

Do we really need cartilage tissue engineering?

Karoliina Pelttari, Anke Wixmerten, Ivan Martin

Departments of Surgery and of Biomedicine, University Hospital Basel, Basel, Switzerland

Summary

The *in vitro* engineering of functionally developed biological cartilage substitutes, based on cells and appropriate structural and soluble factors, is an attractive concept for the clinical treatment of cartilage injuries and degeneration. The field of cartilage tissue engineering has developed strongly in the last few years, bringing together the scientific, clinical and commercial interests of highly interdisciplinary communities. However, engineered grafts are still far from being the standard of care for cartilage repair. In this review we present some of the issues challenging the reproducible engineering of functional cartilage templates starting from human cells. We then discuss the need to identify the mode of action of cartilage tissue engineering approaches, which in turn is expected to define potency markers and quality

controls for grafts capable of inducing durable cartilage regeneration. Finally, we propose the use of engineered cartilage tissues not only as implants to be implemented in the clinic, but also as models to understand mechanisms and processes related to cartilage development and repair. The knowledge generated using these models will be instrumental in moving to the next generation of cartilage repair approaches, namely those inducing regeneration *in situ*, based on the recruitment of resident cells.

Key words: cartilage repair; regenerative medicine; stem cells; tissue manufacturing, chondrogenesis; cell differentiation; aging; nasal chondrocytes; quality control

Introduction

Because of the limited self-healing capacity of cartilage, repair of articular defects caused by degenerative joint diseases or traumatic injuries represents an open clinical challenge. The appearance of lesions is frequently associated with pain, disturbed function and disability, and – if not successfully treated – often results in total replacement of the joint. Some of the most popular treatment options, referred to as marrow-stimulating techniques (e.g., microfracture or subchondral drilling of the bone) are based on the principle of inducing invasion of mesenchymal progenitor cells from the underlying subchondral bone to the lesion site, in order to initiate cartilage repair [1, 2]. In the absence of a material which appropriately “instructs” the mesenchymal progenitors to differentiate into articular chondrocytes in stable fashion, the outcome of these procedures is highly variable and often results in repair tissue composed of fibrocartilage, with limitations in quality and duration as compared to native hyaline tissue [3, 4]. A breakthrough in the field, especially for localised injuries, was the introduction of cell-based repair techniques, such as autologous chondrocyte implantation (ACI) [5]. In this procedure,

in vitro expanded autologous articular chondrocytes are expanded in culture and introduced into the defect site as a cell suspension or in association with a supportive matrix (matrix-assisted ACI, MACI) [6], where they are expected to synthesise new cartilaginous matrix. The clinical outcome of these chondrocyte-based techniques is generally good, as they lead to lessening of symptoms for the patient [7–9], but in many cases results in the formation of fibrous repair tissue with inferior mechanical properties and limited durability [10–14]. A recent study proposed a correlation between the symptomatology of patients treated with ACI and the quality of the repair tissue, suggesting that the persistence of symptoms, e.g. pain or swelling, after surgery reflected the presence of non-hyaline cartilage repair tissue [15]. These observations underline the importance of improving the quality of the generated repair tissue following treatment of cartilage defects.

Regeneration of a hyaline-like tissue could be facilitated by the implantation of a pre-engineered, functional cartilage tissue, as opposed to the delivery of a chondrocyte suspension. This

would involve culturing autologous cells within an environment permitting or supporting chondrogenesis (e.g., a porous three-dimensional – 3D – biomaterial of pre-defined size and shape), generating a graft approaching the biochemical and biomechanical properties of native cartilage. Indeed, the presence of extracellular matrix (ECM) around cells was reported to enhance donor cell retention at the repair site [16] and possibly protect the cells from environmental factors such as inflammatory molecules [17]. Furthermore, pre-cultivation under conditions inducing cell differentiation was shown to support enhanced *in vivo* development of engineered cartilage at ectopic sites in mice [18] and improved cellular response to a compressive deformation conditioning re-

sembling a mild rehabilitation regime [19]. Importantly, from the clinical point of view the improved mechanical stability of the more mature and stable engineered graft would also allow easier surgical handling, application even in critically sized defects [20] and possibly earlier postoperative loading.

In this paper we shall first briefly describe the state of the art in cartilage tissue engineering, highlighting the critical factors and still open scientific and manufacturing challenges. We will then discuss which properties of an engineered cartilage graft are likely to be required to induce cartilage regeneration, and, finally, address the critical question of the potential clinical benefits introduced by cartilage tissue engineering.

Critical factors in cartilage tissue engineering

A typical approach to engineering of autologous cartilage grafts is schematically displayed in figure 1. Several proof-of-principle studies using animal cells have indicated that the approach can generate tissues approaching the biochemical and biomechanical properties of native cartilage [21], and that implantation of such constructs can repair critically sized articular defects [22]. However, attempts to translate such concepts and methods to a clinical-case scenario (e.g., using human cells and reaching a relevant thickness) have revealed crucial factors which still hamper the generation of implants of reproducible quality. Some of the main challenges to be addressed in the process of *human* cartilage engineering are discussed below.

Cell phenotype regulation

As autologous chondrocytes can only be harvested from a small biopsy of articular cartilage in

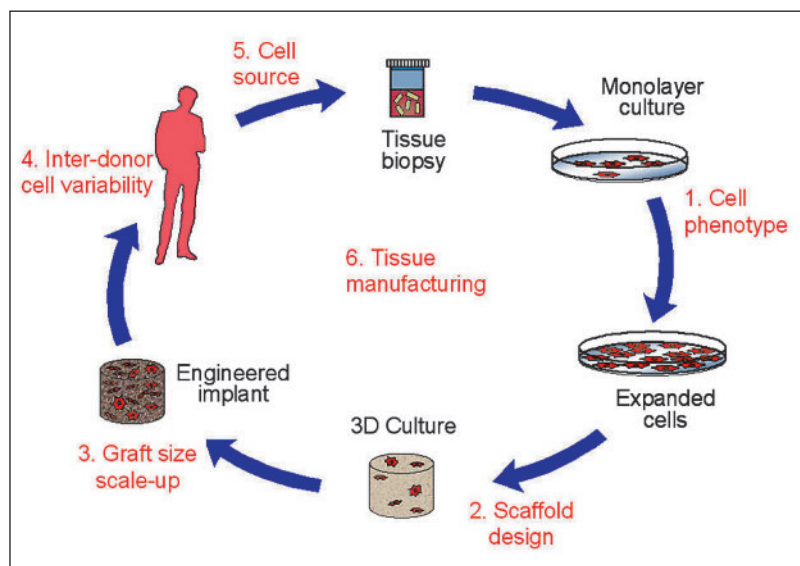
relatively low numbers, typical tissue engineering-based methods so far require monolayer expansion in order to obtain sufficient cell numbers. Isolation of articular chondrocytes from the surrounding cartilage matrix and their subsequent two-dimensional culture, however, is accompanied by cellular de-differentiation, resulting in gradual changes in morphology (i.e., cells lose their spherical shape and acquire an elongated fibroblast-like morphology) and reduction or even total loss of the synthesis of cartilage-specific proteins (i.e., type II collagen or aggrecan) [23–25]. De-differentiated chondrocytes in principle retain the capacity to re-differentiate if transferred into a 3D (e.g., agarose or alginate suspension) environment [26, 27] or cultured at high cell density in medium inducing chondrogenic differentiation [28–30]. However, such a process is often inefficient and the phenotype reached hardly matches that found in native cartilage [31].

To improve the generation of hyaline cartilage *in vitro*, several groups have aimed at preserving the original cell phenotype by preventing de-differentiation during expansion in monolayer. In particular, cells have been cultured on dishes coated with proteins mimicking the ECM, such as fibronectin, type I or type II collagen [32–36], or in environments supporting a 3D cell morphology including gels, scaffolds and porous microcarriers [37–39]. Collectively, however, these studies did not convincingly demonstrate that maintenance of the native phenotype can be achieved in parallel with extensive proliferation, or that a more limited degree of de-differentiation corresponds to a higher quality of the generated tissues.

Instead, it is becoming increasingly clear that de-differentiation per se is not necessarily detrimental, as long as the cells maintain their capacity to re-differentiate. In fact, chondrocytes expanded in the presence of specific growth factors, which enhanced the process of de-differentiation, displayed

Figure 1

Tissue engineering of autologous cartilage grafts. The chart displays the different phases in a typical approach to engineering of autologous grafts (blue arrows) and the most critical factors entailed (1. to 6., discussed in section 2 of this manuscript).



a higher post-expansion cartilage forming ability and could more efficiently respond to chondrogenic stimuli during subsequent culture in a 3D environment [29, 40, 41]. Interestingly, the enhanced de-differentiation in the presence of growth factors was also leading to a multi-lineage differentiation capacity of the expanded cells (e.g., towards osteoblastic or adipocytic phenotypes), suggesting that expansion under appropriate conditions could result in a phenotype and function similar to that of mesenchymal progenitor cells [42], possibly by selective enrichment of putative progenitor cells in the chondrocyte population [43].

Despite these promising descriptive data, it should be clarified that the molecular mechanisms inducing and controlling the processes of chondrocyte de- and re-differentiation are not yet well known, and that the re-differentiated phenotype obtained is in many cases suboptimal.

Scaffold design

The characteristics of the substrate used for 3D culture (i.e., scaffold) may offer a possible way of attaining improved cell differentiation and thus improved cartilage tissue quality. Several materials have been used so far for *in vitro* generation of cartilage tissues, in a broad range of compositions, architectures, porosities, surface topographies and mechanical properties [44, 45]. Beyond some fundamental requirements which need to be provided by a scaffold material (e.g., biocompatibility, adherence by cells, and initial stability) [45, 46], the variety of concepts and models proposed by different groups for the generation of scaffolds for cartilage tissue engineering reflects the fact that understanding of the requirements for regulation of chondrocyte function is still poor. Future interdisciplinary efforts should thus aim at determining which specific feature of a material has what selective effect on chondrocytes or chondrocyte progenitors, in order to identify specific de-

sign criteria for chondroinductive materials. In this regard, it appears that surface wettability, related to initial protein adsorption [47], as well as elasticity of the substrate [48], could have a distinct role which needs to be better understood and exploited.

Graft size scale-up

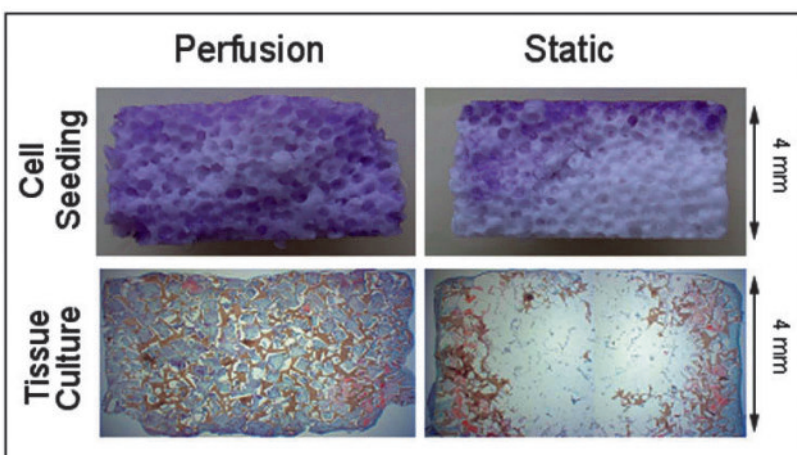
The generation of grafts in a clinically relevant size, i.e. up to 4 mm in thickness, is challenged by the difficulties in achieving uniform cell distribution (i.e., seeding) in the different scaffold areas, as well as efficient nutrition of the seeded cells. Indeed, conventional, static culture conditions typically result in a periphery with cells and a rather necrotic core, with few or no cells. Different types of bioreactor systems, introducing hydrodynamic forces and fluid flow, have been proposed to overcome these limitations [49–51]. In particular, bioreactors perfusing first a cell suspension and subsequently culture medium directly through the pores of the scaffolds have been shown to enable homogeneous and efficient cell seeding on a variety of scaffolds, as well as to provide sufficient nutrition even for cells in the centre of the scaffold [52–54]. This resulted in the uniform deposition of ECM throughout the scaffold, as opposed to static culture leading to a highly heterogeneous tissue with cells and ECM only in the periphery (fig. 2).

Despite these promising results, it is important to stress that the perfusion of fluid within a complex scaffold architecture, which is changing over time due to the deposition of ECM, introduces an additional degree of complexity in the design of a cartilage engineering process, in particular due to interaction between biological, structural and physicochemical parameters [55]. Thus, in order to define suitable operating conditions (e.g., flow rate, oxygen percentage setting) for each scaffold architecture, it will be necessary to develop computational fluid dynamic models (e.g., including shear forces and oxygen diffusion/consumption) which, if combined with experimental data, could ultimately help to predict cell behaviour within distinct scaffold regions, or alternatively to design appropriate scaffold architectures [56, 57].

In addition to the engineering challenges, the up-scaling process for the construct size also requires higher cell numbers in order to reach a critical cell seeding density [58]. Considering the difficulty of obtaining sufficient numbers of cells by expansion from a small autologous biopsy, future strategies should explore the possibility of co-culturing a limited number of chondrocytes together with mesenchymal progenitor cells isolated from other tissues (e.g., bone marrow, fat tissue, skin), which could be appropriately “instructed” by the chondrocytic population [59] (see also section 2.5).

Figure 2

Cell distribution and matrix synthesis under perfusion and static conditions. Seeding of cells in a perfusion-based bioreactor system results in a uniform distribution of cells (here stained purple) throughout the scaffold as compared to conventional static seeding conditions [52]. Subsequent culture under perfusion additionally supports a more homogeneous tissue formation and avoids the formation of a necrotic centre typical of statically cultured cartilage grafts [53].



Management of inter-donor chondrocyte variability

One often underestimated critical factor in cartilage tissue engineering is the quality of the starting material, namely the chondrocytes isolated from different cartilage biopsies. The capacity of isolated cartilage cells to proliferate and – most importantly – to regenerate a tissue is in fact not only dependent on the health state of the joint [60] and on the age of the donor [61], but is also extremely variable between individuals in the same age range and with no history of joint disease [62] (fig. 3). The use of specific growth factors during cell expansion, in conjunction with low percentages of serum, can reduce variability in proliferation and re-differentiation capacity [40], but is not sufficient to guarantee a reproducible quality of the resulting engineered cartilage. This challenge poses the critical question of whether engineered cartilage tissues will ever be considered acceptable grafts for clinical use if their quality cannot be standardised within a rather narrow range.

In this context, several groups have endeavoured to identify markers to predict and possibly control the chondrogenic capacity of chondrocytes. But despite interesting studies targeting expression of a discrete set of genes [63], of surface molecules [64] or of secreted proteins [65], no such reliable markers have yet been identified. Alternatively, use of a different cell source with a higher and more reproducible cartilage-forming capacity could make it possible to reduce the variability in the quality of the generated cartilage grafts.

Cell source selection

Besides the above-mentioned problems surrounding the high variability in cell quality, the use of articular chondrocytes in cartilage tissue engineering is also associated with disadvantages during their procurement, including morbidity at

the donor site. In fact, even a small biopsy harvested from a non-weight bearing site represents an additional injury to the joint and has been reported to be detrimental to the surrounding healthy tissue, possibly increasing the risk of osteoarthritis developing in the future [66, 67]. While it remains controversial whether allogeneic chondrocytes will ever have clinical potential, alternative autologous cell sources based on non-articular tissues have been considered [68]. Mesenchymal progenitor cells from bone marrow, adipose tissue, synovial membrane and other tissues [69–72] are discussed as an attractive and promising alternative cell source, since they display a high proliferation capacity with limited reduction of differentiation potential, and can conveniently be committed *in vitro* towards the chondrogenic lineage [30, 73, 74]. However, chondrogenic differentiation by mesenchymal progenitor cells has been shown to be accompanied by the expression of markers specific for hypertrophic chondrocytes, such as type X collagen and MMP13 [75, 76], typically leading to matrix calcification and vascularisation after ectopic transplantation *in vivo* [77]. These observations indicate potential instability of the acquired chondrocytic phenotype, so that despite their successful application in the repair of osteochondral defects in different animal models [78–80] and humans [81, 82], the safe and long term efficacy of mesenchymal progenitor cells in the regeneration of hyaline cartilage still needs to be demonstrated. The co-culture of differentiated chondrocytes together with mesenchymal progenitors has been proposed as a possible strategy for bypassing this issue. In preliminary studies chondrocytes were able to induce bone marrow-derived stromal cells to differentiate towards the chondrogenic lineage while at the same time reducing the described risk of hypertrophy often observed during the exclusive use of the stromal cell population [83, 84].

As an alternative strategy for avoiding the risk of phenotypic instability of chondrogenically induced progenitor cells, it would be attractive to use already differentiated chondrocytes from non-articular cartilages. For example, nasal septum biopsies can be harvested under local anaesthesia in a minimally invasive procedure and with limited donor site morbidity [85]. Several studies have indicated that human nasal chondrocytes have a higher proliferation rate compared to articular chondrocytes, and after de-differentiation are able to retain their capacity to generate hyaline-like tissues *in vitro* and ectopically *in vivo* [86–89]. The mechanical properties of nasal chondrocyte-based tissue engineered grafts can reproducibly approach those of native cartilage [20], and furthermore the tissue quality seems less dependent on donor age [89] and was reported to be histologically, biochemically and mechanically superior to that generated from articular chondrocytes from matched donors [89]. For nasal chondrocytes to be considered for implantation at

Figure 3

Variability of engineered cartilage quality using articular chondrocytes from different donors. Quality of the cartilage tissues engineered by expanded chondrocytes, here quantified using the content of glycosaminoglycans (GAG)/DNA following pellet culture, depends on the age of the donor but is additionally highly variable between cells from donors in the same age range (see large standard deviations in A). Differences in matrix quality are demonstrated by safranin-O staining for GAG of generated cartilage from cells of a donor with good (B) or bad (C) re-differentiation capacity, within the same age range (40–49 years).

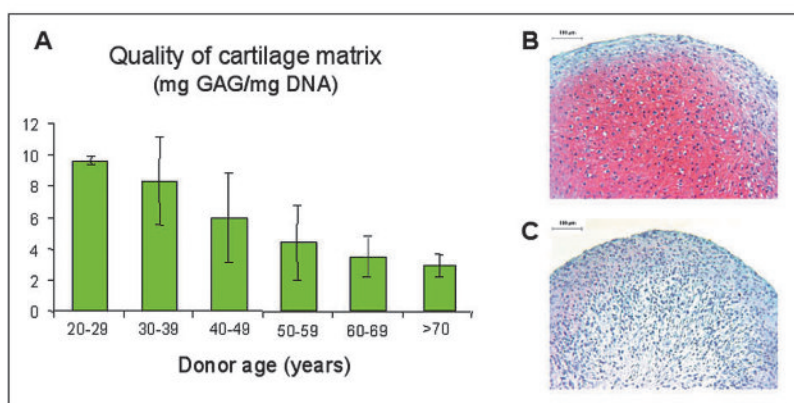
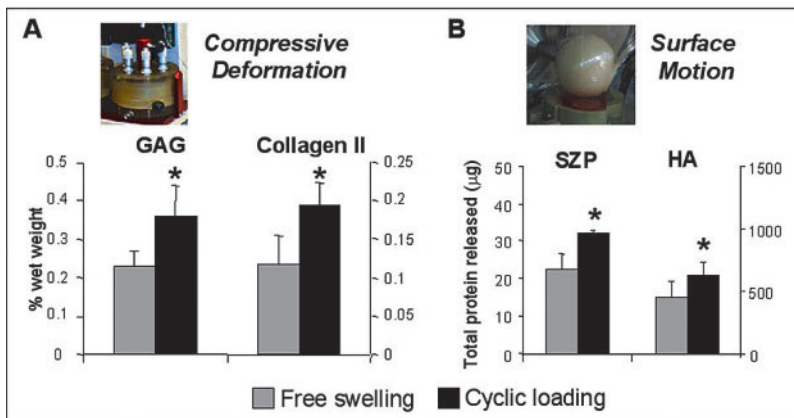


Figure 4

Responsiveness of nasal chondrocytes to mechanical loading. Synthesis of glycosaminoglycans (GAG) and type II collagen (Collagen II) increases in response to compressive mechanical loading on grafts generated from expanded nasal chondrocytes (A). Additionally, the expression of the lubricating factors superficial zone protein (SZP) and hyaluronan (HA) is induced in response to surface motion (B). Asterisks indicate statistically significant differences from the static (free swelling) controls [90].



an articular site, it is crucial not only to determine their chondrogenic capacity but also to assess whether they are responsive to forces associated with joint loading. With this in view, recent experiments demonstrated that cartilage engineered from nasal chondrocytes can respond to physical forces resembling joint loading by up-regulating markers specific for hyaline cartilage, such as glycosaminoglycans and type II collagen, as well as expression of molecules typically involved in joint lubrication (fig. 4) [20, 90, 91], making nasal chondrocytes a highly promising cell source for the repair of articular cartilage defects. Clearly, to demonstrate whether the tissue generated by non-articular chondrocytes from nasal septum is adequate for cartilage repair at articular sites requires further investigations using *in vivo* orthotopic experimental studies [92] and/or *in vitro* loaded models.

Tissue manufacturing

One major obstacle in delivering cartilage tissue engineering products to routine clinical use are the costly, labour-intensive and time-consuming manual processes which are difficult to control and standardise. To be attractive for clinical application, engineered cartilage will need to demonstrate (i) cost-effectiveness and cost-bene-

fits over existing therapies, (ii) absolute safety for patients, manufacturers and the environment, and (iii) compliance with the evolving regulatory framework in terms of quality control and good manufacturing practice (GMP). To meet these targets and translate research-scale production into clinically compatible manufacture, the process could be streamlined and automated within bioreactor systems implementing precisely monitored and tightly controlled conditions [50, 93, 94]. In this respect a promising manufacturing concept is the on-site hospital-based ACTES™ (Autologous Clinical Tissue Engineering System) currently under development by Octane (www.octaneco.com). In this fully automated bioreactor system the patient's cartilage biopsy will be digested and the chondrocytes expanded before being seeded and cultured on an osteoconductive porous scaffold to generate a cartilage graft, with all production phases implemented within a single, closed bioreactor system. This concept could simplify logistical issues surrounding transfer of specimens between locations, reduce the need for large and expensive GMP tissue engineering facilities and minimize operator handling, with the likely final result of reducing the cost of engineered grafts.

An alternative concept for bypassing the bottleneck of tissue manufacturing would be to develop a process whereby cell procurement, scaffold seeding and its transplantation back into the patient would occur during the same surgical operation. Clearly this concept would not make it possible to generate mature cartilage tissue and would rely on the "*in vivo* bioreactor" of our body to develop a functional tissue equivalent from the grafted template. The difficulty of having a sufficiently large number of cells to be implanted could be overcome, for example, by combining a small number of chondrocytes, freshly isolated from a small biopsy, with resident or exogenously delivered mesenchymal progenitor cells. Indeed, it has been reported that non-expanded chondrocytes can induce chondrogenic differentiation of other cell types (see also section 2.5 on cell sources) [59] and that even undigested cartilage tissue, minced into small particles, can be used to repair experimental cartilage defects in large animal models [95, 96].

Potency factors for cartilage tissue engineering

The previous sections of this article have highlighted the rationale for developing engineered cartilage grafts, the advances made in recent years and the still open scientific and technical challenges to be addressed. To translate the opportunities described into effective therapeutic options (e.g., for the treatment of traumatic cartilage injuries), it will be essential to understand

what signals should be delivered at what stage into a joint to promote durable cartilage regeneration. Only with this fundamental knowledge will it be possible to design engineered tissues with a defined mode of action and supported by suitable quality controls for predictable potency. Ultimately, it must be admitted that – although the first clinical report of autologous chondrocyte im-

plantation [5] now dates back more than 15 years – it is not yet established which factors play which role in the cartilage healing processes.

Is cartilage regeneration dependent upon the *quality of the delivered cells*, which play an active role in producing a suitable repair tissue? Or are the functional *properties of the developed extracellular matrix* in the graft the key regenerative signal, possibly by supporting appropriate transduction of mechanical loading [19, 97]? Or is the *profile of cytokines released* by the transplanted cells the essential component in managing local inflammation and stimulating tissue repair? Until scientific

cally grounded answers are found to these crucial questions, it will not be possible to decide whether a quality control for engineered cartilage products should rely on the chondrogenic capacity of the implanted cells, on the mechanical tests of the developed matrix, or on the pattern of factors produced and released during culture. And without a clearly identified mode of action, combined with predictable potency factors, it will not be realistic or sound to introduce a product which is not easily standardised and reproducible for widespread clinical use.

Conclusions

On the basis of the critical considerations presented above, and taking into account the intrinsic difficulties faced in generating and manufacturing engineered cartilage grafts, the rather obvious question to be raised is “Do we really need cartilage tissue engineering?”

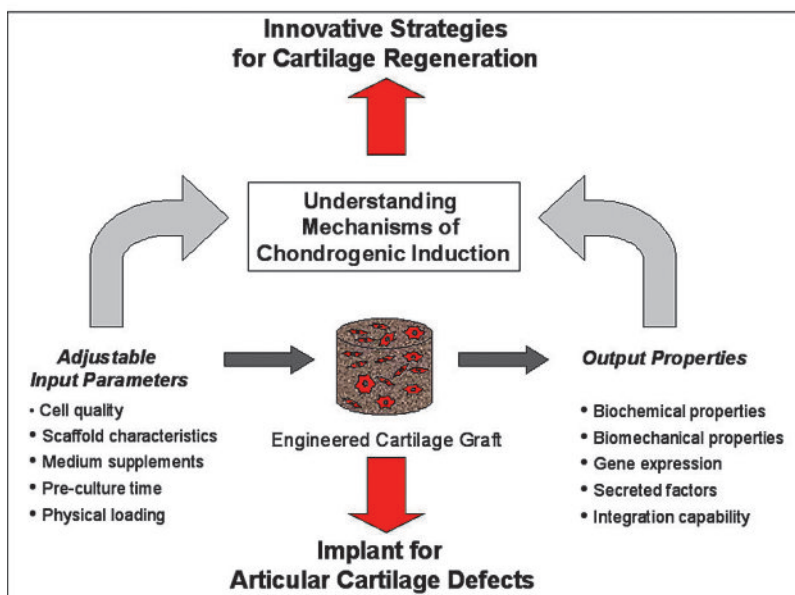
On the one hand, a definite answer can be given only when prospective, randomised and controlled clinical studies have been conducted based on long-term, quantitative outcome measures and in combination with suitable quality controls for the implanted tissues. In this regard, the scientific and clinical communities should be

urged to enter the dimension of this systematic analysis, also giving due consideration to the surgical factors which may strongly affect a clinical result (e.g., whether a concomitant axis correction by osteotomy is performed or not).

On the other hand, and probably even more importantly, cartilage tissue engineering must be considered a necessary powerful tool to model the biological and molecular processes of cartilage development, and to identify the cues required to induce its regeneration. This will be possible by performing hypothesis-driven experiments aiming at the definition of mechanistic relationships between specific culture parameters and the resulting tissue properties (fig. 5). At this stage, the knowledge and understanding gained will be crucial to design alternative, smarter ways to deliver the defined cues in an injured joint, e.g. using drug delivery vehicles, cell-free intelligent scaffolding materials or intraoperative cell processing procedures, combined with appropriate surgical techniques and postoperative rehabilitation regimes. Ultimately, cartilage tissue engineering models will provide the means to operate a paradigm shift, overcoming the traditionally envisaged implantation of preformed grafts and approaching the more modern perspective of regenerative medicine. Paradoxically, then, we do need cartilage tissue engineering to identify cartilage repair strategies which may go beyond cartilage tissue engineering.

Figure 5

Outlook on the need for cartilage tissue engineering. The traditional purpose of generating engineered cartilage is clearly the use as graft for the repair of articular cartilage defects (bottom red arrow). We propose that hypothesis-driven experiments on the cause-effect relationships between modulating parameters (left) and the resulting tissue properties (right) will help to understand general mechanisms of chondrogenesis critical for cartilage development and regeneration. This knowledge will lead to the development of innovative strategies for cartilage regeneration (top red arrow), possibly based on the recruitment of resident cells thanks to the combination of surgical techniques, smart materials and postoperative rehabilitation regimes.



Correspondence:

Prof. Ivan Martin

Institute for Surgical Research and Hospital Management

University Hospital Basel

Hebelstrasse 20

CH-4031 Basel

Switzerland

E-Mail: imartin@ubbs.ch

References

- Levy AS, Lohnes J, Sculley S, LeCroy M, Garrett W. Chondral delamination of the knee in soccer players. *Am J Sports Med.* 1996;24(5):634-9.
- Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone Joint Surg Am.* 1976;58(2):230-3.
- Kreuz PC, Steinwachs MR, Erggelet C, Krause SJ, Konrad G, Uhl M, Sudkamp N. Results after microfracture of full-thickness chondral defects in different compartments in the knee. *Osteoarthritis. Cartilage.* 2006;14(11):1119-25.
- Temenoff JS, Mikos AG. Review: tissue engineering for regeneration of articular cartilage. *Biomaterials.* 2000;21(5):431-40.
- Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med.* 1994;331(14):889-95.
- Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: generations of autologous chondrocyte transplantation. *Eur J Radiol.* 2006;57(1):24-31.
- Bentley G, Biant LC, Carrington RW, Akmal M, Goldberg A, Williams AM, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br.* 2003;85(2):223-30.
- Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am.* 2004;86-A(3):455-64.
- Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res.* 2000;374:212-34.
- Brittberg M. Autologous chondrocyte transplantation. *Clin Orthop Relat Res.* 1999;367(Suppl):S147-S155.
- Grigolo B, Roseti L, De FL, Piacentini A, Cattini L, Manfredini M, et al. Molecular and immunohistological characterization of human cartilage two years following autologous cell transplantation. *J Bone Joint Surg Am.* 2005;87(1):46-57.
- Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. *J Bone Joint Surg Am.* 2003;85-A(2):185-92.
- Roberts S, McCall IW, Darby AJ, Menage J, Evans H, Harrison PE, et al. Autologous chondrocyte implantation for cartilage repair: monitoring its success by magnetic resonance imaging and histology. *Arthritis Res Ther.* 2003;5(1):R60-R73.
- Tins BJ, McCall IW, Takahashi T, Cassar-Pullicino V, Roberts S, Ashton B, et al. Autologous chondrocyte implantation in knee joint: MR imaging and histologic features at 1-year follow-up. *Radiology.* 2005;234(2):501-8.
- Brun P, Dickinson SC, Zavan B, Cortivo R, Hollander AP, Abatangelo G. Characteristics of repair tissue in second-look and third-look biopsies from patients treated with engineered cartilage: relationship to symptomatology and time after implantation. *Arthritis Res Ther.* 2008;10(6):R132.
- Ball ST, Goomer RS, Ostrander RV, Tontz WL Jr, Williams SK, Amiel D. Preincubation of tissue engineered constructs enhances donor cell retention. *Clin Orthop Relat Res.* 2004;420:276-85.
- Francioli S, Miot S, Frueh J, Cavallo C, Jakob M, Grigolo B, et al. Production of cytokines and response to IL-1 β by human articular chondrocytes at different stages of tissue maturation. *Tissue Engineering Part A.* May 2008;14(5):691-943. doi:10.1089/tea.2008.1504.
- Moretti M, Wendt D, Dickinson SC, Sims TJ, Hollander AP, Kelly DJ, et al. Effects of in vitro preculture on in vivo development of human engineered cartilage in an ectopic model. *Tissue Eng.* 2005;11(9-10):1421-8.
- Demarteau O, Wendt D, Braccini A, Jakob M, Schafer D, Heberer M, et al. Dynamic compression of cartilage constructs engineered from expanded human articular chondrocytes. *Biochem Biophys Res Commun.* 2003;310(2):580-8.
- Farhadi J, Fulco I, Miot S, Wirz D, Haug M, Dickinson SC, et al. Precultivation of engineered human nasal cartilage enhances the mechanical properties relevant for use in facial reconstructive surgery. *Ann Surg.* 2006;244(6):978-85.
- Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, et al. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J Orthop Res.* 1999;17(1):130-8.
- Schafer D, Martin I, Jundt G, Seidel J, Heberer M, Grodzinsky A, et al. Tissue-Engineered Composites for the Repair of Large Osteochondral Defects. *Arthritis Rheum.* 2002;46(9):2524-34.
- Benya PD, Padilla SR, Nimmi ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell.* 1978;15(4):1313-21.
- Binette F, McQuaid DP, Haudenschild DR, Yaeger PC, McPherson JM, Tubo R. Expression of a stable articular cartilage phenotype without evidence of hypertrophy by adult human articular chondrocytes in vitro. *J Orthop Res.* 1998;16(2):207-16.
- von der Mark K, Gaus V, von der MH, Muller P. Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. *Nature.* 1977;267(5611):531-2.
- Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell.* 1982;30(1):215-24.
- Bonaventure J, Kadhom N, Cohen-Solal L, Ng KH, Bourguignon J, Lasselin C, Freisinger P. Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. *Exp Cell Res.* 1994;212(1):97-104.
- Holtzer H, Abbott J, Lash J, Holtzer S. The loss of phenotypic traits by differentiated cells in vitro. I. Dedifferentiation of cartilage cells. *Proc Natl Acad Sci U.S.A.* 1960;46(12):1533-42.
- Jakob M, Demarteau O, Schafer D, Hintermann B, Dick W, Heberer M, et al. Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation in vitro. *J Cell Biochem.* 2001;81(2):368-77.
- Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res.* 1998;238(1):265-72.
- Mukaida T, Urabe K, Naruse K, Aikawa J, Katano M, Hyon SH, et al. Influence of threedimensional culture in a type II collagen sponge on primary cultured and dedifferentiated chondrocytes. *J Orthop Sci.* 2005;10(5):521-8.
- Shakibaei M, De SP, Merker HJ. Integrin expression and collagen type II implicated in maintenance of chondrocyte shape in monolayer culture: an immunomorphological study. *Cell Biol Int.* 1997;21(2):115-25.
- Kino-Oka M, Yashiki S, Ota Y, Mushiaki Y, Sugawara K, Yamamoto T, et al. Subculture of chondrocytes on a collagen type I-coated substrate with suppressed cellular dedifferentiation. *Tissue Eng.* 2005;11(3-4):597-608.
- Malda J, Kreijveld E, Temenoff JS, van Blitterswijk CA, Riesle J. Expansion of human nasal chondrocytes on macroporous microcarriers enhances redifferentiation. *Biomaterials.* 2003;24(28):5153-61.
- Brodtkin KR, Garcia AJ, Levenston ME. Chondrocyte phenotypes on different extracellular matrix monolayers. *Biomaterials.* 2004;25(28):5929-38.
- Barbero A, Grogan SP, Mainil-Varlet P, Martin I. Expansion on specific substrates regulates the phenotype and differentiation capacity of human articular chondrocytes. *J Cell Biochem.* 2006;98(5):1140-9.
- Kimura T, Yasui N, Ohsawa S, Ono K. Chondrocytes embedded in collagen gels maintain cartilage phenotype during long-term cultures. *Clin Orthop Relat Res.* 1984;(186):231-9.
- Gugala Z, Gogolewski S. In vitro growth and activity of primary chondrocytes on a resorbable polylactide three-dimensional scaffold. *J Biomed Mater Res.* 2000;49(2):183-91.
- Malda J, van Blitterswijk CA, Grojec M, Martens DE, Tramper J, Riesle J. Expansion of bovine chondrocytes on microcarriers enhances redifferentiation. *Tissue Eng.* 2003;9(5):939-48.
- Francioli SE, Martin I, Sie CP, Hagg R, Tommasini R, Candrian C, et al. Growth factors for clinical-scale expansion of human articular chondrocytes: relevance for automated bioreactor systems. *Tissue Eng.* 2007;13(6):1227-34.
- Martin I, Suetterlin R, Baschong W, Heberer M, Vunjak-Novakovic G, Freed LE. Enhanced cartilage tissue engineering by sequential exposure of chondrocytes to FGF-2 during 2D expansion and BMP-2 during 3D cultivation. *J Cell Biochem.* 2001;83(1):121-8.
- Barbero A, Ploegert S, Heberer M, Martin I. Plasticity of clonal populations of dedifferentiated adult human articular chondrocytes. *Arthritis Rheum.* 2003;48(5):1315-25.
- Dowthwaite GP, Bishop JC, Redman SN, Khan IM, Rooney P, Evans DJ, et al. The surface of articular cartilage contains a progenitor cell population. *J Cell Sci.* 2004;117(6):889-97.
- Woodfield TB, Bezemer JM, Pieper JS, van Blitterswijk CA, Riesle J. Scaffolds for tissue engineering of cartilage. *Crit Rev Eukaryot Gene Expr.* 2002;12(3):209-36.
- Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noel D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. *Trends Biotechnol.* 2009;27(5):307-14.
- Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage.* 2002;10(6):432-63.
- Miot S, Scandiucci de FP, Wirz D, Daniels AU, Sims TJ, Hollander AP, et al. Cartilage tissue engineering by expanded goat articular chondrocytes. *J Orthop Res.* 2006;24(5):1078-85.

- 48 Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006;126(4):677-89.
- 49 Freed LE, Hollander AP, Martin I, Barry JR, Langer R, Vunjak-Novakovic G. Chondrogenesis in a cell-polymer-bioreactor system. *Exp Cell Res*. 1998;240(1):58-65.
- 50 Martin I, Wendt D, Heberer M. The role of bioreactors in tissue engineering. *Trends Biotechnol*. 2004;22(2):80-6.
- 51 Schulz RM, Wustneck N, van Donkelaar CC, Shelton JC, Bader A. Development and validation of a novel bioreactor system for load- and perfusion-controlled tissue engineering of chondrocyte-constructs. *Biotechnol Bioeng*. 2008;101(4):714-28.
- 52 Wendt D, Marsano A, Jakob M, Heberer M, Martin I. Oscillating perfusion of cell suspensions through three-dimensional scaffolds enhances cell seeding efficiency and uniformity. *Biotechnol Bioeng*. 2003;84(2):205-14.
- 53 Wendt D, Stroebel S, Jakob M, John GT, Martin I. Uniform tissues engineered by seeding and culturing cells in 3D scaffolds under perfusion at defined oxygen tensions. *Biorheology*. 2006;43(3-4):481-8.
- 54 Khan AA, Suits JM, Kandel RA, Waldman SD. The effect of continuous culture on the growth and structure of tissue-engineered cartilage. *Biotechnol Prog*. 2009;25(2):508-15.
- 55 Zhou S, Cui Z, Urban JP. Nutrient gradients in engineered cartilage: metabolic kinetics measurement and mass transfer modeling. *Biotechnol Bioeng*. 2008;101(2):408-21.
- 56 Boschetti F, Raimondi MT, Migliavacca F, Dubini G. Prediction of the micro-fluid dynamic environment imposed to three-dimensional engineered cell systems in bioreactors. *J Biomech*. 2006;39(3):418-25.
- 57 Cioffi M, Kuffer J, Strobel S, Dubini G, Martin I, Wendt D. Computational evaluation of oxygen and shear stress distributions in 3D perfusion culture systems: macro-scale and micro-structured models. *J Biomech*. 2008;41(14):2918-25.
- 58 Chen AC, Nagrampa JP, Schinagl RM, Lottman LM, Sah RL. Chondrocyte transplantation to articular cartilage explants in vitro. *J Orthop Res*. 1997;15(6):791-802.
- 59 Hendriks J, Riesle J, van Blitterswijk CA. Co-culture in cartilage tissue engineering. *J Tissue Eng Regen Med*. 2007;1(3):170-8.
- 60 Tallheden T, Bengtsson C, Brantsing C, Sjogren-Jansson E, Carlsson L, Peterson L, et al. Proliferation and differentiation potential of chondrocytes from osteoarthritic patients. *Arthritis Res Ther*. 2005;7(3):R560-R568.
- 61 Loeser RF, Shanker G, Carlson CS, Gardin JE, Shelton BJ, Sonntag WE. Reduction in the chondrocyte response to insulin-like growth factor 1 in aging and osteoarthritis: studies in a non-human primate model of naturally occurring disease. *Arthritis Rheum*. 2000;43(9):2110-20.
- 62 Barbero A, Grogan S, Schafer D, Heberer M, Mainil-Varlet P, Martin I. Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. *Osteoarthritis Cartilage*. 2004;12(6):476-84.
- 63 Dell'Accio F, De BC, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis Rheum*. 2001;44(7):1608-19.
- 64 Grogan SP, Barbero A, az-Romero J, Cleton-Jansen AM, Soeder S, Whiteside R, et al. Identification of markers to characterize and sort human articular chondrocytes with enhanced in vitro chondrogenic capacity. *Arthritis Rheum*. 2007;56(2):586-95.
- 65 Peltari K, Lorenz H, Boeuf S, Templin MF, Bischel O, Goetzke K, et al. Secretion of matrix metalloproteinase 3 by expanded articular chondrocytes as a predictor of ectopic cartilage formation capacity in vivo. *Arthritis Rheum*. 2008;58(2):467-74.
- 66 Lee CR, Grodzinsky AJ, Hsu HP, Martin SD, Spector M. Effects of harvest and selected cartilage repair procedures on the physical and biochemical properties of articular cartilage in the canine knee. *J Orthop Res*. 2000;18(5):790-9.
- 67 Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. *Arthroscopy*. 2002;18(7):730-4.
- 68 Chung C, Burdick JA. Engineering cartilage tissue. *Adv Drug Deliv Rev*. 2008;60(2):243-62.
- 69 Friedenstien AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet*. 1970;3(4):393-403.
- 70 Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, et al. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood*. 1980;56(2):289-301.
- 71 Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7(2):211-28.
- 72 De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. 2001;44(8):1928-42.
- 73 Barry F, Boynton RE, Liu B, Murphy JM. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res*. 2001;268(2):189-200.
- 74 Sekiya I, Vuorio JT, Larson BL, Prockop DJ. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc Natl Acad Sci U.S.A.* 2002;99(7):4397-402.
- 75 Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng*. 1998;4(4):415-28.
- 76 Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, et al. Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. *Arthritis Rheum*. 2003;48(2):418-29.
- 77 Peltari K, Winter A, Steck E, Goetzke K, Hennig T, Ochs BG, et al. Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. *Arthritis Rheum*. 2006;54(10):3254-66.
- 78 Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy*. 2007;23(2):178-87.
- 79 Im GI, Kim DY, Shin JH, Hyun CW, Cho WH. Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow. *J Bone Joint Surg Br*. 2001;83(2):289-94.
- 80 Guo X, Wang C, Zhang Y, Xia R, Hu M, Duan C, et al. Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. *Tissue Eng*. 2004;10(11-12):1818-29.
- 81 Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transplant*. 2004;13(5):595-600.
- 82 Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, et al. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage*. 2007;15(2):226-31.
- 83 Mo XT, Guo SC, Xie HQ, Deng L, Zhi W, Xiang Z, et al. Variations in the ratios of co-cultured mesenchymal stem cells and chondrocytes regulate the expression of cartilaginous and osseous phenotype in alginate constructs. *Bone*. 2009;45(1):42-51.
- 84 Bigdeli N, Karlsson C, Strehl R, Concaro S, Hyllner J, Lindahl A. Co-culture of Human Embryonic Stem Cells and Human Articular Chondrocytes Results In Significantly Altered Phenotype and Improved Chondrogenic Differentiation. *Stem Cells* 2009.
- 85 Siegel NS, Gliklich RE, Taghizadeh F, Chang Y. Outcomes of septoplasty. *Otolaryngol Head Neck Surg*. 2000;122(2):228-32.
- 86 Naumann A, Dennis JE, Aigner J, Cotichia J, Arnold J, Berghaus A, et al. Tissue engineering of autologous cartilage grafts in three-dimensional in vitro macroaggregate culture system. *Tissue Eng*. 2004;10(11-12):1695-706.
- 87 Tay AG, Farhadi J, Suetterlin R, Pierer G, Heberer M, Martin I. Cell yield, proliferation, postexpansion differentiation capacity of human ear, nasal, and rib chondrocytes. *Tissue Eng*. 2004;10(5-6):762-70.
- 88 Kafienah W, Jakob M, Demarteau O, Frazer A, Barker MD, Martin I, et al. Three-dimensional tissue engineering of hyaline cartilage: comparison of adult nasal and articular chondrocytes. *Tissue Eng*. 2002;8(5):817-26.
- 89 Rotter N, Bonassar LJ, Tobias G, Lebl M, Roy AK, Vacanti CA. Age dependence of biochemical and biomechanical properties of tissue-engineered human septal cartilage. *Biomaterials*. 2002;23(15):3087-94.
- 90 Candrian C, Vonwil D, Barbero A, Bonacina E, Miot S, Farhadi J, et al. Engineered cartilage generated by nasal chondrocytes is responsive to physical forces resembling joint loading. *Arthritis Rheum*. 2008;58(1):197-208.
- 91 Grad S, Lee CR, Gorna K, Gogolewski S, Wimmer MA, Alini M. Surface motion upregulates superficial zone protein and hyaluronan production in chondrocyte-seeded three-dimensional scaffolds. *Tissue Eng*. 2005;11(1-2):249-56.
- 92 Vinatier C, Gauthier O, Masson M, Malard O, Moreau A, Fellah BH, et al. Nasal chondrocytes and fibrin sealant for cartilage tissue engineering. *J Biomed Mater Res A*. 2008;176-85.
- 93 Ratcliffe A, Niklason LE. Bioreactors and bioprocessing for tissue engineering. *Ann N.Y. Acad Sci*. 2002;961:210-5.
- 94 Matin I, Smith T, Wendt D. A roadmap for the bioreactor-based translation of tissue engineering strategies into clinical products. *Trends Biotechnol*. 2009;27(9):495-502.
- 95 Frisbie D, Lu Y, Colhoun H, Kawcak M, Binette F, McIlwraith C. In vivo evaluation of a one step autologous cartilage resurfacing technique in a long term equine model. *Transactions of the 51st Annual Meeting of the Orthopaedic Research Society*. 2005, vol. 30, p. 1355.
- 96 Lu Y, Dhanaraj S, Wang Z, Kong W, Bradley D, Binette F. A novel intra-operative approach to treat full thickness articular defects with chondrocyte-loaded implants. *Transactions of the 51st Annual Meeting of the Orthopaedic Research Society*. 2005, vol. 30, p. 1363.
- 97 Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. *J Cell Sci*. 1995;108(Pt 4):1497-508.