by encapsulating, for example, anti-inflammatory drugs and releasing them at the target site.[42,47] We anticipate that local drug delivery systems of anti-inflammatory drugs will become important tools to antagonize inflammation during RA and will enable cell-based and cell-free tissue engineering.

In this context, the immunomodulatory functions of MSCs with antiproliferative and anti-inflammatory effects are promising. As reviewed by others,[17,48] MSC-mediated immunomodulation requires activation by immune cells. After activation, MSC-mediated immunosuppression primarily acts through the secretion of soluble factors, whose expression is regulated by crosstalk with various target cells. Suppression is initiated by autologous and allogeneic MSCs, an important aspect, as so far it is not known whether MSCs from patients with different types of arthritis display normal or impaired qualities. MSCs from patients with RA have features of early senescence, reduced

clonogenic and proliferative activity, but normal multilineage and immunosuppressive properties.[25] MSCs modulate the host immune response mainly by inhibiting T-cell proliferation. As T-cell response is one important event in inflammation during arthritis, MSC administration is a prospective treatment option. In this scenario, damaged joint tissues could be substituted by MSC-based MACI and the new tissue could be protected. However, in a preclinical collagen-induced arthritis experimental mouse model of RA—in which mice were immunized with collagen and received an intraperitoneal injection of MSCs—results were contradictory.[17,48] Some groups observed a reduced destruction of joint tissues, proliferation of T-helper cells and secretion of inflammatory cytokines; others found no or even negative effects.[17,48] New data indicate that the outcome is related to the time of injection and/or the current immune status of the animal. In this context, an interesting but still open question is, if and how the immunomodulatory features of MSC changes during differentiation in general, and especially in the inflammatory milieu. Based on the present knowledge, we believe that the establishment of MSC mediated anti-inflammatory treatment strategies is very complex but if successful, it may become a powerful tool to treat chronic joint diseases.

Regulatory nature

Presently, tissue engineering faces a crucial phase to implement cell-based therapies in routine clinical practice. In the USA, cell products are regulated by the FDA as human cells, tissues, and cellular and tissue-based products.[49,50] In Europe, cell-based tissue engineering is regulated under the new regulation of advanced therapies medicinal products (ATMP) from the European Medicines Agency.[49] The consequences thereof will be essential for commercialization and a broad clinical availability. This pharmaceutical regulatory framework is relevant to all allogeneic and autologous cell-based treatments of injured or diseased tissues (Figure 1), which includes new products, those that are currently under development and existing products such as ACI or MACI.

We consider the new ATMP regulation as an important step forward. Before that, regulation for cell-based products varied markedly between countries, and development and commercialization was hard to plan for the European health market. Although

research in the past decade has proven the regenerative potential of cell-based therapies, this field has only gradually increased its success in the health market with so far mainly smaller companies developing these products.[51] It seems obvious that pharmaceutical regulation is an expensive burden for small and medium-sized enterprises. Furthermore, manufacturing and logistics of living pharmaceutical products is still a rather novel technology and a challenging effort for small and even larger companies. Currently, with ChondroCelect® (TiGENIX, Leuven, Belgium), an ACI product,[52] only one cell product is available commercially with approval according to the new ATMP regulation in 2009. Most other cell-based tissue engineering products are marketed according to preceding national regulatory requirements.

Consequently, only limited experience is available for the development of new therapies under the new regulatory framework. The general effect on the costs for development of cell-based products and also for the routine manufacturing in clean rooms are not yet clear. Considering the enormous efforts in stem cell research for regeneration of joint tissues, it seems important to carefully evaluate the regulatory issues on cell products early in research and address the essential requirements for safety, quality and feasibility from a regulatory perspective.[53] This approach could help to promote translation of research beyond preclinical and clinical studies towards a broad availability of such new treatments in the near future.

Conclusions

Today, autologous chondrocytes (ACI, MACI) or *in situ* recruited MSCs are routinely clinically used for the regenerative treatment of injured or early osteoarthritic damaged joint tissue. The importance of administration of MSCs expanded *in vitro* from bone marrow, synovium or adipose tissue is still under investigation. However, 10 years after the proof of concept that intraarticular MSC injection delays progression of OA, no MSC-based cell product exists. One possible reason is that *in vitro* differentiated MSCs from synovium failed to form ectopic stable cartilage *in vivo*. Another reason, also relevant for pluripotent cells, might be regulatory affairs in the context of stem cell product approval. We believe the field in general faces a crucial phase to translate cell-based strategies into clinical practice. Health insurances are reluctant and require a long-term socioeconomic benefit

of novel treatments. In Europe, the consequences of the new ATMP regulatory framework will be essential for commercialization and broad clinical availability.

In situ tissue engineering products (both passive and active approaches) are promising treatment options for patients with cartilage defect or chronic cartilage degeneration. The importance of tissue engineering will dramatically increase if large lesions caused by OA or RA can be regenerated. Furthermore, targeted controlled delivery of anti-inflammatory drugs will be of utmost importance for inflammatory conditions in the joint that are accompanied by impaired cartilage regeneration abilities and degradation of new formed cartilage. Moreover, we believe that, in the more distant future, the immunomodulatory function of MSCs could be used in modified MACI and *in situ* tissue engineering approaches to alter the surrounding joint milieu in such a way that cell-based tissue formation and subsequent protection is possible. Clearly, such approaches are complex, but very powerful, in the context of regenerative treatment.

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Author contributions
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