Cell Seeding Densities in Autologous Chondrocyte Implantation Techniques for Cartilage Repair

Casper Bindzus Foldager¹,²,³, Andreas H. Gomoll¹, Martin Lind⁴, and Myron Spector¹,²

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. Although the evidence for ACI-related treatments for cartilage repair is continuously being debated, 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 \( \text{cm}^2 \).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-\( \text{cm}^2 \) average-sized defects and 5.2 \( \times 10^6 \) cells in 5.7-\( \text{cm}^2 \) 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 \( \leq 7 \text{cm}^2 \), 2 vials for defects 7 to 14 \text{cm}^2, and 3 vials for defects \( >14 \text{cm}^2 \). 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 \text{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

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**Table 1**

<table>
<thead>
<tr>
<th>Company</th>
<th>Cell Line</th>
<th>Scaffold</th>
<th>Seeding Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carticel</td>
<td>Autologous</td>
<td>Collagen</td>
<td>( \leq 7 \text{cm}^2 ): 1 vial, 7 to 14 \text{cm}^2: 2 vials, &gt;14 \text{cm}^2: 3 vials</td>
</tr>
<tr>
<td>CartiGro</td>
<td>Autologous</td>
<td>Chondro-Gide</td>
<td>1.0 to 1.5 ( \times 10^6 ) cells/cm(^2)</td>
</tr>
</tbody>
</table>
Cartilage 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/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.19,20

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

### Table 1. Cell Seeding Densities Used in Available Clinical Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Name</th>
<th>Company</th>
<th>Cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>ChondroCelect</td>
<td>TiGenix</td>
<td>0.8-1.0 × 10^6/cm^2</td>
</tr>
<tr>
<td></td>
<td>CartiCel</td>
<td>Genzyme</td>
<td>≥2.0 × 10^6/cm^2^</td>
</tr>
<tr>
<td></td>
<td>CartiGro</td>
<td>CellGenix/Geistlich</td>
<td>1.0-1.5 × 10^6/cm^2^</td>
</tr>
<tr>
<td>Scaffold carrier</td>
<td>BioCart II</td>
<td>ProChon</td>
<td>0.5 × 10^6/cm^2</td>
</tr>
<tr>
<td></td>
<td>BioSeed-C</td>
<td>Biotissue</td>
<td>4 × 10^6/cm^2</td>
</tr>
<tr>
<td></td>
<td>Hyalograft C</td>
<td>Anika</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>MACI</td>
<td>Genzyme</td>
<td>1 × 10^6/cm^2</td>
</tr>
<tr>
<td></td>
<td>NeoCart</td>
<td>Histogenics</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Novocart 3D</td>
<td>Tec</td>
<td>0.5-3.0 × 10^6/cm^2</td>
</tr>
<tr>
<td>Gel type</td>
<td>Cartipatch</td>
<td>TBF Tissue Engineering</td>
<td>&gt;10 × 10^6/mL</td>
</tr>
<tr>
<td></td>
<td>Chondron</td>
<td>Sewon CellOnTech</td>
<td>12 × 10^6/vial</td>
</tr>
<tr>
<td></td>
<td>CaReS</td>
<td>Arthro Kinetics</td>
<td>N/A</td>
</tr>
<tr>
<td>Other</td>
<td>Chondrosphere</td>
<td>co don</td>
<td>2-14 × 10^6/cm^2</td>
</tr>
<tr>
<td></td>
<td>deNovo NT</td>
<td>Zimmer</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>CAiS</td>
<td>DePuy Mitek</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Note: N/A = not applicable.

*Dependent on defect size. Each vial contains 14 million cells. One vial is used for defects 0 to 7 cm²; 2 vials for defects 7 to 14 cm²; and 3 vials for defects >14 cm².
treatment with a collagen-based scaffold (CaReS), showing comparable clinical outcomes after 2 years. 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.

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/cm² 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. In a randomized study of defects larger than 4 cm², MACI (Genzyme Europe) provided significantly better outcomes when compared with microfracture.

NeoCart (Histogenics, Waltham, MA) was used in a small series of 8 patients with full-thickness cartilage defects in the distal femur. 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/cm². Twenty-two patients with osteochondral defects (average, 4.8 cm²) 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.

**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 cm². 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.

Cartipatch (TBF Tissue Engineering, Mions, France) is a product in which monolayer-expanded autologous chondrocytes are embedded into an agarose-alginic acid hydrogel at a density of at least 10 × 10^6 cells/cm³. Selmi et al. presented the first clinical outcome in a small prospective multicenter study on 17 patients with 2-year follow-up. 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. 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. 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/cm² 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.

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).

A demonstration of true regeneration of articular cartilage has been shown in the fetal lamb. 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. In terms of morphology, a dramatic decrease in chondrocyte density is seen throughout this maturation. 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 \( \times 10^6 \) 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 \( 2.35 \times 10^6 \) 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, \( 3.2 \times 10^6 \) 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 fowl54 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 cells55 to a central core.56 This aggregation caused an increase in mesenchymal cell packing density55,57 without an increase in cell proliferation57,58 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 chondrogenitor 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)-ß, 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 MSCs62 and chondrocytes63,64 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 (\( \sim 20,000 \) cells/mm\(^3\)) displayed greater amounts of cartilage than constructs that displayed less contracture and, hence, cell density (\( \sim 5,000 \) 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 proliferation65; expression of cartilage-specific genes66; glycosaminoglycan (GAG) and type II collagen content57,66; mechanical properties68; and cartilage-like morphology of the resulting tissue.69 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/cm². Introducing
biomaterials as cell carriers, similar benefits of high cell
densities have been suggested by Francioli et al. when cul-
tured 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 cm³) from 1.2 × 10⁷ to 2.2 × 10⁷ led
to a significant increase in extracellular matrix (ECM) pro-
duction.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 × 10⁶ cells/cm² compared to 10 × 10⁶ cells/cm².73
However, other authors have found that if chondrocytes are
packed too close together, matrix synthesis may be inhib-
ited, 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 × 10⁶ cells/mL
stimulated higher chondrogenic transcription factor (sox9)
levels than a density of 7 × 10⁶ cells/mL in alginate-embedded
chondrocytes in vitro.75 In addition, an in vitro study by
Chiang et al. found no effects of increased cell density with
ACI in a porcine cartilage defect model.76 Although con-
flicting, 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 consider-
ations 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 peristem-covered
defect have been found to be unequally dispersed due to
gravity, which results in a heterogeneous chondrocyte den-
sity in the defect after implantation.77

Advantages of a lower cell density for cartilage repair
procedures include smaller biopsy size and decreased cul-
ture 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 conse-
quence 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 expres-
sion, which is seen in their prolonged culture in mono-
layer.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 inter-
ventions 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 sur-
geon variability, and rehabilitation also contribute signifi-
cantly 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
therapy 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 prod-
uct, 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 × 10⁶ cells in a 4-cm² defect with an average depth of
2 mm, the cell concentration in the suspension should be at
least 15 × 10⁶ 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 evi-
dence 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 evi-
dent 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 ten-
dency in the preclinical literature toward favoring high den-
sities, 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 × 10⁶ chondrocytes/cm², 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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References


