AUTOLOGOUS CHONDROCYTE IMPLANTATION

Damaged articular cartilage has a limited capacity for self-repair. Patients with cartilage lesions usually suffer from disability and symptoms such as pain, swelling, locking and malfunction of the joint and if these lesions are left untreated it may lead to osteoarthritis (OA). Autologous chondrocyte implantation (ACI) is a therapy widely used for the treatment of isolated cartilage defects. The original technique (first generation technique) is based on an implantation of a suspension of in-vitro expanded chondrocytes into the defect, beneath a sealed cover of periostium flap. Since the technique was introduced in 1987 by Brittberg et al, more than 45,000 patients have been treated worldwide.

The second generation ACI technique that followed includes the use of a bi-layer collagen membrane instead of the periosteal flap. The use of a collagen membrane simplifies the surgical procedure and reduces complications such as periosteal hypertrophy.

Further technological advances led to the development of the third generation technique that involves both in-vitro expanded chondrocytes and a scaffold. After the culture is expanded in flasks, the cells are seeded onto a membrane or a biodegradable scaffold before implantation. The scaffold may function as a carrier for the cells or as a structure to build up the 3D environment for the cells.

- Recently, the fourth generation technique has been described. This generation involves the transplantation of cells directly after isolation or the transplantation of cartilage fragments.

Attempts have been made to predict chondrogenesis and repair capacity but currently no clinical marker exists.
Donor selection

The indication for ACI treatment is young patients with large (>2 cm²) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. The patient is examined arthroscopically and the location, depth and size of the defect and the quality of the surrounding cartilage are evaluated. ACI is not indicated for patients with severe osteoarthritis, active rheumatoid arthritis, active autoimmune connective tissue diseases or patients with concomitant malignancies².

Procurement

The ACI technique includes a two-stage procedure with an initial harvest of a cartilage biopsy that is sent for chondrocyte culture, followed by a second stage operation that includes the cell implantation. A full-thickness cartilage biopsy (mean 260 mg) is harvested from a low-weight-bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with biopsy medium, packed and sent to a high-quality Good Manufacturing Practice laboratory for further processing, together with 15 × 9 ml autologous blood collected from the patient. The serum is used in the culture process as an additive to the culture medium.

Processing and storage

1st and 2nd generation ACI

Chondrocytes are isolated from the cartilage by mechanical mincing followed by treatment with collagenase. After the digestion process, in which the chondrocytes are released from the matrix, the cells are seeded in culture flasks. Around 2102 cells/mg of cartilage are isolated. The mean number of cells isolated per patient at our laboratory is 5.3 × 10⁵ cells. After a total culture time of 14 to 21 days, the right numbers have usually been reached and the implantation of the cells can be performed. This cell-product is aseptically filled in syringes or vials. The syringes or vials are then packed for shipment to the operating theatre, where the cells are injected into the defect, either under a flap of periosteum or a collagen membrane, with a recommended treatment dose of approximately 1 to 2 million cells/cm².

3rd generation ACI

The third generation includes culture-expanded chondrocytes that are seeded into a scaffold, which functions as a three-dimensional carrier system for the cells to grow and produce cartilage-specific proteins. The culture period in the scaffold is approximately 14 days, resulting in a total culture time of around 5 weeks, including the expansion phase for the chondrocytes (Figure 1). The cell-loaded membranes are packed in a specific container and transported to the operating room, where the scaffold is implanted into the defect arthroscopically (Figure 1 a-d).
4th generation ACI

There are two techniques that can be classified as the 4th generation of ACI; direct isolation of chondrocytes, performed during an operation as a single-stage procedure\(^4\) and cartilage fragment implantation (Cartilage Autograft Implantation System)\(^5\). With the direct operating room isolation technique, the surgeon uses a cell processor in the operating theatre to isolate the patient’s own chondrocytes from cartilage biopsies and harvest bone marrow, seed the scaffold with those two cell types and prepare it for implantation during a single, minimally invasive surgical procedure. This technique is more time consuming in the operating room compared to a ready-to-go implantation from a second stage 3rd generation ACI. The Cartilage Autograft Implantation System technology is based on harvesting cartilage pieces which are reimplanted into the cartilage lesion in small fragments on a fibrin gel covered membrane. Chondrocytes migrate out from the fragmented cartilage to start the repair process\(^6\).

The cell bank

Cells can be frozen directly after isolation from the cartilage biopsies or after primary expansion. The material is stored in cryo tubes at a temperature of -192°Celsius. The cells could theoretically be stored for hundreds of years, but the authors do not store their specimens for more than 10 years.

Release criteria

There are several aspects that are controlled prior to the release of the product. The morphology of the cells can be easily controlled. The sterility of the cell culture media is controlled with the Bact/Alert system 24 hours before implantation. The viability of the cells in suspension is determined using trypan blue. The number of cell divisions is controlled due to the fact that chondrocytes cannot differentiate beyond 8 population doublings. This is important because the redifferentiation capacity diminishes with high levels of cell doubling. The number of cells per vial or per square centimetre of scaffold is determined and should be not less than \(1 \times 10^6\) cells per vial or per square centimetre of scaffold.

Endotoxins are lipopolysaccharides derived from the membrane of gram-negative bacteria and can cause illness. A common laboratory testing assay for detecting the presence of endotoxin is the Limulus Amebocyte Lysate assay. The levels must be at or below acceptable levels for product release.

Chondrocyte specific quality controls

Purity

The chondrocytes are checked to ensure there are no possible contaminants in the product, such as synoviocyte cells from the joint capsule. There can also be other types of impurities such as bone cells. A representative batch of cells are also validated for mRNA expression of synoviocyte-specific genes which should be low.

Identity

Cells for implantation are checked to ensure that they are indeed chondrocytes. Identity markers should include mRNA markers of chondrogenic lineage like sox9. Since cells are dedifferentiated during culture more specific markers of differentiation are not tested.

Potency

The potency of the cells are checked to determine their chondrogenic potential and thus their capacity for repair. Attempts have been made to predict chondrogenesis and repair capacity\(^7\), but currently no clinical potency marker exists.

Traceability

Implantations are traced to the implanted individual by the order and reply system between the hospital and the culture laboratory. Reference samples are biobanked.

Regulatory aspects

An advantage of using autologous chondrocytes is that one does not require manipulation cues to induce differentiation. Due to the advanced therapy medicinal product regulations in the European Union, the cultured chondrocytes have known safety profiles and efficacy parameters\(^7\).

In the USA, the Food and Drug Administration regulates the use of cultured chondrocytes and so far, only Carticel is approved, a 1st generation ACI in which cells in suspension are used under a periosteal membrane. In Europe, the European Medical Agency assesses which cells are authorised for use in the European Union and its conditions of use. Currently a 1st generation ACI product, Chondroselect\(^8\), and a 3rd generation ACI product, MACI\(^9\) have been...
approved for use in Europe. Several other 3rd generation products are being studied and/or used with special and most often temporary amendment authorisations.

SUMMARY

Biological approaches to cartilage repair involve the use of cells. Rationally, chondrocytes (cartilage cells) present the most effective way of providing such treatments. Today, there are different techniques to isolate cells for cell expansion. One well-studied approach is in-vitro cell expansion based on a two-stage procedure, with first step being to harvest cells and the second step to implant them. The latest version of cell implantation uses direct isolation technology, cells are then seeded onto a matrix and implanted directly as a one-step procedure. An even simpler way to activate chondrocytes, is the cartilage fragment method, where the chondrocytes migrate out from fragmented harvested cartilage when seeded on an implanted matrix, also performed as a single-step procedure. The first approach has the advantage of producing a large number of viable cells, while the two other techniques are based on a primary isolate and a much lower number of cells for chondrogenesis. With further research, the future will tell the most viable option when using chondrocytes for cartilage repair.

References