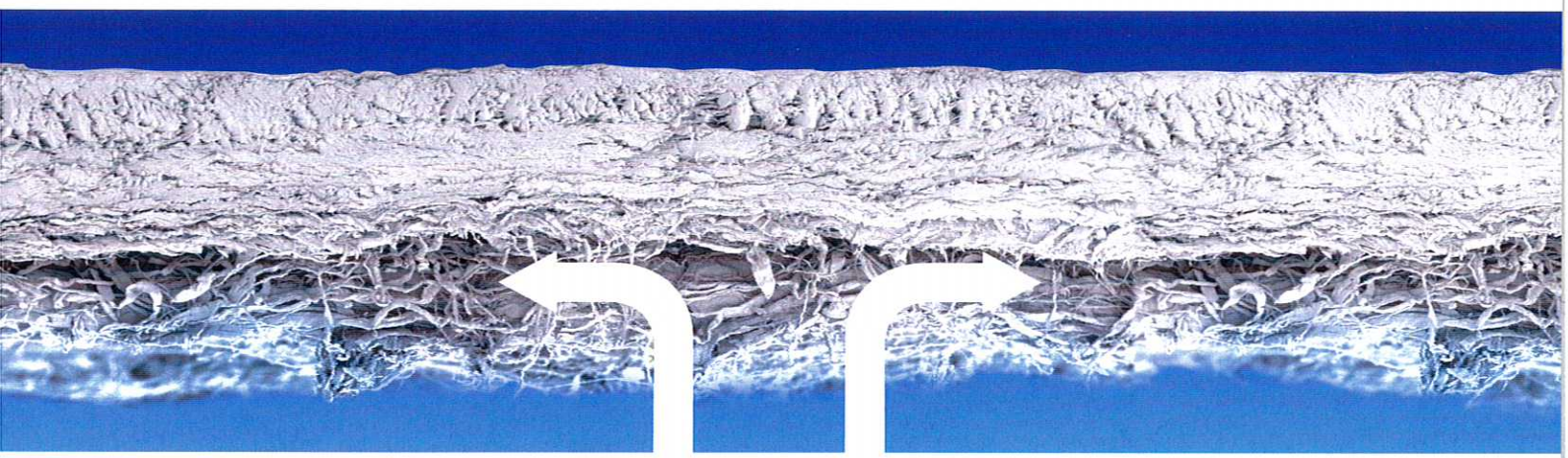
Chondro-Gide®



Collagen Membrane for Articular Cartilage Repair

Geistlich
Biomaterials

Articular Cartilage

Articular cartilage has a limited regeneration capacity, due to the absence of bloodvessels and the low mitogenic potency of the chondrocytes. Repair is due to infiltration of stem cells from the bone marrow, through perforation of the subchondral plate. The reparative tissue consists of fibrocartilage, which has not the same biomechanical properties as hyaline cartilage, usually leading to a fast breakdown of the newly formed tissue. When the subchondral plate remains intact, no repair is possible. Depending on its location and size the defect will progress, resulting in osteoarthritis. To prevent this devasting process, the aim of articular cartilage surgery is to restore the structure and function of hyaline cartilage.

The capacity for spontaneous repair of articular cartilage in the adult is limited.

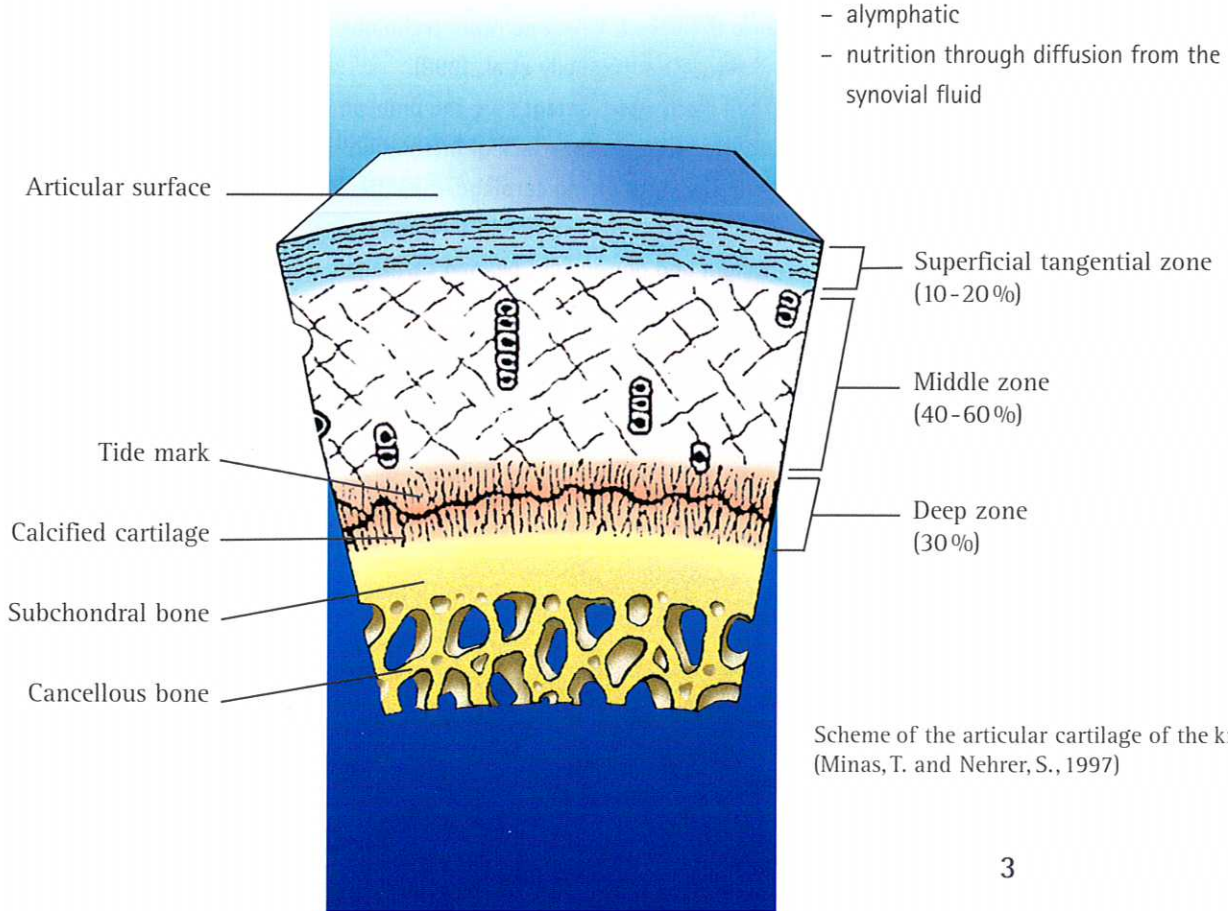
Injuries of the articular cartilage that do not penetrate the subchondral bone do not heal and progress to degeneration of the joint.
Injuries that penetrate the subchondral bone undergo repair through the formation of suboptimal fibrocartilage.

The Articular Cartilage
(Buckwalter et al. 1994)

Composition:
- chondrocytes (1 to 10%)
- extracellular matrix (proteoglycans, glycoproteins, collagen, lipids and electrolytes)
- water (65 to 80%)

The collagen architecture contributes to the tensile strength and stiffness of cartilage (Brower and Hsu 1969); its compressibility is due to the binding of proteoglycan with water molecules, which produces a rigid structure. Type II collagen predominates (90-95%), smaller amounts of types V, VI, IX, X and XI collagen are also present.

Characteristics of articular cartilage:
- avascular
- aneural
- alymphatic
- nutrition through diffusion from the synovial fluid



Scheme of the articular cartilage of the knee (Minas, T. and Nehrer, S., 1997)

Methods for Articular Cartilage Repair

The goal of articular cartilage repair is to restore the integrity of the joint surface and to provide full range of pain-free motion, restore function, reduce disability and postpone or alleviate the need for prosthetic replacement. (Jackson, Scheer et al., 2001)

Treatment procedures
(Nehrer, et al. 2000; Felson, et al., 2000).

- Abrasion arthroplasty
- Subchondral drilling
- Microfracture
- Osteochondral transplantation (OCT)
- Autologous chondrocyte transplantation (ACT)
- Periosteal or perichondrial grafting

Bone marrow stimulation repair techniques

- **Abrasion arthroplasty (Magnuson, 1941)**
- **Subchondral drilling (Pridie, 1959)**
- **Microfracture (Steadman, 1999)**

These procedures are recommended for lesions smaller then 2 cm^2. They lead to the formation of hyaline-like cartilage and fibrocartilage, which does not have the same mechanical properties as hyaline cartilage (Buckwalter 1997). The clinical outcome after 2 years is satisfying in 80%, however beyond this time the results decline.

Transplantation techniques

- **Osteochondral auto- and allografts (OCT)**

These grafts are used in osteochondral defects, less then 3 cm^2. Their advantage is that they introduce hyaline cartilage into the defect, which no other technique is able to do (Hangody et al., 1998).
Their main disadvantages are the problem of incongruency, their lack of integration with the surrounding cartilage, the difference in cartilage thickness between weight and non-weight bearing cartilage and donor side morbidity.

- **Perichondrial or periosteal grafting**

Procedures to induce chondrogenesis such as transplantation of periosteum (O'Driscoll 1999) and perichondrium (Homminga, Bulstra et al. 1990) are based on the presence of the cambium layer. Preservation of this layer during surgery may be difficult and the quality of the flap has a strong interindividual variability, which affects the postoperative outcome. Other problems include the risk of calcification, subsequent ossification of the graft, and delamination of the cartilage from the subchondral bone plate (Minas and Nehrer, 1997).

- **Autologous chondrocyte transplantation (ACT)**

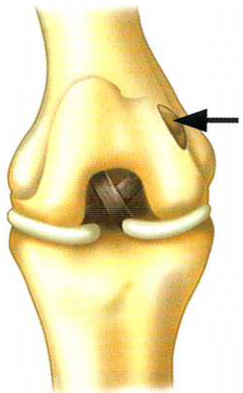
Autologous chondrocytes are cultured in vitro and subsequently reimplanted into the defect, with the aim of regenerating a larger amount of hyaline cartilage (Brittberg, 1994; Steinwachs, 1999).

ACT

Periost is used as a cover to hold the cultivated autologous chondrocytes in the articular cartilage defect. The presence of the cambium layer, with precursor cells that have been shown to differentiate into chondrocytes and to produce hyaline-like cartilage is an advantage of the periost. However, the role of these cells in the production of new cartilage when compared to the cultivated autologous cells seems to be neglectible, as hyaline cartilage formation is found near the subchondral bone (Peterson, 2000).

Treatment steps of ACT

1. Harvesting of normal articular cartilage from non-weightbearing sites of the knee during arthoscopy

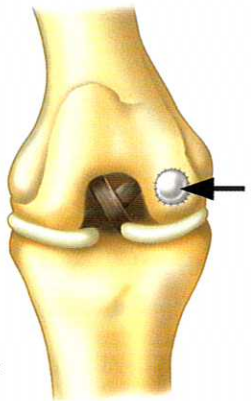


2. Extraction of chondrocytes and in vitro cultivation during 2-6 weeks



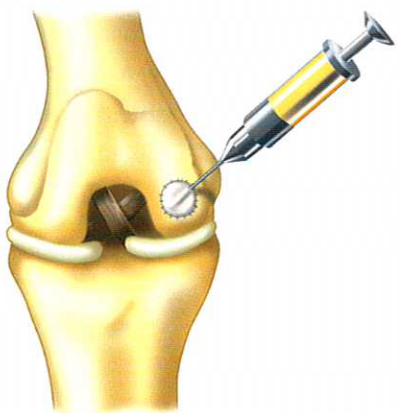
3. Arthrotomy, debridement of the cartilage defect, preparation of periost

4. Suturing of the periost over the defect and sealing which fibrin glue



5. Test for leakage with normal saline

6. Injection of 2–5 x 10^6 cultivated dedifferentiated chondrocytes under the periost



Indications of ACT

(Peterson, Steinwachs, 2001)

- physiologically young patient
- absence of OA, metabolic discease, osteomyelitis, carcinoma
- absence of misalignment
- treatment of symptomatic unipolar grade III or grade IV Outerbridge classification injuries, size > 2 cm^2

Membrane grafting in ACT is performed in order to hold the cultivated cells in place. In this bioactive chamber the dedifferentiated chondrocytes are expected to produce new hyaline cartilage.

Disadvantages of using periost/ perichondrium may include

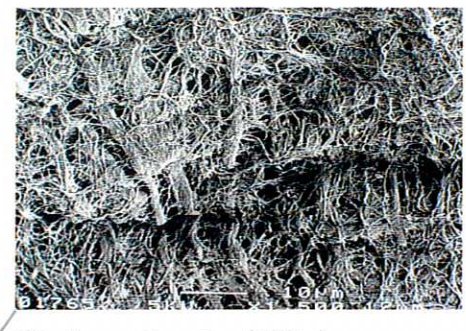
- second operation site required
- difficult isolation leading to longer operation time
- quality differences between patients and surgeons
- hyperthrophic changes (Horas, Schnettler et al. 2000)
- detachment
- delamination (Driesang, Hunziker 2000)
- pain and discomfort for the patient

Design of Bilayer Collagen Membrane

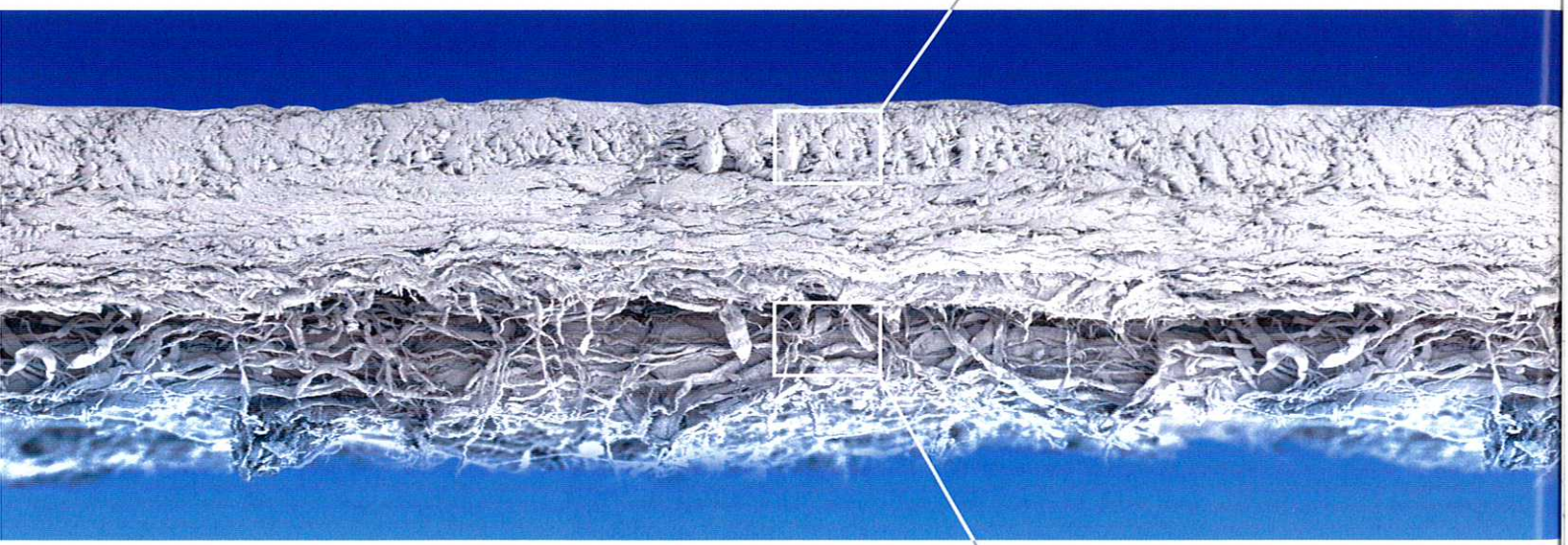
To avoid the mentioned disadvantages of periost transplantation in ACT, a resorbable collagen membrane, Chondro-Gide® has been developed.

The membrane consists of collagen types I and III. A unique, patented manufacturing procedure results in a membrane with a **bilayer structure**, with one compact and one porous side. The compact membrane layer which has a smooth surface is cell occlusive, preventing the injected cultivated cells from diffusing into the synovial fluid and also protecting them from mechanical impact. The other layer of the membrane consists of collagen fibers in a loose, porous arrangement that favours cell invasion and attachment. The collagen matrix stimulates the autologous cultured cells to differentiate into the chondrocyte phenotype and to produce collagen II and GAG (Fuss et al., 2000; Ehlers et al., 1999).

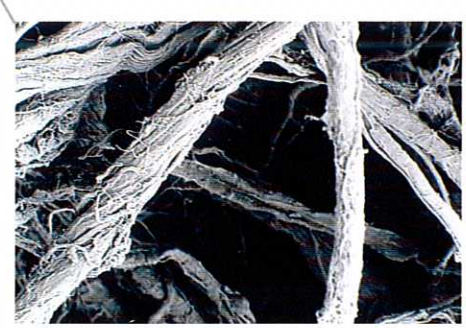
The arrangement of the fibers results in high tensile strength and resistance to tearing. It can therefore be held in position by sutures or pins, which prevent membrane mobilization under mechanical loading.



EM of smooth surface (1500 x)



EM of bilayer design



EM of porous surface (1500 x)

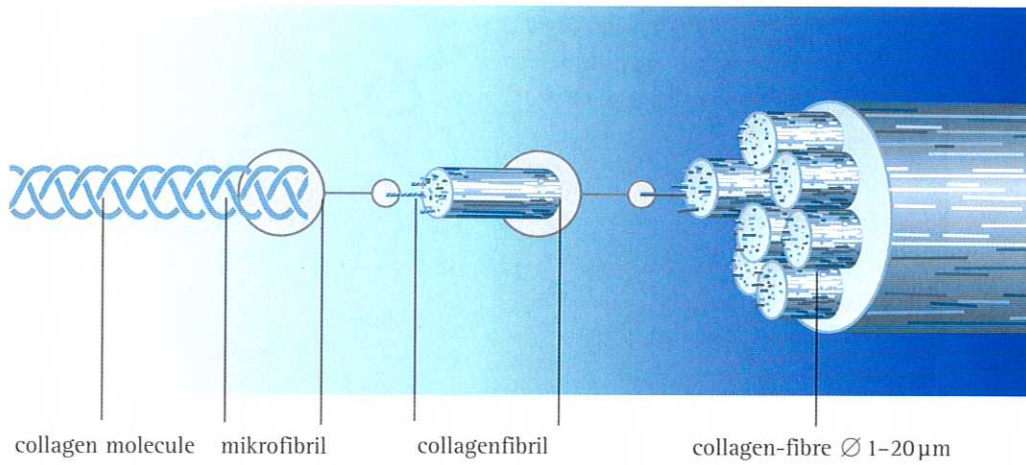
Advantages of using Chondro-Gide® compared with periost

- No need of second operation site, leading to shorter OP time
- No quality differences between patients and surgeons
- No donor side morbidity
- Easy handling even when wet, resistant to tearing
- Reduced risk of hypertrophy

Collagen

Collagen

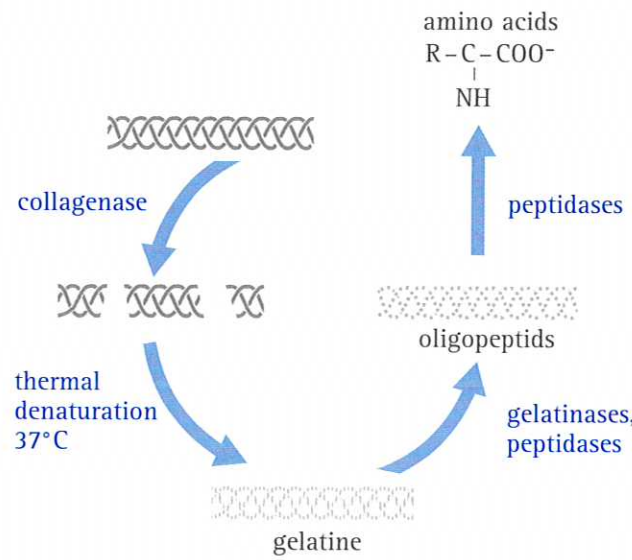
Collagen is a main structural protein in vertebrates. At present twenty different collagens have been identified. All collagens contain a unique triple helix however the length of the helix and the nature and size of nonhelical portions of the molecule vary from type to type. Collagen shows a high degree of similarity between different species. Collagen is an important component of the cartilage. (Encyclopedia of polymer science and engineering, vol. 3, second edition, 1985).



Scheme of collagen structure

Resorption

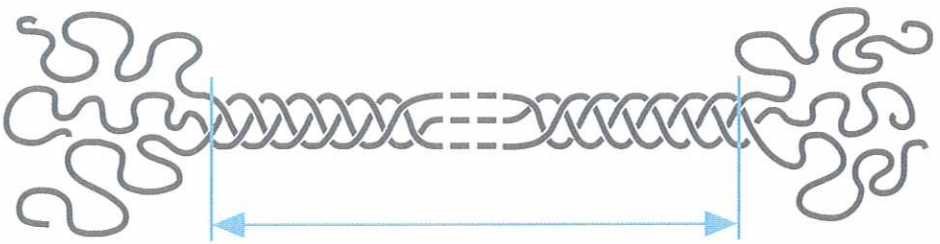
Because Chondro-Gide® is manufactured from pig-collagen, it is naturally resorbed. Resorption of Chondro-Gide® starts with the action of collagenase, which splits the collagen at specific sites. The resulting collagen fragments are temperature sensitive and are denatured to gelatine at 37° C. Gelatinases and proteinases are then responsible for the further breakdown into oligopeptides and finally single amino acids.

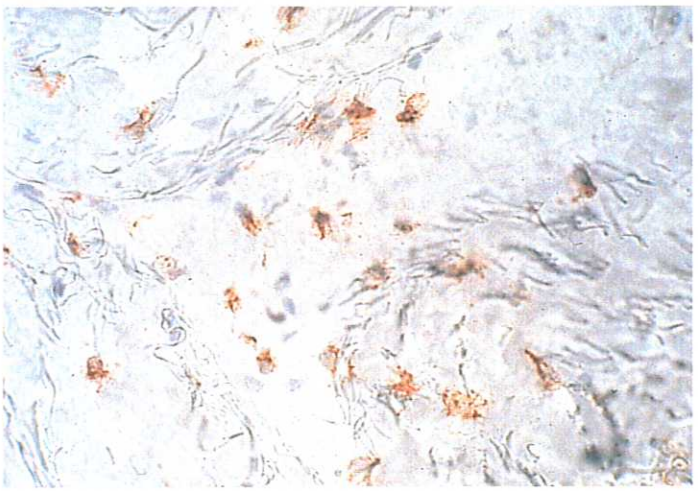


Denaturation of Chondro-Gide®

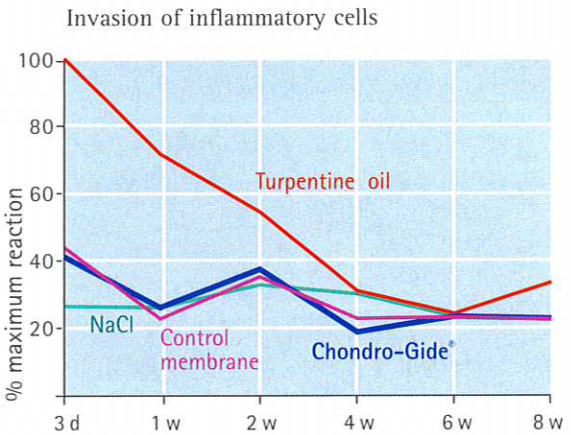
Antigenicity

A further important criterion in assessing the properties of biomaterials is the determination of antigenicity. Collagen is only weakly immunogenic (Sabelman, 1985). With the telopeptides being of greatest importance for the antigenicity of the protein, as these are cleaved during the production of Chondro-Gide®, the purified membrane has a low immunogenic potential.

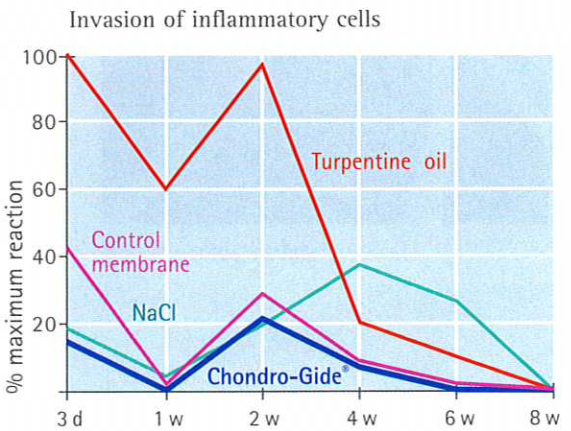




Antigenicity test: The accumulation of inflammatory cells in the implantation area was investigated and marked by monoclonal antibodies. 2 weeks after implantation of Chondro-Gide® there was no accumulation (e.g. macrophages, brown) in comparison with the control group (surgery with saline). Histology Prof. Cohen, University at Buffalo, New York



Graph showing cellular reaction in the antigenicity test: accumulation of macrophages (marked with monoclonal antibodies) in the implantation area. Turpentine oil = positive control
NaCl = negative control
control membrane = CollaCoat®, Calcitek



Graph showing cellular reaction in the antigenicity test: accumulation of monocytes

Safety

Chondro-Gide® is obtained from porcine collagen. The manufacturing process involves several steps before the bilayer design is achieved. According to EC-guidelines to reduce the risk of viral or bacterial contamination, a strong alkaline treatment for several hours is included in the processing steps. Afterwards the structural quality of each membrane is meticulously controlled. Standardized processes under clean room conditions, rigorous in-process and end controls, guarantee a high quality biological product.

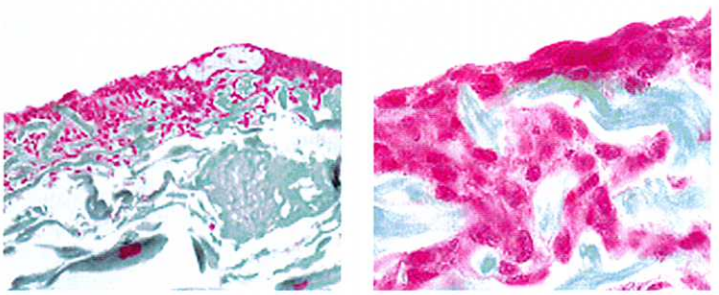
CE



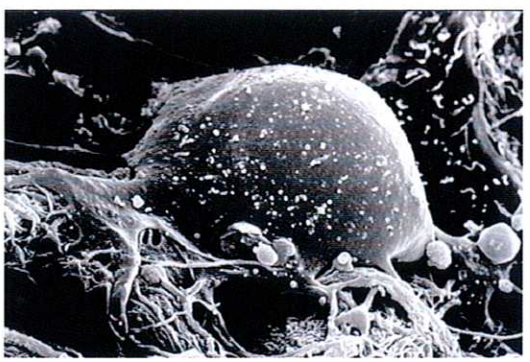
Clean room processing of Chondro-Gide®

In vitro study

Isolated chondrocytes were cultured in vitro and after 6 weeks grown on the three dimensional collagen matrix Chondro-Gide®. The cultured dedifferentiated cells transformed to the chondrocyte phenotype in the collagen matrix and produced GAG and collagen (Ehlers et al., 1999).



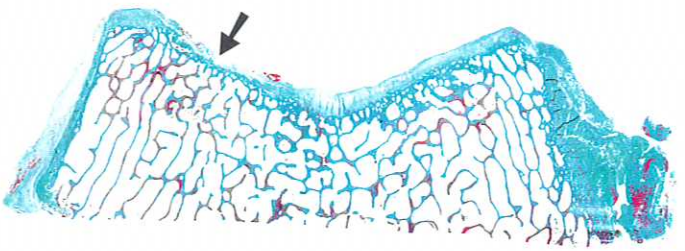
Chondrocytes grown on a collagen I/III scaffold (Chondro-Gide®), Masson-Goldner. Original magnification x 100 and x 200.



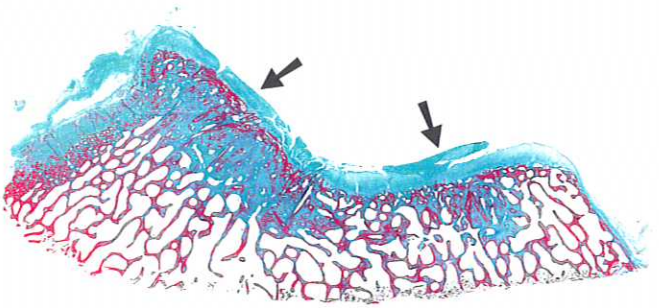
SEM of a chondrocyte grown on the Chondro-Gide® membrane. Magnification x 4030.

In vivo study

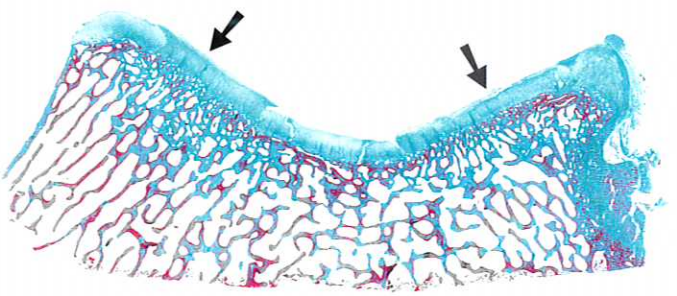
In a sheep study performed by PD Dr Behrens and Dr Russlies, Lübeck, the ACT with periost and Chondro-Gide® was compared. Defects without treatment served as control. One year after operation a histological evaluation was done by Dr. Ehlers, Lübeck (see photographs). No difference was found between the two treatment groups. A further quantitative analysis is going on at the moment.



Control defect – no treatment (arrow). Almost no formation of new tissue. Compare with adjacent healthy cartilage on the right.



Defect treated with periost + ACT. Notice the new formation of tissue to the same level as the surrounding cartilage and the more compact formation of subchondral bone under the defect.

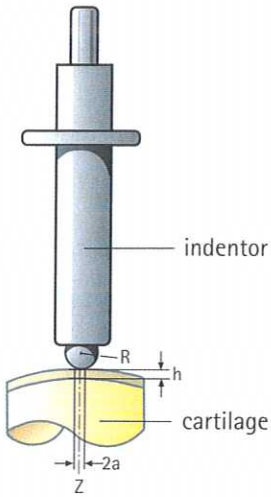


Defect treated with Chondro-Gide® and ACT. As with periost, there is a more compact organization of subchondral bone under the defect. The defect was filled with new tissue to the same level as the surrounding cartilage.

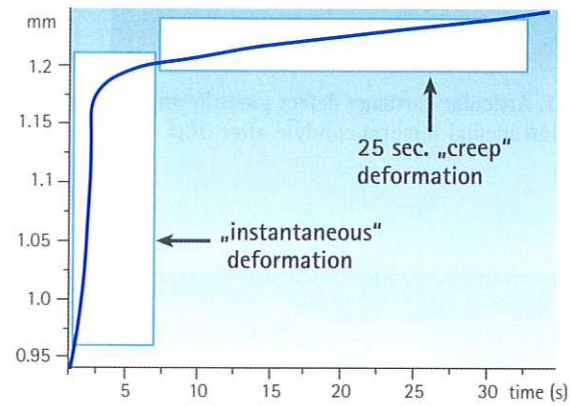
Biomechanics

In the same sheep study the biomechanical property of the new formed cartilage at four different defect sites was examined (Dr Russlies, Lübeck).

Schematic presentation of the indentor. The end has a spherical shape, with a radius of 1.5 mm. A constant force of 0.5 N was exerted on the cartilage, at room temperature irrigation.

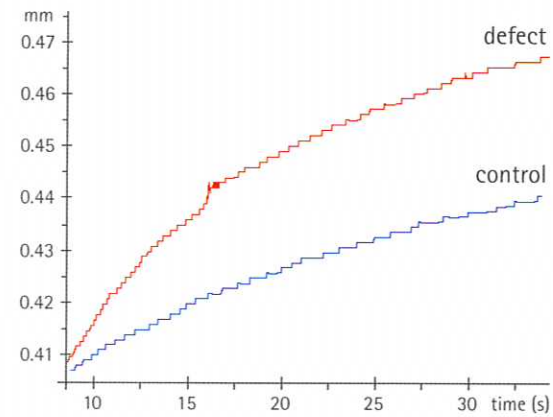


Indentation Graph



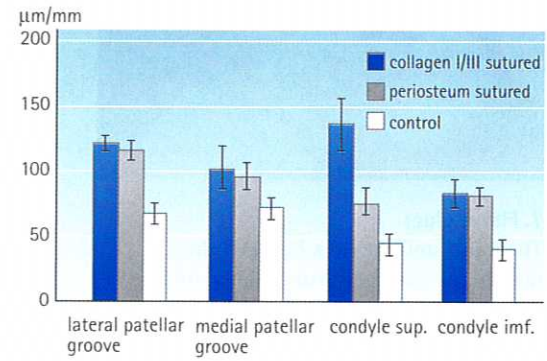
After indentation, an instantaneous deformation is seen in the first five seconds, followed by a creep deformation.

25 sec. Creep Deformation



The creep deformation is different between the normal healthy cartilage from the contralateral knee and the repaired cartilage.

Creep Index

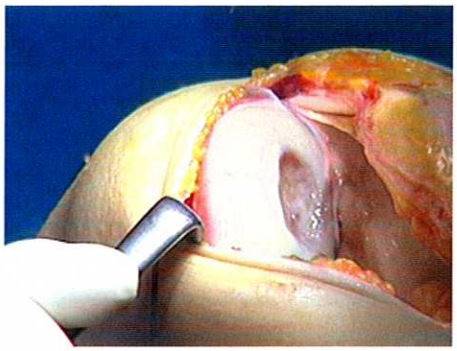


The creep index can be calculated by dividing the creep deformation of 25 seconds by the thickness of the articular cartilage.

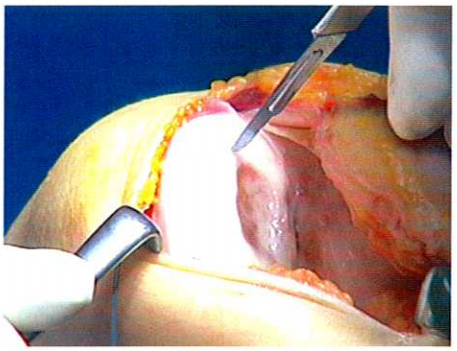
Conclusion: the repaired cartilage is less resistable then the normal healthy cartilage, but there is no statistical difference between the repair techniques.

General guidelines for ACT using Chondro-Gide®

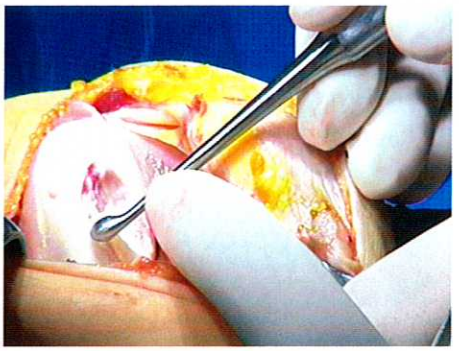
Case: Dr. M. R. Steinwachs, Freiburg



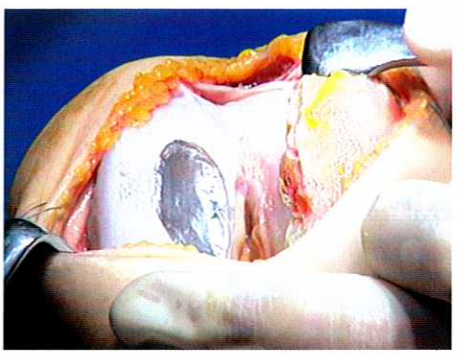
1. Articular cartilage defect grade IV at the left medial femoral condyle after OCD.



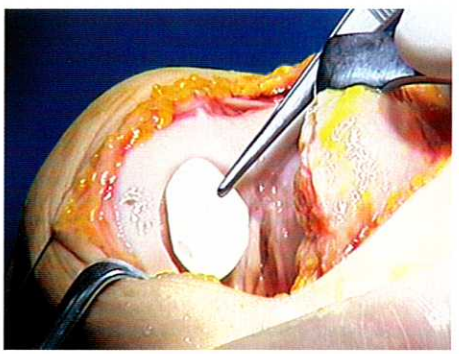
2. Dissection of the defect:
The defect must be carefully cut in an oval or round shape using a scalpel without penetrating the subchondral bone.



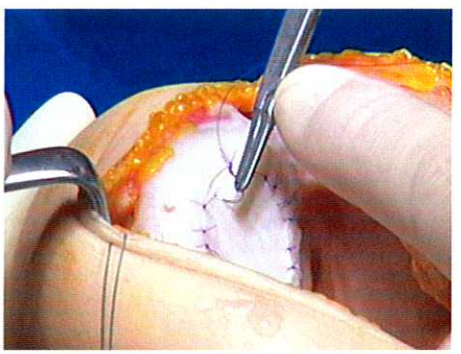
3. Debridement:
Degenerative and detached cartilage has to be completely removed. The walls of the defect should be perpendicular to the subchondral bone.



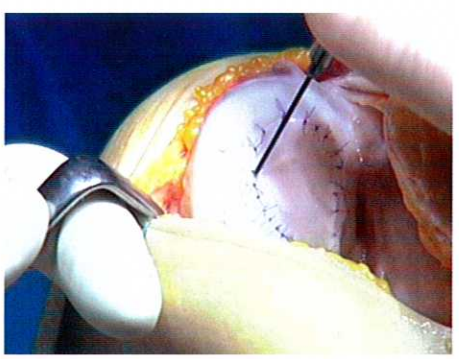
4. Creation of a template:
Measurement of the defect size with the help of a template. The imprint is subsequently cut out and marked at the proximal end.



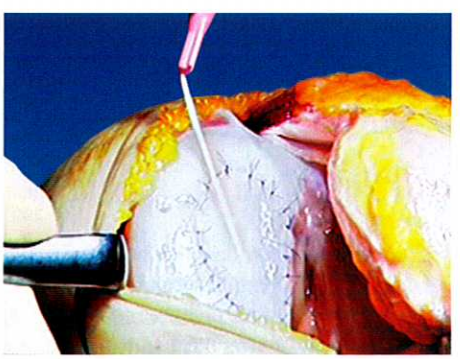
5. Dissection of the membrane:
The Chondro-Gide® is cut along the template in the correct size. Then the membrane is transferred into a Ringer solution filled small container using non-toothed tweezer to avoid penetration.



6. Suturing procedure:
Suture the Chondro-Gide® membrane with the porous side facing the subchondral bone to the surrounding healthy cartilage. An inside-out suture technique should be applied.



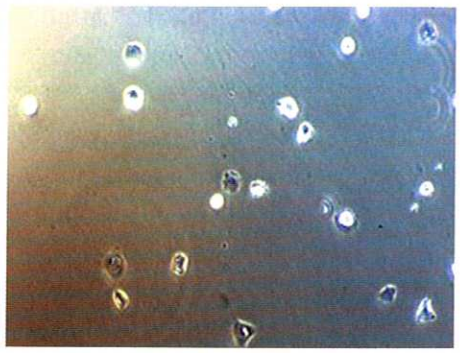
7. Fibrin glue:
The knots and the area between the membrane and the surrounding cartilage are covered with fibrin glue. The superior opening has to remain uncovered.



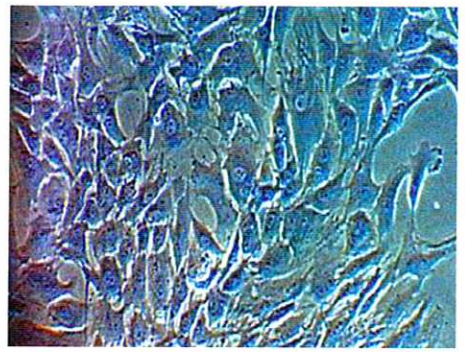
8. Check for watertight integrity / Injection of the cell suspension:
Sterile Ringer solution is injected underneath the Chondro-Gide® using a plastic catheter. In cases of leakage additional sutures are inserted. After removal of the injected liquid, the cell suspension is injected.

ACT cell culturing

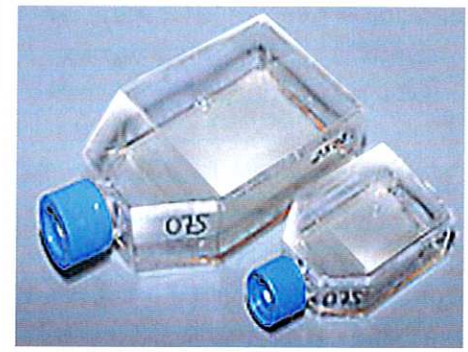
Pictures by: Dr. M. R. Steinwachs, Freiburg



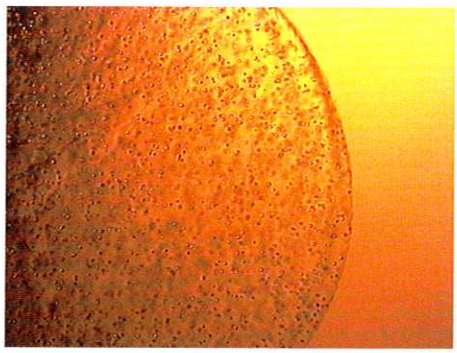
The isolated chondrocytes are cultivated in vitro for 2-6 weeks.



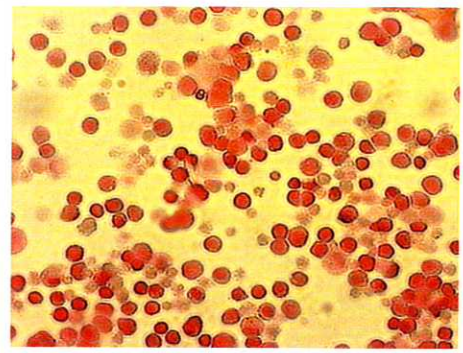
Human chondrocytes show a fibroblast phenotype after 1 week in monolayer culture.



Culture flasks.



Redifferentiated human chondrocytes in an alginate bead.



Safranin O staining showing GAG production of the cultured human chondrocytes in vitro.

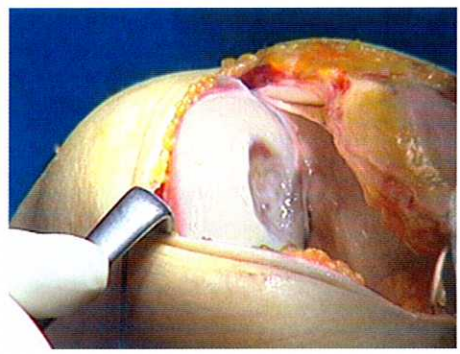
Clinical cases

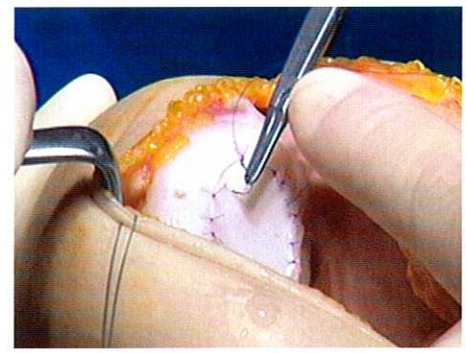
All cases: Dr. M. Steinwachs, Freiburg



Case 1:

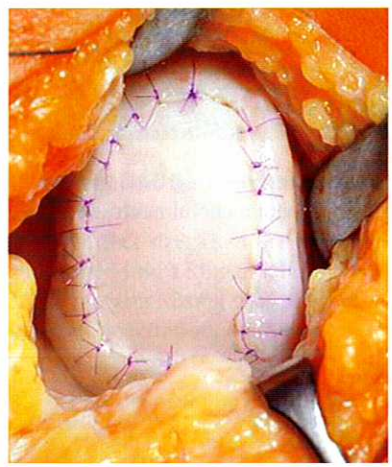
24 year old female with a grade IV cartilage defect at the medial left femur condyle after OCD.

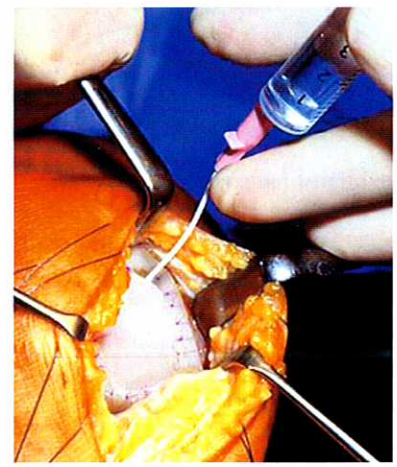




Case 2:

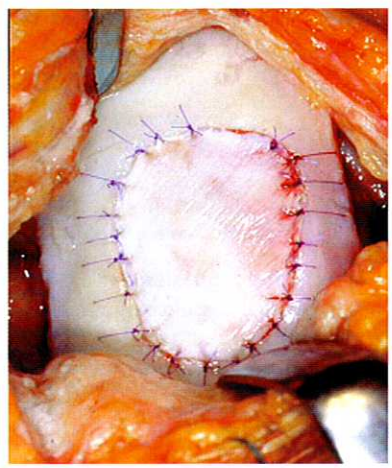
36 year old female with a 6 cm^2 defect size at the medial femoral condyle of the right knee covered with Chondro-Gide® membrane.

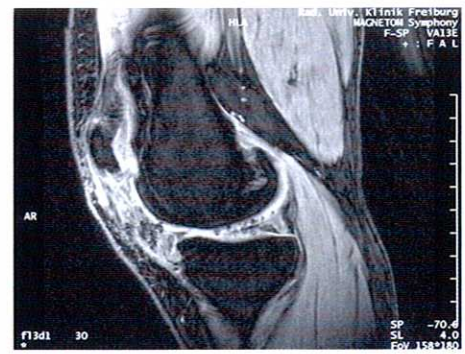




Case 3:

27 year old man with a traumatic cartilage lesion at the right medial femur condyle. The MRI control 6 months later shows that the defect is completely filled.





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