THE CHONDROCYTE; STILL VALUABLE FOR CARTILAGE REPAIR

HISTORY AND NEW ACHIEVEMENTS

– Written by Mats Brittberg, Sweden

INTRODUCTION

Musculoskeletal injuries with traumatized tissues are resulting in a bleeding, blood clot formation and an ingrowth of repair cells into the blood clot scaffold[1]. Cartilage being devoid of blood vessels and nerves will not have a chance of such repair with just a few cells migrating into the defect and no messenger to instruct a start of a reparative process[1]. Numerous attempts have been performed to increase the reparative ability of cartilage with most of them involving bone marrow stimulation (BMS) and by that induction of a bleeding with subsequent blood clot scaffolding to attract cells from the bone marrow to repair the defects[2,3,4].

Small defects have been treated by BMS alone while larger defects have been augmented by artificial scaffolds to improve the filling[2,3,4,5,6]. For long, it has been thought that the bone marrow cells are repair cells while recent studies tell us that those cells are more of medicinal signaling cells stimulating the cartilaginous surrounding and synovia[7]. Recently, Arnold Caplan suggested a change of the name of MSCs from mesenchymal stem cells to Medicinal Signaling Cells to better reflect the fact that these cells home in on sites of injury or disease and secrete bioactive factors[7].

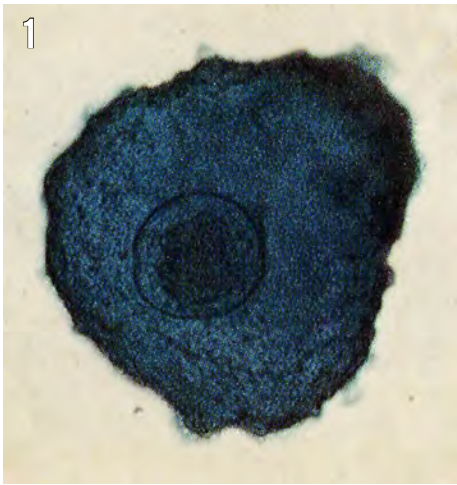
The chondrocyte (Figure 1), the one and only cell in cartilage is being responsible for all matrix production and would be the most natural cell to use when to repair cartilage defects[8,9]. When chondrocytes are separated from their matrix, the cells could divide, proliferate and become more in numbers[10]. Enzymatic digestions of cartilage and in vitro cell expansions are used when to culture chondrocytes to use them as cell source for cartilage repair[10,11].

The clinical use of chondrocytes

The first clinical use of chondrocytes for clinical cartilage repair was performed in Gothenburg, Sweden in 1987 (Brittberg et al 1994)[12].There exist today long-term results up to 20 years with good results based on the 1st generation ACI with chondrocytes in suspension implanted under a periosteal membrane[13-16].

Today in 2021 we have now 4 generations of ACI:

- 1st generation ACI: Chondrocytes in suspension injected under a living periosteal membrane[12] (Figure 2).
- 2nd generation ACI with cells in suspension injected under a collagen membrane[17].
- 3rd generation of ACI with cells either grown on a surface carrier[18] or cells grown in a porous matrix/scaffold[19]. To this generation also scaffold-free ACI is categorized[20].
- 4th generation ACI is when chondrocytes are in different ways implanted as one-stage procedures. Examples are when chondrocytes are directly isolated and mixed with directly isolated autologous MSCs[21] or allogeneic MSCS seeded in a matrix[22]. Fourth generation ACI are also variants of particulated or minced autologous or allogeneic cartilage on scaffolds



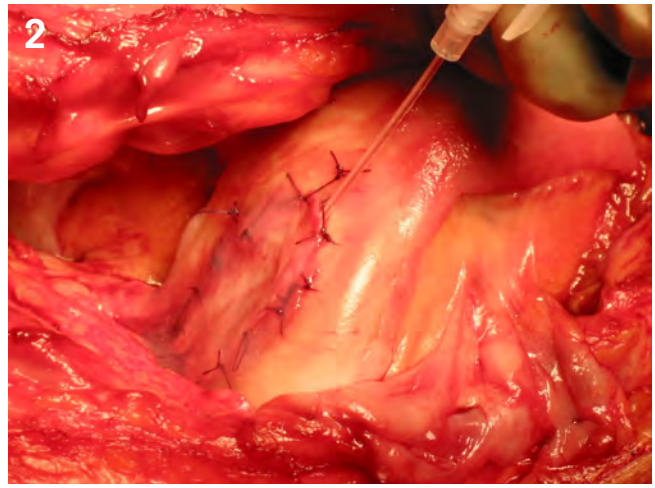


Figure 1: A chondrocyte in cell culture. Alcian-Blue.

Figure 2: Chondrocyte implantation under a periosteal flap-1st generation ACI.

(CAIS[23], CAFRIMA[24], AutoCart®[25], and DeNovo®[26]).(Figure 3 a and b).

Cell therapies including cultured chondrocytes are examples of cell manipulations and such modulations are requiring special regulatory frameworks developed by FDA[27] in USA and EMEA[28] in Europe. The way to be approved for cell therapies is long and very expensive and many companies involved in cartilage repair have tried to find cell therapies not involving cell manipulations and by that much easier to use for the surgeons with less costs. Subsequently, today very few in vitro expanded chondrocytes techniques are available for the patients. Commercially available ACI Gen III techniques today in 2021 are:

- MACI®[29]-Vericel USA.
- Bioseed-C®[30] – BioTissue Germany.
- CaRes- Arthro®[31] Kinetics Biotechnology GmbH (Austria).
- Chondrosphere®[32] (spherox)-CoDon Gemany.

In clinical trials:

- Hyalograft-C-HS[33].
- NeoCart®[34]-Histogenics (USA).
- NovoCart®[35]-Aesculap biologics.

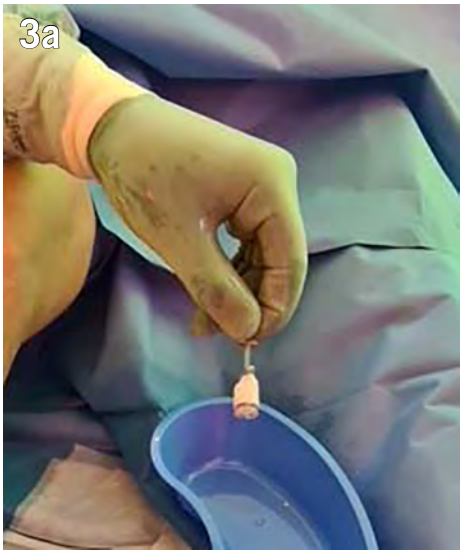
Other chondrogeneic cells

Instead of using manipulated cells, the companies have focused on the use of different chondrogeneic cells for repair, cells that can be used as one-stage procedures. Both chondrocytes but also cells not being pure chondrocytes could be used for cartilage repair.

Non chondrocyte Chondrogeneic cells are:

- Bone Marrow-Derived Stem Cells[36,37].
- Adipose-Derived Stem Cells[38].
- Synovial Membrane-Derived Stem Cells[39].
- Muscle-Derived Stem Cells[40].
- Peripheral Blood Stem Cells[41].
- Menstrual blood progenitor cells[42].

Those adult stem cells have limited self-renewal capacities. Furthermore, as a person ages, these cells exhibit decreased proliferation rates and lessened chondrogeneic differentiation potential.



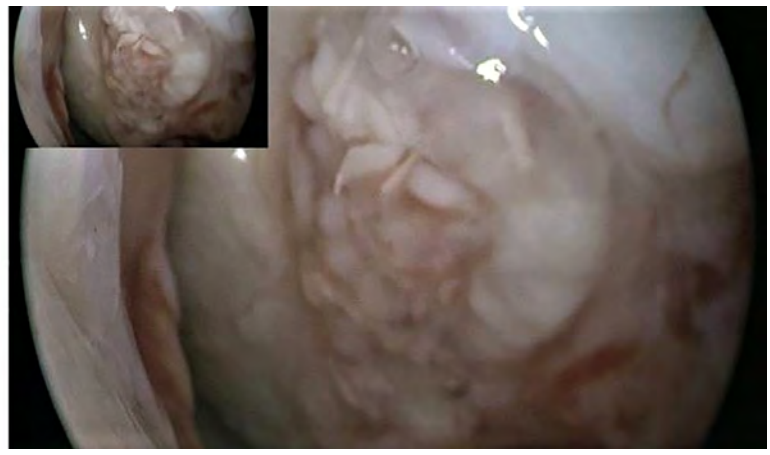


Figure 3: (a) Cartilage fragments have been harvested trans-arthroscopic with a shaver and a special cartilage fragment collector (Graftnet collector-Arthrex).The fragments are shown in the collector and will be implanted into the joint. (b) A cartilage defect, first as empty defect and then after filling with cartilage fragments in fibrin glue.

Furthermore, also extra embryonic sources of cells to be used exists such as[43]:

- Wharton's Jelly Stem Cells.
- Umbilical Cord Blood Stem Cells (BMP-2, BMP-6).
- Amniotic Fluid Stem Cells.
- Placenta-Derived Mesenchymal Stem Cells.

A study compared human MSCs derived from bone marrow, Periosteum, Synovium, skeletal muscle and adipose tissue[44]. The study revealed that synovium-derived MSCs exhibited the highest capacity for chondrogenesis, followed by bone marrow-derived and periosteum-derived MSCs.

Furthermore, it has been shown that culture-expanded chondrocytes have the potential[45] :

- to form cartilage in in vitro pellet mass cultures,
- to form adipose cells in dense monolayer culture,
- to form a calcium-rich matrix in an osteogenic assay.

Important finding was, however that in contrast with MSCs, chondrocytes formed cartilage only and not bone with in the study used in vivo osteochondrogenic assay[45].

In another study, Karlsson et al[46] compared articular chondrocytes and iliac crest derived MSCs and allowed them to differentiate in so called pellet mass cultures. Significantly decreased expression of collagen type I was accompanied by increased expression of collagen types IIA and IIB during differentiation of chondrocytes, indicating differentiation towards a hyaline phenotype[46]. Chondrogenesis in MSCs on the other hand resulted in up-regulation of collagen types I, IIA, IIB, and X, demonstrating differentiation towards cartilage of a mixed phenotype[46]. These findings suggest that chondrocytes and MSCs differentiated and formed different subtypes of cartilage, the hyaline and a mixed cartilage phenotype, respectively[46] and the bone marrow stem cells are prone to produce bone instead of cartilage. Such a finding is important to know about as when surgeons are doing bone marrow stimulation like micro-fracturing (MFX) with a risk of too much bone ingrowth. Some factors that promote chondrogenesis while inhibiting hypertrophic changes from MSCs might be necessary for the cartilage engineering from non-chondrocyte MSCs.

THE FUTURE ACI

Combinations of chondrocytes and MSCs

New findings demonstrate that co-culturing human MSCs with human articular chondrocytes in HA-hydrogels enhances the mechanical properties and cartilage specific ECM content of tissue-engineered cartilage[47]. However, co-culture decrease the expression of collagen type X by MSCs, which is an important marker of MSC.

Initially, it was thought that when mixing chondrocytes with MSCs, the MSCs were recruited by the chondrocytes to go into a chondrogeneic lineage. Recent studies instead show that MSCs are functioning as medicinal signaling cells to stimulate the chondrocytes for a stronger repair response[7].

Subsequently when to repair a cartilage defects at least for larger defects, chondrocytes are needed in some form. With the complicated regulations regarding chondrocyte cultures, the possibility of using direct isolation of chondrocytes mixed with MSCs as one-stage procedures has open new doors for cartilage repairs[21,22].

One-stage ACI techniques are called ACI 4th generation. In the INSTRUCT study, the surgeons harvested bone marrow cells from iliac crest and mixed them with chondrocytes directly isolated in the OR[21]. The cell mixture was then injected in a scaffold for a direct cartilage lesion implantation. In a 24 months study in 40 patients, good lesion fill and sustained clinically important and statistically significant improvement were found in all patient-reported outcome scores throughout the 24-month study. Hyaline-like cartilage was observed on biopsy specimen in at least 22 of the 40 patients[21].

Another such one-stage procedure is the IMPACT study[22] where instead of direct isolation of chondrocytes, chondrocytes with surrounding pericellular matrix is isolated as chondrons. The chondrons are then mixed with allogeneic MSCs and injected in fibrin glue into the defect. Using allogenic MSCs, no signs of a foreign body response or serious adverse reactions were recorded after 5 years. The majority of patients showed statistically significant and clinically relevant improvement in the KOOS and all its subscales from baseline to 60 months[22].

Minced cartilage derived ACI

However, even if those above described techniques are one-stage procedures, they involve cell isolations through minor manipulations and cells with osteogenic potential that may influence the degree of chondrogenesis. A simpler, one-stage procedure is then to use particulated or fragmented cartilage for repair. The initial technique called CAIS was studied in two RCTS[23,48] showing in both studies significant improvement of the patients treated by cartilage fragments in resorbable scaffold versus microfracture. Unfortunately, the company developing CAIS decided not to launch the technique for further use commercially. Instead, other companies have used the technology to develop modified versions of CAIS with fragments in different scaffolds.

Williams and co-workers[49] have identified a population of chondroprogenitor cells

Image: Illustration.

from the surface zone of bovine articular cartilage using differential adhesion to fibronectin[49]. This population of cells can form large numbers of colonies from a low seeding density and is capable of extended culture without losing the chondrogenic phenotype and they are subsequently cartilage progenitor cells[49].

Therefore, these populations are expected to be extra interesting potential cell sources for cartilage repair as being cartilage pluripotent "stem cells". Migratory ability enables cartilage-derived pluripotent cells to migrate to the injured site and repair cartilage damage. Even stem cells from human OA cartilage also have the potential for cartilage repair. Koelling et al[50] observed that also cartilage progenitor cells from late stage OA knee joints regained a round chondrocyte-like phenotype and exhibited collagen type II mRNA expression as well as collagen type II protein expression in a 3D-alginate culture without any chondrogenic supplementation[50].

Based on such findings, the use of fragmented cartilage is of increasing interest as it has been shown in laboratory experiments that new cartilage tissue is formed in direct connection to the fragments. Endogenous cartilage progenitor cells migrate from the fragments into the surrounding scaffolding material to start new matrix production. With special harvest instruments mini fragments are produced which could be put onto different scaffolds and be implanted fixated with a biological glue[25].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Marmotti et al[51] have shown that there is an age-dependent and time-dependent chondrocyte migration. A significant difference ($P < 0.05$) was observed between young and older donors[51]. Furthermore, it has also been shown that at one month high cellularity and intense extracellular matrix (ECM) production could be seen and that a two months, ECM was positive for collagen type II[52]. Furthermore, the matrix production is influenced by the degree of fragmentation and Bonasia et al[53] found that a chondral paste of fragments with size < 0.3 mm performed best in histology comparisons[53].

Juvenile chondrocytes have shown in vitro superior capabilities of producing cartilage extracellular matrix [54]. With the knowledge of chondral fragments for cartilage repair, now also allogeneic juvenile cartilage fragments have been introduced for chondral repair[26,55].

3D-printing of chondrocytes in Bio-ink with Biopens

The concept of 3D-printing involves a construct production having a control over spatial resolution, shape, and mechanical properties[56]. When to repair a cartilage defect, a gradient repair is important where cells in different layers may be able to via cross-talking develop a good quality repair. Many of the 3D printing concepts involve several cell types and different materials for the bone and cartilage layer. Most often an osteochondral repair approach is best with a 3D printing addressing the bone defect with printing of bone cells into layers of bone substitute materials like hydroxyapatite and followed by different chondrocytes printed layered between a more cartilage specific matrix materials like hyaluronic acid[57,58].

The status of the Chondrocyte for cartilage repair in randomized controlled trials

Randomized controlled clinical trials (RCTs) are considered to be the gold standard for evidence-based medicine. Subsequently, RCTs are important in also cartilage repair methods, steering the surgeons to use well-controlled and validated methods. In 2019, Matar and Platt[59] published a paper on RCTs in orthopedic reseach[59]. The authors included 1078 RCTs across seven most commonly performed elective procedures. Unfortunately, cartilage repair procedures were not included in their review. Of the

seven procedures studied, only 16% of the RCTs reported significant findings.

However, from 2003 to 2021, 21 RCTs have been performed[23,48,60-79]. Sixteen of those RCTs involved different generations of ACI versus other cartilage repair techniques[23,48,63-78]. In 9 of those 16 studies, ACI showed significant superiority in different parameters studied versus the other cartilage repair method[23,48,65,70-74,76]. Ten of those studies involved different generations of ACI versus bone marrow stimulation without scaffold (MFX (9) and abrasion arthroplasty (1)[23,48,68,69,70-74,76]. ACI was significantly better in different parameters than BMS without scaffold in 8/10 studies[23, 48, 70-74, 76] (Figure 4).

How and when to use chondrocytes for a cartilage repair?

There are numerous algorithms to use for cartilage repair. Most often the surgeons trend to overestimate the size of the lesions to repair.

The mean size width of the both condyles in a man is a little less than 9 cm[90]. A defect with a size of 1 cm located centrally on a condyle is subsequently quite a large defect to repair.

The authors' suggestions of methods to use for a cartilage defect are:

- BMS (like MFX or drilling) for small defects 0.5 cm²
- Augmented BMS (with a scaffold) for small-medium sized defect 0.6-2 cm²
- Alternative also for re-operations in such defects if a simple BMS has been done before
- ACI-one stage with autologous or allogeneic chondral fragments >1cm²
- ACI-two stage with cultured chondrocytes > 2 cm²
- ACI-one stage with mixed chondrocytes and MSCs > 2 cm²
- Above Cell based treatments for re-operations > 1 cm²
- Osteochondral Allografts for extra-large defects like condylar replacements

It is also important not to forget that unloading osteotomies are useful in combination with local repairs.

Furthermore, as mentioned earlier in the text, the activities of chondrocytes are depending on the patients' age. A local cartilage repair can be done for local trauma defects, local degenerative lesions but may also be used for a local well-defined lesion in an early OA joint. However, local repairs are not used in a full established osteoarthritic joint.

Third Party Testing

ACI Gen I versus Gen II and III
= *Schneider et al 2003*
= *Bartlett et al*
= *Gooding et al*
= *Zeifang et al 2010*

ACI Gen I versus Mosaicplasty
= *Horas et al 2003*
\+ *Bentley et al 2003*
= *Dozin et al 2005*

ACI Gen III versus Mosaicplasty
\- *Clavé et al 2016*

ACI Gen I versus MFX
= *Knutsen et al 2004*
= *Lim et al 2012*
\+ *Vanlauwe et al 2011*

ACI Gen III versus MFX / Abrasion Arthroplasty
\+ *Visina et al 2004*
\+ *Basad et al 2010*
\+ *Crawford et al 2012*
\+ *Saris et al 2014*
\+ *Niemeyer et al 2019*

ACI Gen II versus AMIC (Scaffold + bone marrow stimulation)
= *Fossum et al 2019*

ACI Gen III versus AMIC (Scaffold + bone marrow stimulation)
\- *Akgun et al 2015*

ACI Gen III with scaffold free ACI in comparison to three different cell concentrations
= *Becher et al 2017*

ACI Gen IV versus MFX
\+ *Cole et al 2011*
\+ *Spalding et al 2011*

Figure 4: A summary of the RCTs done with ACI versus different other methods from 2003-2021.

Chang and co-workers[81] have detected multipotent mesenchymal progenitor cells in human articular cartilage of all ages. Of interest to know is that chondral progenitor cells accounted for 94.69%±2.31%, 4.85%±2.62%, and 6.33%±3.05% of cells in articular cartilage obtained from fetuses, adults, and elderly patients, respectively (P<.001)[81]. Furthermore, fetal mesenchymal progenitor cells had the highest rates of proliferation measured by cell doubling times and chondrogenic differentiation as compared to those from adult and elderly patients[81]. With that in mind, the repair quality is expected to become better, the younger patient that is treated but ACI may be used in elderly patients still having in total a healthy cartilage as there also exist cartilage progenitor cells but with less chondrogeneic differentiation ability.

> ***The chondrocytes are the masters of the cartilaginous tissue and they are subsequently still most valuable to use when to repair a traumatized cartilage.***

CONCLUSION

The chondrocytes are the masters of the cartilaginous tissue and they are subsequently still most valuable to use when to repair a traumatized cartilage. DNA methylation is essential for normal development and is associated with a number of key processes[82]. Besides what has been mentioned about the strong chondrogeneic ability of primary chondrocytes compared to MSCs of different origins, Bomer et al[82] have nicely shown that In vitro engineered neo-cartilage tissue from primary chondrocytes exhibits a DNA methylation landscape that is almost identical (99% similarity) to autologous cartilage, in contrast to neocartilage engineered from bone marrow-derived mesenchymal stem cells (MSCs).

I still believe that we will use chondrocytes for a biological repair in the future but with fewer manipulations of the cells due to the strict regulations world-wide making cell expansion and culture expensive. Different variants of one-stage procedure will appear more and more with both autologous or allogeneic cells and even mixtures. The dream goal is a full cartilage regeneration still not achieved in a clinical setting. However, when to reach as near as possible regeneration, true committed chondrocytes and chondral progenitor cells seem still to be the best choice in 2021.

References
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