Cell Seeding Densities in Autologous Chondrocyte Implantation Techniques for Cartilage Repair

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Abstract

Cartilage repair techniques have been among the most intensively investigated treatments in orthopedics for the past decade, and several different treatment modalities are currently available. Despite the extensive research effort within this field, the generation of hyaline cartilage remains a considerable challenge. There are many parameters attendant to each of the cartilage repair techniques that can affect the amount and types of reparative tissue generated in the cartilage defect, and some of the most fundamental of these parameters have yet to be fully investigated. For procedures in which *in vitro*–cultured autologous chondrocytes are implanted under a periosteal or synthetic membrane cover, or seeded onto a porous membrane or scaffold, little is known about how the number of cells affects the clinical outcome. Few published clinical studies address the cell seeding density that was employed. The principal objective of this review is to provide an overview of the cell seeding densities used in cell-based treatments currently available in the clinic for cartilage repair. Select preclinical studies that have informed the use of specific cell seeding densities in the clinic are also discussed.

Keywords

cartilage repair, autologous cartilage implantation, cell seeding density

Introduction

Articular cartilage injuries in the knee are common, and despite 2 decades of intensive research, the treatment remains challenging.[1,2] Depending on factors such as defect location and size, patient age, activity level, comorbidities, and defect chronicity, the surgeon can choose from among an array of treatment options, including microfracture, scaffold-supported microfracture, osteochondral autograft and allograft transplantation, and autologous chondrocyte implantation (ACI)–related techniques.[3] The use of cultured autologous chondrocytes for implantation under a periosteal membrane (i.e., ACI) as originally proposed by Grande *et al*.[4] and clinically applied by Brittberg *et al.*[5] has given rise to a large number of related treatments, which occasionally are introduced to the clinic with limited preclinical evidence. These treatments are either "second-generation" ACI techniques using a synthetic membrane as a substitute for the periosteum or "third-generation" ACI methods using synthetic membranes or biodegradable, porous sponge-like scaffolds or gels as carriers for the chondrocytes. In addition, modifications of the growth and selection of the chondrocytes used in ACI have been introduced into the clinic, including implementation of protocols to investigate the chondrogenic potential of cells prior to their implantation and matrix-free growth of the cells to form spheroids.[6-8]

A critical question for all cell-based therapies, which has not yet been definitively answered, is how the exogenous cells contribute to chondrogenesis: do the cells themselves proliferate, retaining their phenotype, and directly synthesize the cartilaginous matrix? Or do the exogenous cells serve as regulators of endogenous progenitor cell differentiation and function? In either of the hypothesized cases, the cell density could serve as an important parameter in determining the outcome of the procedure by maintaining the chondrogenic phenotype and synthesizing matrix, which will be discussed later. Relative to the amount

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of research performed within the field of cartilage repair, this issue has previously received very little attention.[9] Although the evidence for ACI-related treatments for cartilage repair is continuously being debated,[10] systematic reviews of the clinical outcomes and complications of many of these treatments in human trials have been published elsewhere and will not be discussed here.[11-13]

Of the many factors that might affect the outcome of the cell-based treatments, one that has received little attention is the number of cells injected, or incorporated into a scaffold and implanted, into the defect. The number of cells can be provided in terms of cell density by volume, based on the estimated articulating surface occupied by the lesion and assumed cartilage thickness. At times, the cell density may be presented on the basis of the area of the lesion alone as judged by the surgeon. Finally, there are times when the area density may be based on the surface area within the lesion available for cell attachment (e.g., base and walls of the defect). The objectives of the present review are to address the issues surrounding the criteria for the selection of the chondrocyte seeding density for articular cartilage repair and to provide an overview of the cell seeding densities used in commercially available cell-based treatments that are currently approved for clinical use in the United States and other countries. Data for the latter were obtained from the websites or representatives of the companies that process the cells. The goal is to provide a basis for the informed decision of the number of cells to be used and future studies to address these critical unanswered questions.

While the focus of this review is on the number of cells per unit area, assessed by the surgeon, that are employed in cell-based cartilage repair procedures, it is clear that other factors (outside the scope of this review), which can affect the "quality" (including phenotype, biosynthetic activity, survivability) and homogeneity of the cells being used, are also important: the culture medium employed for the cell expansion *in vitro*, including the use of autologous or bovine serum and growth factor supplementation; the oxygen concentration; time in culture; and type of tissue culture dishware, including bioreactors. The "optimum" cell number for a specific defect is clearly interrelated with the quality and homogeneity of the cells. Just as there has been relatively little work relating the cell number to the clinical outcome, so too have there been few studies relating the quality and homogeneity of the cells to effectiveness in facilitating cartilage repair.

Clinical Treatments

In the original work of the ACI procedure with periosteal cover, Brittberg *et al.* used an injection of 2.6 to 5.0 $\times$ 10^6 cells under the periosteal flap in defects with a mean size of 3.1 cm^2.[5] In concordance with this study, subsequent investigations using this procedure most often describe an indirect seeding density by the number of cells in the syringe, when mentioned at all. An average number of cells in the defect can then be approximated by the average defect size. The calculated seeding density used is generally close to 1 $\times$ 10^6 cells/cm^2. In 2002, Peterson *et al.* reported good or excellent results in 50 of 61 patients 24 months after surgery and good or excellent results in 51 of 61 patients after 5 to 11 years.[14] Peterson *et al.* also reported the outcome of 58 patients with 2 to 11 years' follow-up in 2003.[15] At 24 months' follow-up, 91% of the patients had a good (22/58) or excellent (31/58) outcome. In these 2 Swedish studies, 4.5 $\times$ 10^6 cells in 3.4-cm^2 average-sized defects and 5.2 $\times$ 10^6 cells in 5.7-cm^2 average-sized defects were used, respectively.

Since Peterson *et al.*'s original work, additional related products have become available that use chondrocytes. An overview of these technologies follows below; the cell seeding densities are provided in **Table 1**. While this refers only to the densities at the time of shipment from the manufacturer, the subsequent handling before implantation might significantly affect the viability and quality of the cells.

Cell Suspensions with Cover

Carticel (Genzyme, Cambridge, MA) is a first-generation periosteum-covered autologous chondrocyte product based on work by Brittberg and Peterson.[5] It has demonstrated its efficacy in multiple studies.[16-18] To simplify the surgical procedure and avoid hypertrophy-related complications, the procedure is now performed mostly with a collagen patch cover. This approach showed a decrease in hypertrophy-related reoperations in 101 patients compared to a cohort of 300 patients who had undergone periosteum-covered ACI.[19] The finished Carticel (Genzyme) vial contains approximately 12 million cells, and 1 vial is provided for defects $\leq$7 cm^2, 2 vials for defects 7 to 14 cm^2, and 3 vials for defects >14 cm^2. In a study using a collagen membrane as a carrier for Carticel-cultured (Genzyme) cells, cells were seeded onto the scaffold in the operation room with recommended cell densities as described above.[20]

The CartiGro (Stryker, Montreux, Switzerland) procedure utilizes cells cultured by CellGenix (Freiburg, Germany) in conjunction with a Chondro-Gide (Geistlich Biomaterials, Wolhusen, Switzerland) scaffold. Chondro-Gide (Geistlich Biomaterials) consists of a porcine-derived collagen type I/III matrix that is used as a common scaffold for cultured chondrocytes in Europe. A vial of up to 12 $\times$ 10^6 cells is provided to the surgeon to seed a Chondro-Gide (Geistlich Biomaterials) matrix of 12 cm^2. The company recommends a seeding density of 1.0 to 1.5 $\times$ 10^6 cells/cm^2, although some surgeons tend to use densities up to 3.0 $\times$ 10^6 cells/cm^2 for smaller defects. Because the final product is

Table 1. Cell Seeding Densities Used in Available Clinical Treatments

Treatment	Name	Company	Cell density
Cover	ChondroCelect	TiGenix	0.8-1.0 × 10^6/cm^2
	Carticel	Genzyme	≥2.0 × 10^6/cm^2[a]
	CartiGro	CellGenix/Geistlich	1.0-1.5 × 10^6/cm^2
Scaffold carrier	BioCart II	ProChon	0.5 × 10^6/cm^2
	BioSeed-C	Biotissue	4 × 10^6/cm^2
	Hyalograft C	Anika	N/A
	MACI	Genzyme	1 × 10^6/cm^2
	NeoCart	Histogenics	N/A
	Novocart 3D	Tetec	0.5-3.0 × 10^6/cm^2
Gel type	Cartipatch	TBF Tissue Engineering	>10 × 10^6/mL
	Chondron	Sewon CellOnTech	12 × 10^6/vial
	CaReS	Arthro Kinetics	N/A
Other	Chondrosphere	co.don	2-14 × 10^6/cm^2
	deNovo NT	Zimmer	N/A
	CAIS	DePuy Mitek	N/A

Note: N/A = not applicable.
[a]Dependent on defect size. Each vial contains 14 million cells. One vial is used for defects 0 to 7 cm^2, 2 vials for defects 7 to 14 cm^2, and 3 vials for defects >14 cm^2.

assembled in the operation room by the surgeon, the density might therefore vary according to the defect location, size, and previous experiences of the surgeon.

ChondroCelect (TiGenix, Leuven, Belgium) is an autologous chondrocyte product used in characterized chondrocyte implantation (CCI).[21-23] It differs from other cell-based therapies through the use of the so-called ChondroCelect (TiGenix) score; the cell culture is given a quality score derived from several gene markers, and implantation is not recommended below a certain threshold. A cell density of 0.8 to 1.0 × 10^6 cells/cm^2 is used under a periosteal cover. While structural regeneration showed by histology was significantly better for CCI compared to microfracture, no difference in clinical outcome was found after 18 months.[6] However, after 36-month follow-up, the authors found that the clinical outcome using CCI was significantly better than by microfracture.[24]

Scaffold Carriers

BioCart II (ProChon, Ness Ziona, Israel) is a technique that uses a fibrin-hyaluronan scaffold (CartiMate) and a fibroblast growth factor (FGF) variant to enhance the proliferation rate and the chondrogenic potential of chondrocytes. In this treatment, approximately 0.5 × 10^6 cells/cm^2 are seeded onto the scaffold 3 to 4 days prior to surgery. A preliminary study on 8 patients showed an improvement in outcome after 1-year follow-up, and another study using MRI T2-mapping and dGEMRIC has shown relaxation times close to those of native cartilage at 15- to 27-month follow-up.[25,26]

BioSeed-C (Biotissue Technologies, Freiburg, Germany) uses a biodegradable polylactate scaffold as a carrier. Chondrocytes with a density of 20 × 10^6 cells/cm^3 are seeded onto the scaffold, with the dimensions 2 × 3 × 0.2 cm corresponding to 4 × 10^6 cells/cm^2. Two- and 4-year follow-up results on 79 patients showed a significant improvement in International Knee Documentation Committee (IKDC) score that was maintained up to 4 years postoperatively.[27,28] Erggelet *et al.*[29] retrospectively compared the use of BioSeed-C (Biotissue Technologies) to periosteum-covered ACI and concluded that the treatments were equally effective for focal cartilage defects. Zeifang *et al.*[30] published a randomized controlled trial comparing BioSeed-C (Biotissue Technologies) to periosteum-covered ACI and found no differences in IKDC score, Tegner activity score, and Short Form-36 score between the groups at 1 and 2 years' follow-up. They did, however, observe better outcomes in the periosteum group in the Lysholm and Gillquist scores.

Hyalograft C (Anika Therapeutics, Bedford, MA) is a hyaluronic acid–based scaffold seeded with chondrocytes. Marcacci *et al.* were the first to report the midterm clinical outcome using Hyalograft C (Anika Therapeutics) in a multicenter case series of 141 patients with 2 to 5 years' follow-up.[31] Patients reported a significant improvement from baseline in functional outcome and subjective knee assessment. Gobbi *et al.* published 2 case series with follow-up times from 1 to 5 years.[32,33] The first study showed improvement in 29 of 32 patients after 2 years, but their second study demonstrated a decline in IKDC score within 2 to 5 years in 34 patients. Two cohort studies have been published comparing Hyalograft C (Anika Therapeutics) to microfracture, showing a significantly higher IKDC score at 5 years using Hyalograft C (Anika Therapeutics),[34] and to

treatment with a collagen-based scaffold (CaReS), showing comparable clinical outcomes after 2 years.[35] Additionally, 2 MRI follow-up studies showed complete filling in 15 of 23 patients after 2 years and in 26 of 40 patients after a minimum of 5 years, respectively.[36,37]

MACI (Genzyme Europe, Naarden, the Netherlands), or matrix-assisted chondrocyte implantation, is often used as a general term to refer to any chondrocyte-seeded scaffold treatment. MACI (Genzyme Europe) uses a bovine-derived collagen type I/III scaffold as a chondrocyte carrier and, like many of the other treatments in this review, is not available in the United States. Chondrocytes are seeded at a density of 1.0×10^6 cells/cm^2 onto the scaffold 4 days prior to implantation, and the results have shown that 8 of 11 patients reported they did "better" or "much better" after surgery and that clinical scores showed significantly improved outcome after 5 years.[38] In a randomized study of defects larger than 4 cm^2, MACI (Genzyme Europe) provided significantly better outcomes when compared with microfracture.[39]

NeoCart (Histogenics, Waltham, MA) was used in a small series of 8 patients with full-thickness cartilage defects in the distal femur.[40] Harvested chondrocytes from a biopsy were expanded and seeded into a 3-dimensional bovine type I collagen honeycomb matrix for culture in a bioreactor setting including hydrostatic pressure followed by static culturing. They found a significant decrease in visual analog scale (VAS) score for pain and that 7 of 8 patients had improved outcome from baseline on IKDC.

Novocart (Tetec, Reutlingen, Germany) is a culturing technique for ACI, while Novocart 3D (Tetec) is a collagen–chondroitin sulfate scaffold seeded with chondrocytes with a density of 0.5 to 3.0×10^6 cells/cm^2. Twenty-two patients with osteochondral defects (average, 4.8 cm^2) due to osteochondritis dissecans (OCD) underwent restoration of the bony defect with autologous cylinder bone grafts from the iliac crest with concurrent Novocart 3D (Tetec) treatment to restore the articular surface. The average follow-up was 16 months, and patients showed significant improvement compared to baseline.[41]

Gel-Type Carriers

CaReS (Arthro Kinetics, Berlin, Germany) is the combination of autologous chondrocytes seeded in a 3-dimensional collagen type 1 gel (rat tail cartilage). Because cells are seeded directly into the gel and are never kept in 2-dimensional culture, no cell dedifferentiation occurs. Maus *et al.* reported on the application of this product in 13 patients with OCD lesions of the knee, with an average lesion size of 8.1 cm^2. After an average follow-up of approximately 3 years, patients demonstrated significant improvements in pain and function. One graft failed, and the patient was treated with marrow stimulation.[42]

Cartipatch (TBF Tissue Engineering, Mions, France) is a product in which monolayer-expanded autologous chondrocytes are embedded into an agarose-alginate hydrogel at a density of at least 10×10^6 cells/cm^3. Selmi *et al.* presented the first clinical outcome in a small prospective multicenter study on 17 patients with 2-year follow-up.[43] The patients improved significantly in IKDC score from baseline after both 1 and 2 years, and cartilage histology results showed predominantly hyaline cartilage in 8 of 13 patients (69%).

For the Chondron (Sewon CellOnTech, Seoul, South Korea) treatment, a vial of 12×10^6 cells is provided for implantation after mixing with a fibrin gel. Choi *et al.* reported the outcome of a series of 98 patients divided into 2 groups.[7] Based on telephone interviews, they found a significant improvement from baseline in patient-reported outcome, with the results in the >25-month group being significantly better than the 13- to 25-month group.

Other Cell Implantation Treatments

Chondrosphere (co.don, Teltow, Germany) utilizes autologous chondrocytes grown in the patient's own serum; cells are initially expanded in monolayer and then are transferred into a suspension culture.[44] During the subsequent 2 weeks, the chondrocytes form small (~500-800 μm in diameter) spheroids of immature cartilage matrix. These spheroids are implanted at a density of approximately 3×10^6 cells/cm^2 without any additional fixation and adhere to the subchondral bone. This approach differs from the above by *in vitro* formation of condensed chondrocyte spheres and early matrix formation prior to implantation. A study of 36 patients followed for 12 months demonstrated significant improvements in IKDC, Western Ontario and McMaster Universities Arthritis Index (WOMAC), and Lysholm scores. Nine patients underwent second-look arthroscopy, which showed excellent integration and fill.[8]

As an alternative to implantation of *in vitro*–cultured chondrocytes, implantation of a morselized autologous cartilage biopsy containing both cells and matrix is being investigated as a 1-step procedure known as Cartilage Autograft Implantation System (CAIS, DePuy Mitek, Raynham, MA). Cole *et al.* compared this to microfracture and found significantly better clinical outcome at 12 and 24 months using CAIS (DePuy Mitek).[45]

A demonstration of true regeneration of articular cartilage has been shown in the fetal lamb.[46] The development of articular cartilage from the fetal through juvenile to adult state is a process that yields several structural and biological differences at each state.[47] In terms of morphology, a dramatic decrease in chondrocyte density is seen throughout this maturation.[48] A recent approach for cartilage repair, deNovo NT (Zimmer, Warsaw, IN), aims at integrating the benefits of immature cartilage into a clinical treatment modality by using allogenous particulate juvenile cartilage

with live cells. The first report on the outcome of 4 patients with 2-year follow-up showed improvement in clinical outcome measures.[49]

Selected Preclinical Studies

The methodology for isolating and growing chondrocytes for cartilage repair procedures and decisions regarding the dose of cells to be employed are based on many years of *in vitro* experimentation and animal studies, informed to some extent by the cell number density in normal articular cartilage. While the principal focus of this review is on the number of cells employed in the clinic, a brief review of select preclinical studies can be instructive in providing a context into which to place the current clinical implementation of chondrocyte cell therapy.

The seeding densities from 0.5 to 12×10^6 cells/cm^2 that have been used in clinical studies can be viewed in the context of the mean cell density found in native cartilage. One study found the chondrocyte density in adult human cartilage to be approximately 23,500 cells in a cross-sectional area of 1 mm^2 (corresponding to 2.35×10^6 cells/cm^2), depending on the location.[50] Another study measuring density as cells per volume found approximately 24,000 cells/mm^3 only in the superficial layer and between 7,000 and 10,000 cells/mm^3 in the deeper layers.[51] In addition, Stockwell found a mean density of 14,100 cells/mm^3, with variation between the different joints.[52] Based on *in vitro* experiments and the above histomorphometric findings, suggestions have been made that, as a rule of thumb, 3.2×10^6 cells/cm^2 should be used to fill defects in articular cartilage with a mean thickness of 2.3 mm.[53]

An important issue, as noted above, when comparing relevant seeding densities among studies is the measure of the number of cells per surface area or per volume. While the term *density* generally refers to a per-volume value, reports often use this term to describe the number of cells implanted per surface area of the defect. In theory, the use of surface area best applies when it is the cell attachment to a substrate (e.g., the base and walls of a defect, or the struts and walls of a scaffold) that is being addressed. This area available for cell attachment is different than the articulating area encompassed by the defect, as assessed by the surgeon. The use of volume implies that the cells will display a 3-dimensional distribution within the defect or scaffold, perhaps as they settle by gravity onto each other. Calculations estimating the effects of cell seeding density, based on surface area or volume, on cartilage formation are confounded by the changing number of cells in the defect due to the contributions of cell proliferation, migration, and apoptosis, which can take effect within days after seeding.

In the absence of clinical studies relating seeding density to outcome, it is useful to turn to the *in vitro* and preclinical literature. Although extrapolating *in vitro* findings to *in vivo* behavior is generally uncertain, cell culture experiments investigating the effects of cell density on chondrogenesis can serve as useful guides to experimental parameters to be investigated *in vivo*. The importance of cell density in chondrogenesis was initially observed in some of the earliest studies of cartilage formation *in vivo*. Studies in embryonic fowl[54] revealed that "mesenchyme condenses to form a compact mass of cells which marks the site of future cartilage." "Condensation" (i.e., the increase in cell density to a critical mass) occurred *in vivo* as a result of the migration of previously dispersed cells[55] to a central core.[56] This aggregation caused an increase in mesenchymal cell packing density[55,57] without an increase in cell proliferation[57,58] and was associated with an increase in cell-cell contacts.[59,60] Of interest is that these *in vivo* observations related to the condensation of chondroprogenitor cells in their native 3-dimensional matrix *in vivo* are reflected in *in vitro* findings of mesenchymal stem cells (MSCs) in the "pellet" assay.[61] In the pellet assay, MSCs in suspension are added to a tube and centrifuged to concentrate the cells. When grown in medium supplemented with dexamethasone, ascorbate, and transforming growth factor (TGF)–β, the cell concentrate forms a pellet, which undergoes further densification by a contractile process. The condensed pellet then undergoes chondrogenesis.

Similar observations relating cell packing density to chondrogenesis have been made in studies *in vitro* in which MSCs[62] and chondrocytes[63,64] were seeded into sponge-like collagen scaffolds. In the investigation of chondrocyte-seeded scaffolds, constructs compliant enough to allow contraction resulting in increased cell packing densities (~20,000 cells/mm^3) displayed greater amounts of cartilage than constructs that displayed less contracture and, hence, cell density (~5,000 cells/mm^3).[21] Other prior studies employing cell-seeded scaffolds *in vitro* have shown an array of benefits of employing high cell densities on chondrogenesis, such as increased: cellular proliferation[65]; expression of cartilage-specific genes[66]; glycosaminoglycan (GAG) and type II collagen content[67,68]; mechanical properties[68]; and cartilage-like morphology of the resulting tissue.[65] These *in vivo* and *in vitro* findings collectively demonstrate the importance of cell seeding density in chondrogenesis.

In vitro and preclinical data on chondrocytes show conflicting results about the effect of increasing cell density in cartilage repair. In the earliest studies of *in vitro* chondrocyte culturing by Handley and Oakes, it was found that to maintain the chondrogenic phenotype and avoid dedifferentiation, high-density cultures were necessary.[69,70] In these studies, "high-density" chondrocyte cultures in both monolayer and multilayer with an initial seeding density of 250,000 cells/cm^2 showed increased proliferation and a much lower amount of elongated fibroblast-like cells after prolonged culture compared to "low-density" cultures with

an initial seeding density of 40,000 cells/cm^2. Introducing biomaterials as cell carriers, similar benefits of high cell densities have been suggested by Francioli *et al.* when cultured on a collagen type II scaffold, and Mahmoudifar *et al.* found that increasing the cell number on a 4.75-mm-thick scaffold (volume, 0.84 cm^3) from 1.2×10^7 to 2.2×10^7 led to a significant increase in extracellular matrix (ECM) production.[71,72] Buckley *et al.* performed mechanical testing of agarose gel-embedded chondrocytes in 2 concentrations and found that with a fixed agarose concentration, there was a significant decrease in the dynamic modulus using 40×10^6 cells/cm^2 compared to 10×10^6 cells/cm^2.[73] However, other authors have found that if chondrocytes are packed too close together, matrix synthesis may be inhibited, and thus, the synthesis rate of the ECM may decrease as confluence is approached.[74] This is in line with another study that showed that a cell density of 4×10^6 cells/mL stimulated higher chondrogenic transcription factor (sox9) levels than a density of 7×10^7 cells/mL in alginate-embedded chondrocytes *in vitro.*[75] In addition, an *in vivo* study by Chiang *et al.* found no effects of increased cell density with ACI in a porcine cartilage defect model.[76] Although conflicting, most of the *in vitro* work demonstrates the benefits of high-density cultures both for human and animal studies in maintaining chondrocyte differentiation, and suggestions on the optimal seeding density based on these considerations have been made.[53] However, while *in vitro* studies are limited in dealing with all the issues that present in the clinic, these suggestions have been somewhat ignored, and clinical effects of using higher cell seeding densities remain unknown. Another complicating factor in deciding seeding density is that implanted cells in a periosteum-covered defect have been found to be unequally dispersed due to gravity, which results in a heterogeneous chondrocyte density in the defect after implantation.[77]

Advantages of a lower cell density for cartilage repair procedures include smaller biopsy size and decreased culture time, and although donor site morbidity relative to biopsy size is still controversial, there is no question that shortening of culture time will decrease the costs of *in vitro* growth of the cells. Another potentially favorable consequence of shortening the culture time is the limitation of the dedifferentiation of the chondrocytes, marked by the shift in collagen type from II to I, and the decrease in sox9 expression, which is seen in their prolonged culture in monolayer.[78,79] However, while not yet fully understood, it has been proposed that these changes with culture time are not a result of actual dedifferentiation and that redifferentiation of these chondrocytes can be obtained by different interventions including reimplantation.[80-83]

Several factors influence the cells during *in vitro* culture such as culture time, culture environment (oxygen, culture medium, growth factors, surface), seeding density, and quality of the harvested chondrocytes.[21-23,75,84,85] Thus, implementing these factors for the *in vitro* culture can influence the quality and the differentiated state of the implanted cells even in low-density cultures.[86] In addition, clinical factors such as patient selection, patient and surgeon variability, and rehabilitation also contribute significantly to the outcome.

Discussion

No clinical studies have specifically investigated the effect of cell seeding density on the amount and type of reparative tissue and clinical outcome, and the preclinical data remain inconclusive in determining to what extent cartilage repair is affected by seeding density. The optimal cell seeding density may vary with seeding efficiency (i.e., the number of cells that survive the implantation process), distribution of cells in the defect, and the use of cell carriers/scaffolds as opposed to injection of a suspension under a covering membrane. Investigation of the specific influence of cell density on the clinical outcome of scaffold-supported ACI treatment is also confounded by the contribution of both chemical and physical properties of the scaffold, including surface area and pore characteristics.[87] Hence, the optimal cell density may also be scaffold dependent.

Regulating the cell concentration in the final cell product, consisting of cultured cells in a suspension, to deliver a specific number of cells for a particular defect is important and relies on the surgeon's estimate of the volume of the defect. Thus, if the surgeon wants to implant a minimum of 12×10^6 cells in a 4-cm^2 defect with an average depth of 2 mm, the cell concentration in the suspension should be at least 15×10^6 cells/mL. This issue is of importance for both the surgeon as well as the cell culture company.

The clinical indications of ACI-related procedures are continuously being debated, and some argue that the evidence for treating these patients is too sparse.[10] While the aim should always be improvement of the patients' joint function and quality of life, only a limited number of studies integrate several objective outcome measures such as MRI, indentometry, and histology, which are useful in correlating the biological response to our interventions.[88,89]

When reviewing the clinical literature, it becomes evident that few studies report the cell seeding density that was employed. In addition, comparing the outcome of cartilage repair treatments is also hampered by the selective use of the many different clinical functional outcome measures. There is no evidence that patients treated with chondrocyte implantations in a high density have better outcomes than when treated with low-density ACI. Hence, despite the tendency in the preclinical literature toward favoring high densities, the clinical effect is unknown. In addition, the cells are not equally distributed within the defect, and thus, some parts of the treated defect might have high densities and other parts low densities. Future preclinical studies and

clinical investigations in particular are indeed needed to more substantially investigate proper cell seeding densities with direct comparisons of seeding densities with objective evaluation measurements such as histology and MRI. Future findings could potentially limit the *in vitro* culture time and biopsy size and provide a better clinical outcome.

Conclusion

In the absence of systematic evaluations of the effects of cell density and clinical outcome, many clinicians continue to use 1 to 2×10^6 chondrocytes/cm^2, which, despite its lack of evidence and the fact that most *in vitro* studies point toward benefits of higher densities, has been associated with favorable clinical outcomes and also nearly approximates the densities found in native adult articular cartilage.

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Declaration of Conflicting Interests

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