Discurso de Investidura como Doctor Honoris Causa de la Universidad Católica San Antonio de Murcia

"Aportación de los Cultivos Celulares (Condrocitos) en las Lesiones de las Articulaciones Dañadas"

"CARTILAGE REGENERATION FROM ACI to MACI to ICC to IPC"

Stephen P. Abelow, M.D., F.A.C.S

Murcia, 12 de junio de 2015

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- Su Eminencia Reverendísima Cardenal Antonio Cañizares Llovera, Obispo de Valencia.

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Aportación de los Cultivos Celulares (Condrocitos) en las Lesiones de las Articulaciones Dañadas

CARTILAGE REGENERATION FROM ACI to MACI to ICC to IPC

Stephen P. Abelow, M.D., F.A.C.S

Articular cartilage is a connective tissue that covers joint surfaces. It has important biological and biomechanical properties. With a coefficient of friction of 0.002, articular cartilage is 1000 more times slippery than ice-on-ice.[1] It allows minimal friction between opposing joint forces with movement and articular cartilage distributes loads on the joints over wide areas and minimizes peak stresses on subchondral bone.[2]

95% of the collagen content of articular cartilage is Type II collagen. This provides the cartilaginous framework and tensile strength. The Type II collagen has a half-life of approximately 25 years and is thusly very stable.[1]

Articular cartilage has no blood vessels (avascular), no nerves (aneural) and no lymphatics (alymphatic) and thusly has a limited capacity for intrinsic repair or regeneration. This is complicated by the fact that the chondrocytes (the basic cell of adult cartilage which synthesizes extracellular matrix) is surrounded in a thick extracellular matrix and the chondrocytes are unable to migrate from the uninjured matrix to a zone of injury. (Fig 1) Chondrocytes produce a Type II collagen.

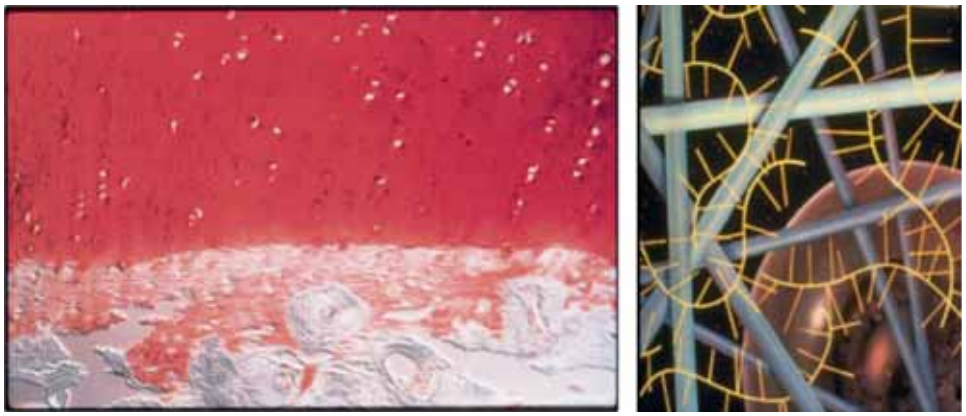


Figure 1

Buckwalter and Mankin reported that cartilage lesions that do not compromise the subchondral bone have difficulty healing. Full thickness injuries that violate the subchondral bone can form a fibrocartilaginous tissue (or endochondral bone).[3]

The goal of any cartilage restoration procedure is to restore the articular surface by matching the histological, biochemical and biomechanical properties of normal hyaline cartilage, improve patients symptoms and function, and prevent or slow the progression of focal chondral injury to end-stage arthritis.

The goal of improving the healing of cartilage lesions by means of autologous chondrocyte implantation and tissue engineering is the current goal,

TREATMENT MODALITIES FOR CARTILAGE

Joint lavage has been utilized with the idea of rinsing the joint of debris and catabolic enzymes. There is no regeneration of hyaline cartilage. Variable short-term results have been reported but no statistically significant long-term improvements have been reported.

Debridement has been utilized to remove mechanical symptoms from a loose chondral flap, loose bodies, degenerative cartilage, osteophytes, or for synovectomy. No attempt to repair or replace damaged articular cartilage is made. This is largely a palliative procedure and any initial good symptom relief often declines with time.

Marrow stimulating techniques such as abrasion arthroplasty, drilling, or microfracture were conceived to allow mesenchymal stem cells and other healing bioactive elements access to a damaged area in order to stimulate a healing response in the cartilage. The problem with these techniques is that they provide a fibrocartilaginous fill of the cartilage defect. This fibrocartilage-regenerated tissue has less Col II, more Col I and less aggrecan than normal hyaline cartilage.[4] A Level II, systematic review of 15 Level I and II

studies by Goyal, et. al. in 2013 reported good clinical outcomes at short-term follow-up for the treatment of small lesions and patients with low postoperative demands. Younger patients showed better clinical outcomes. They reported "Beyond 5 years postoperatively, treatment failure after microfracture could be expected regardless of lesion size."[5]

Osteochondral autograft (OATS/ Mosaicplasty) is the transference of an osteochondral plug of bone and cartilage from an area of low stress to an area of damaged cartilage. These have been used successfully in moderate to large sized cartilage defects (1.5-3cm. diameter). Concerns with Oats / Mosaicplasty procedures are that they "Rob Peter to pay Paul," donor site morbidity, malangulation, malrotation, and autografts that are either too proud or too countersunk. If several bone plugs are used, there can be dead spaces between the circular grafts. There can be different thickness and mechanical properties of the donor and recipient articular cartilage. (e.g. Knee joint cartilage 3-6mm thick; Talar joint cartilage 0.89mm thick). The short-term result of autologous osteochondral transfer seems to be good to excellent in many cases. (Fig 4)

Figure 4

For larger cartilage defects *osteochondral allografts* have been successfully performed. This is one stage procedure and can be utilized for deep bone loss. The allografts should be harvested within 24 hours of donor death when they are 100% viable and can be stored at 40C for up to 28 days. The allografts should not be frozen. Freezing of chondral allografts leads to chondrocyte death and is not appropriate for graft preservation. Cell viability does decline after 5 days. Tissue matching and immunologic suppression is unnecessary. Bugbee et. al. reported an 86% survivorship at ten years follow-up (92 patients; Cohort Level III study).[6] According to Dr. Bugbee one should expect 1-3 mm of subsidence and 28% get 4-5 mm of subsidence.[7]

The indications for cartilage replacement surgical techniques are symptomatic deep lesions characterized by the International Cartilage Repair Society (ICRS) Grade 3: deep greater than 50% cartilage depth and down to but not through the chondral bone and Grade 4: subchondral bone exposed (with lesions extending through the subchondral bone plate or deeper into the trabecular bone). There should be no uncorrected malalignment or instability and no significant osteoarthritis. (Fig. 2,3)

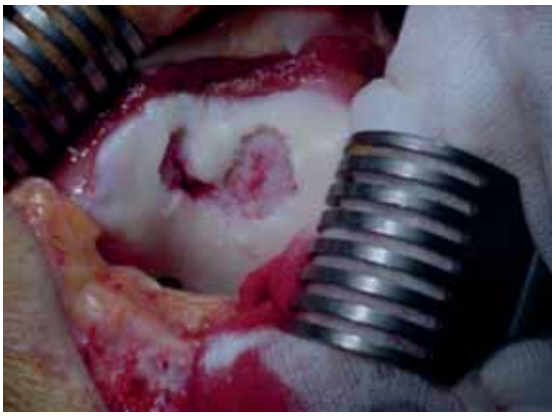


Figure 2

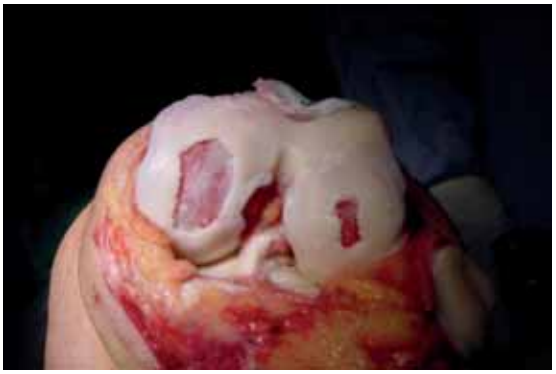


Figure 3

AUTOLOGOUS CHONDROCYTE IMPLANTATION

Autologous chondrocyte implantation (ACI) is the implantation of *in vitro* cultured autologous chondrocytes using a periosteal tissue cover after expansion of the isolated chondrocytes.

Autologous chondrocyte implantation (ACI) was first reported by Brittberg and coworkers in Gothenburg, Sweden in 1994,[8] has been successfully utilized in the knee and ankle joint. This procedure has yielded 80-90% good to excellent results in cases of isolated articular cartilage injuries and osteochondritis dissecans on the femoral condyles of the knee.

ACI is a two-stage process. Articular cartilage chondrocytes are harvested by either arthroscopic or open techniques. The chondrocytes are cultured in vitro for 3 to 5 weeks, expanded and reimplanted by arthrotomy. A periosteal graft must be harvested and sutured in place over the chondral defect in a "water tight" manner (2-3 mm apart). The cultured autologous chondrocytes are then injected onto the defect under the periosteal patch and the arthrotomy incision is closed. This often requires a wide arthrotomy incision to be made to allow for proper suturing of the periosteal graft. Complications include graft hypertrophy, delamination of defect, and intraarticular adhesions.[9,10] (FIG. 5)

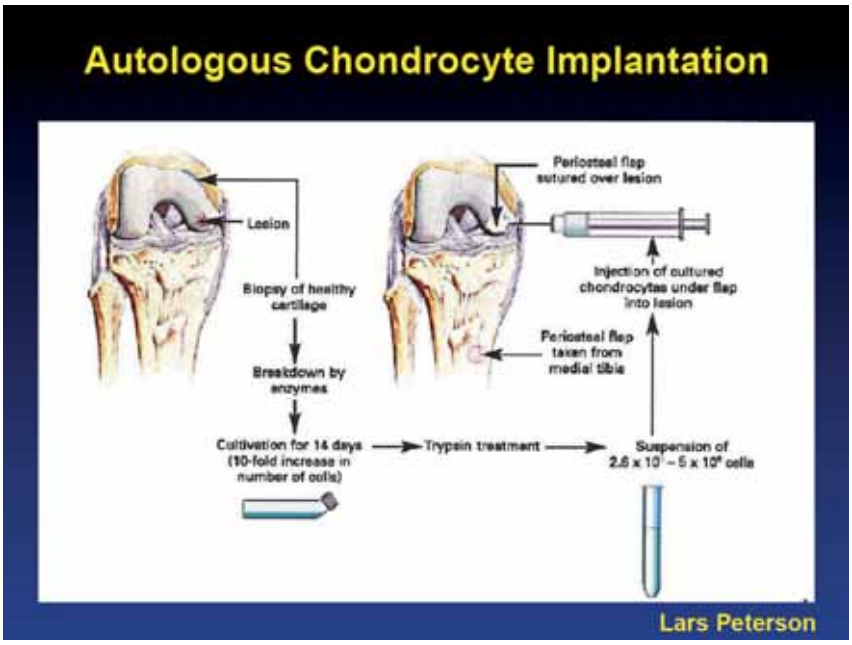


Figure 5

152 cases of ACI were performed from 1996 thru 2001. Average size of the defect was 6.1cm (0.25cm-13.5). There were 146 knees and 6 ankles (medial femoral condyle 64). Average age was 30 years (12-54 years). Results (retrospective Level 5) 3 to 8 years follow-up: 82% good to excellent 13% fair; 5% poor. There was one significant case of periosteal delamination in an elite level football player.

COLLAGE COVERED AUTOLOGOUS CHONDROCYTE IMPLANTATION (CACI)

Harvesting and suturing of a periosteal patch in autologous chondrocyte implantation is technically demanding and time consuming. Problems such as periosteal patch quality, symptomatic periosteal hypertrophy, and delamination have led to the development of biocompatible and bioabsorbable membranes to cover the chondral defect. A bilayer absorbable porcine collagen I/III membrane (Chondro-Guide, Geistlich Biomaterial, Wolhuser Switzerland) has been used instead of a periosteal patch. The membrane is degraded by enzymatic division (collagenase) and the resultant collagen-fragments denature at 37 degrees C. to gelatin. (Figs 6,7)

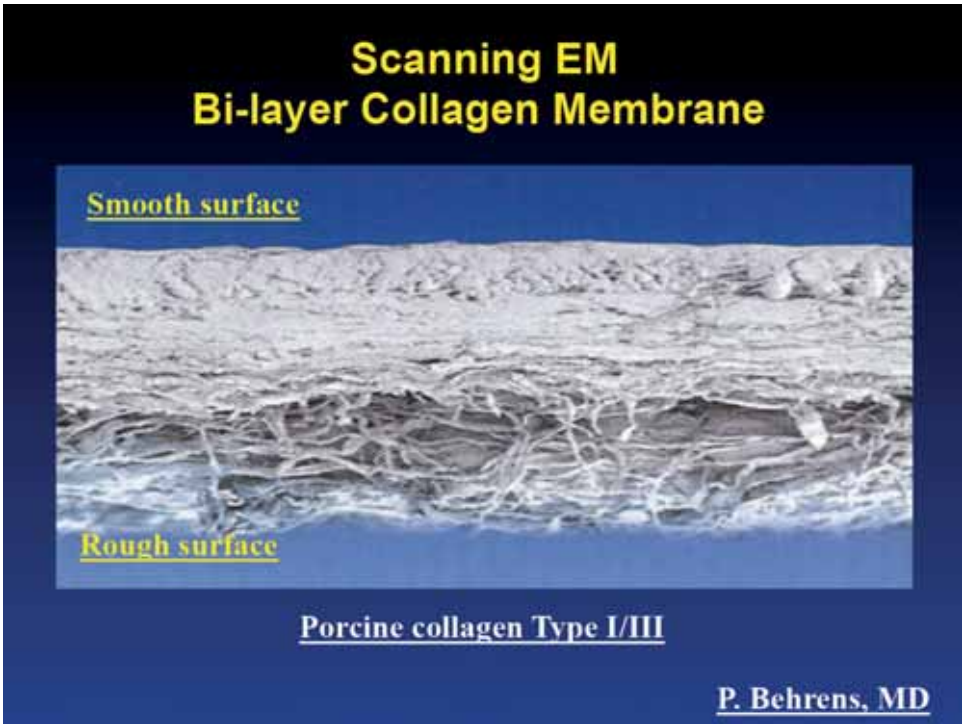


Figure 6

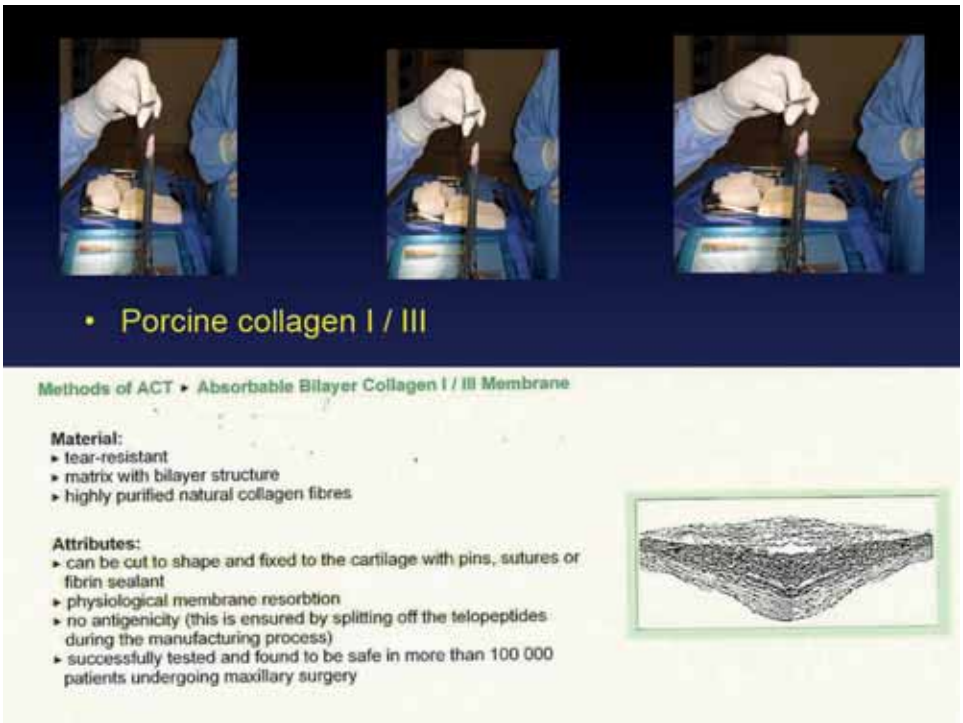


Figure 7

Is a periosteal patch necessary? Steinwachs in a prospective study reported on 63 patients with a collagen membrane (Chondro-Gide) ACI.[11] 88% reported good to excellent results three years after surgery. There was no case of membrane hypertrophy. In another study, 100 patients underwent ACI with a periosteal patch with 78% reported good to excellent results.[12]

MATRIX / MEMBRANE-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI)

MACI is a third generation chondrocyte implantation process. MACI is a new biotechnology allowing the impregnation of autologous cultured chondrocytes onto a highly purified porcine collagen I / III membrane (Vericell, Cambridge, MA). The MACI implant can be fixed to the chondral defect by fibrin glue (with little or no suture necessary), suture, or bioabsorbable pins or tacks. The procedure can be performed arthroscopically or by mini-arthrotomy. No periosteal graft is needed. (Fig. 8)

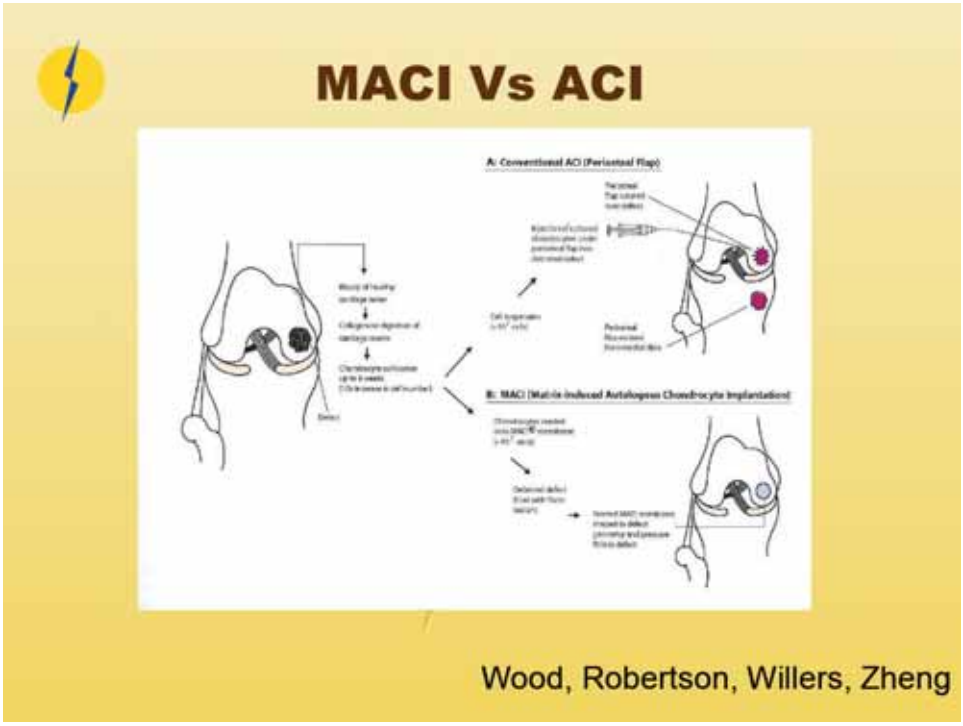


Figure 8

OPEN MACI TECHNIQUE

Initially chondrocytes are harvested arthroscopically from a non weight-bearing area of the ipsilateral knee (200-300 mg of healthy cartilage). (Fig. 13)

Figure 13: Chondrocytes in cell culture

The chondrocytes are then cultured, expanded *in vitro* (in 3-5 weeks) and then impregnated on an absorbable three-dimensional bilayered, purified porcine collagen I/III membrane. The bilayer structure has a smooth side that is non-porous acting as a natural barrier and faces the joint. Chondrocytes are seeded on the porous side of the matrix. The membrane is tear resistant and can be easily templated, trimmed, and cut to shape. The membrane is not self-adherent and can be "rolled-up" and handled with standard arthroscopic instrumentation allowing for arthroscopic implantation of the membrane.[13,14,15] The membrane is non-antigenic (telopeptides are split during the manufacturing process) and is bioabsorbable. The bioabsorbable membrane can be fixed to the cartilage defect with fibrin glue, pins or suture. (Fig. 7,8)

Utilizing mini-arthrotomy or arthrotomy techniques the cartilage defect is debrided and curetted with a sharp ring curette to remove the calcified fibrous cartilage layer without penetrating the subchondral bone. (Avoid bleeding of the subchondral bone!) (Fig 9)

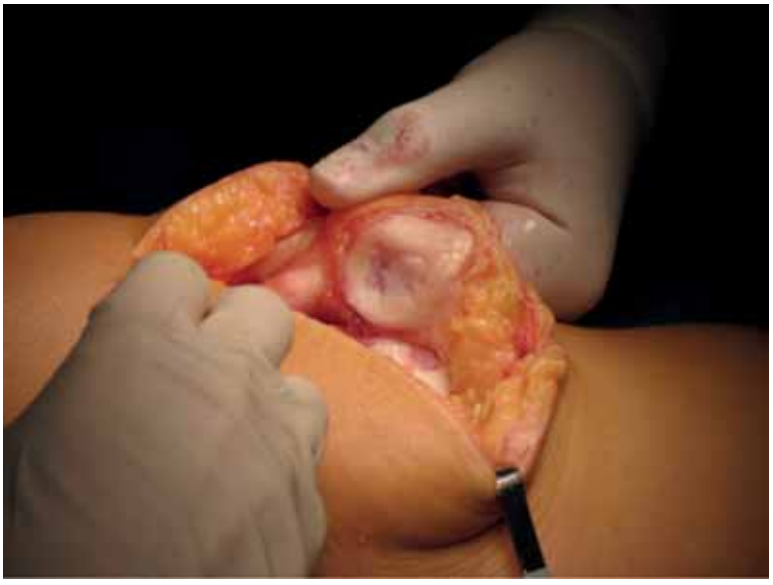


Figure 9: Chondral defect of the patella

A stable cartilage rim with sharp vertical walls of healthy cartilage is created. (Note: all "damaged" cartilage should be debrided back to a healthy stable border). (Figs 9,10,11,12) Intralesional osteophytes, if any should be

removed. The chondral defect is measured and templated. (Figs 14,15) The MACI membrane is cut to the proper shape with a scalpel or scissors. (Figs 15,16)

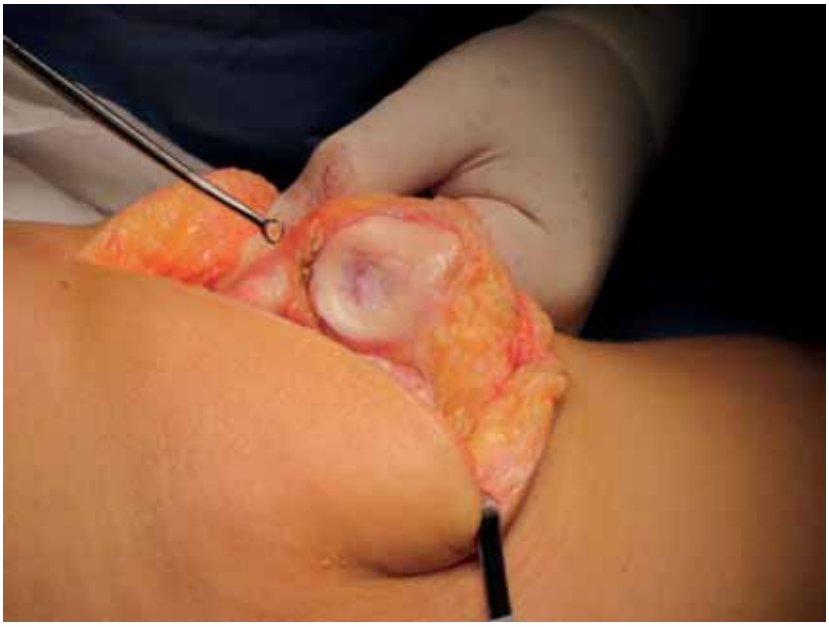


Figure 10: Chondral defect of patella curetted

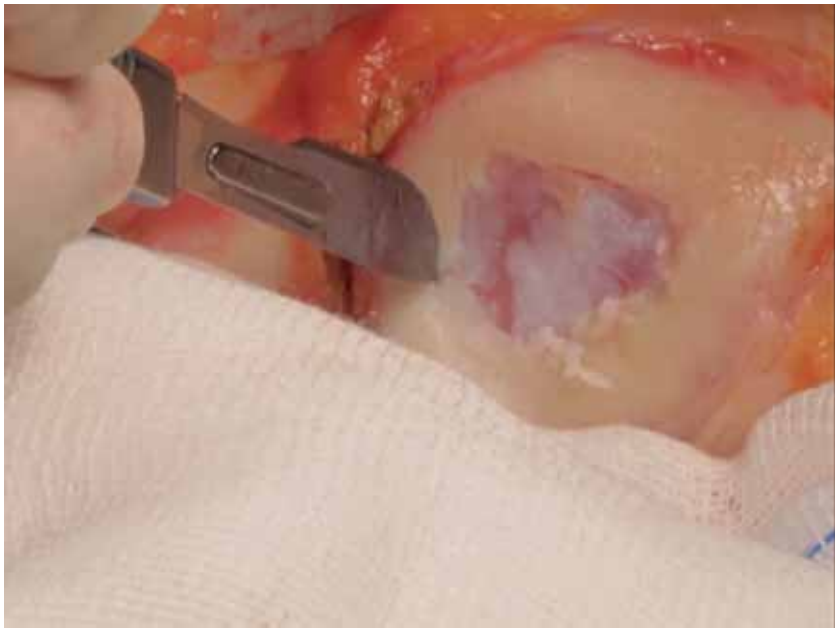


Figure 11: Create a stable rim with stable walls

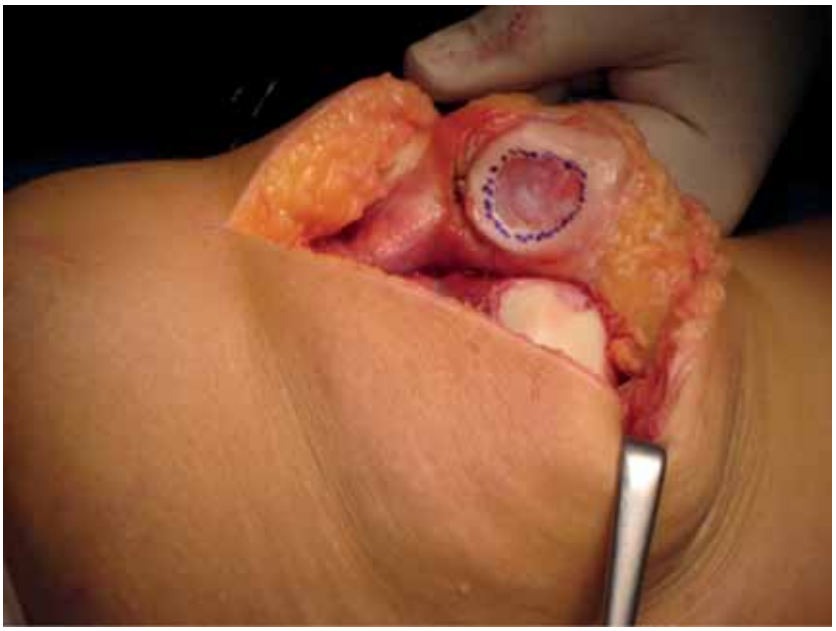


Figure 12: Cartilage lesion patella with stable vertical wall

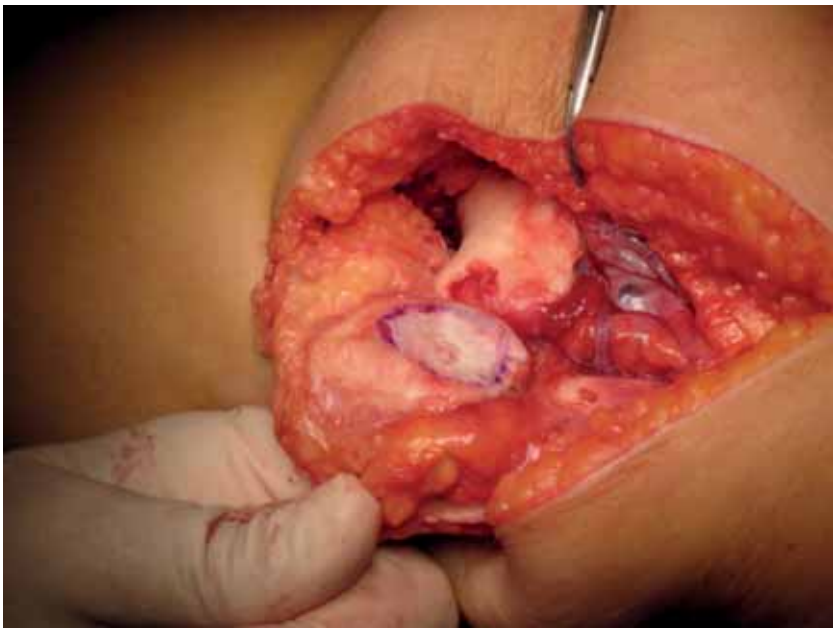


Figure 14: Lesion of patella templated

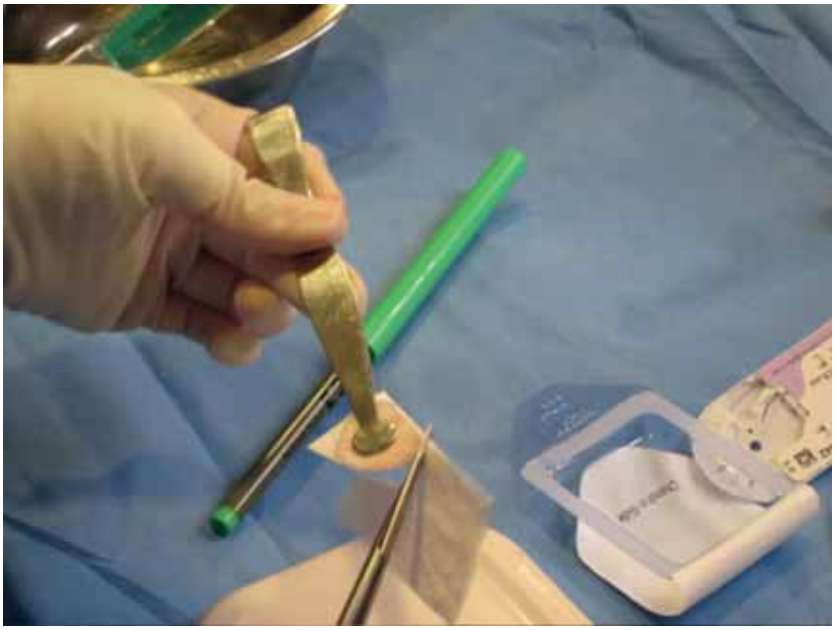


Figure 15: Chondro-Gide membrane cut to size of lesion

Figure 16: Trimming the Chondro-Gide membrane

The membrane is then fixed with fibrin glue (Tisucol, Baxter, Spain). Suture is used for the patella (fig. 18)

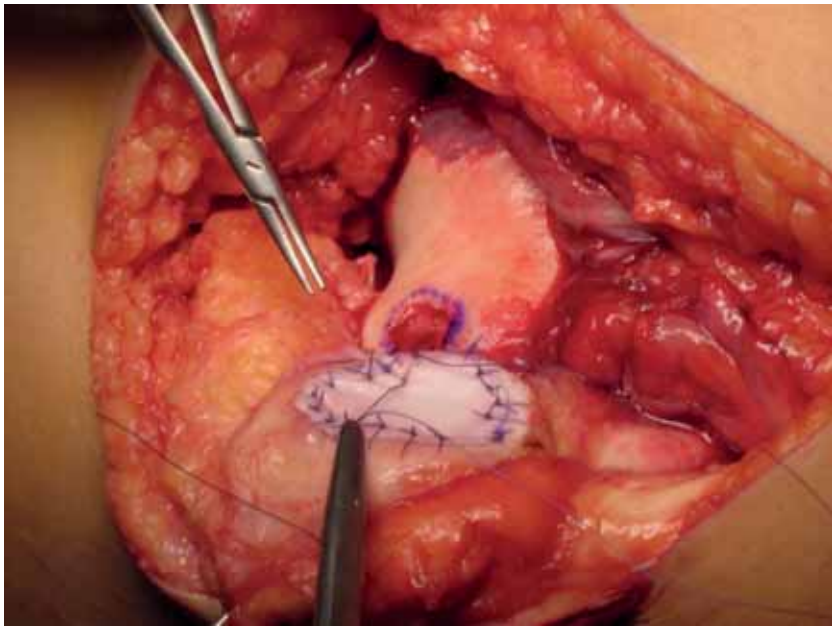


Figure 18: ICC membrane sutured in place

Postoperatively the patient is placed in a soft dressing and placed on continuous passive motion (when available) for 8 weeks. The patient is kept to partial weight bearing activity for 8 weeks. Larger and more central lesions are kept partial weight bearing for 12 weeks.

ARTHROSCOPIC MACI TECHNIQUE[13,14,15]

After previous biopsy and culturing of chondrocytes, a standard arthroscopy is performed thru a specially designed arthroscopic cannula and the cartilage defect is curetted using sharp ring curettes to remove the calcified cartilage layer. A stable rim with sharp vertical walls of healthy cartilage is created. Using a flexible ruler, a standard probe and a specially designed arthroscopic caliper, the size of the lesion is calculated. A template is created (using packaging from a suture pack or rubber drain) and placed in the cartilage defect to test for size.

Utilizing a “dry scope” the area of the cartilage defect is visualized (ambient air, no insufflation). Instrumentation has been developed at the Clinica CEMTRO that allows the MACI membrane to be pierced in its center and then placed in the center of the cartilage defect. The membrane is then pushed down the cannula with a slotted articulated inserter and held in place by the arthroscopic “skewer”. Fibrin glue is then placed under the MACI membrane, and the membrane is smoothed out using an articulated “T” smoother/tamper. The excess glue is removed, and the membrane contours to the cartilage defect while the fibrin glue is setting. Mini suture anchors or absorbable “pins’ can be used if a more secure fixation is required for stability. The joint is taken through a range of motion to insure the graft is stable. (Fig. 19,20)

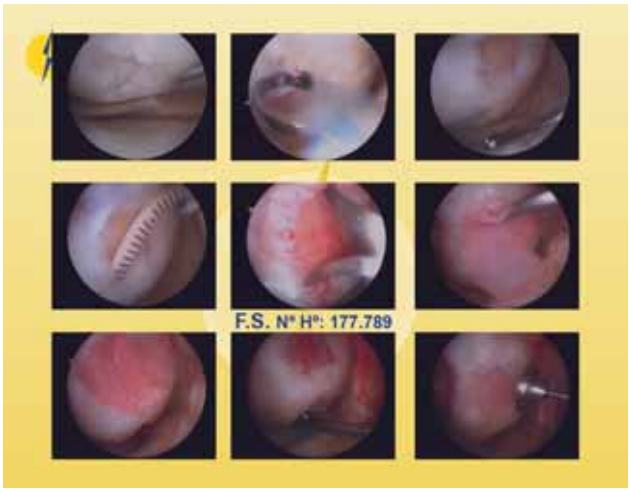


Figure 19

Figure 20

ICC
(Figures 9-18)

The MACI membrane as currently supplied is now 3x5 cm and is seeded with 1 million chondrocytes per cm^2 for a total of 15 million chondrocytes. (Previously the membrane size was 4x5cm^2 with a total of 20 million chondrocytes.)

If one were to treat a 3x2 cm^2 lesion of the patella with the traditional MACI technique, 6 million chondrocytes would be utilized and 9 million chondrocytes would "literally" be thrown away. The same lesion treated with traditional ACI would potentially have 12 million cells at the site of the cartilage lesion, which is double the amount of chondrocytes delivered to the same sized lesion treated with MACI. Since one side of the membrane is not porous, the MACI membranes really cannot be stacked upon one another. (Fig. 24)

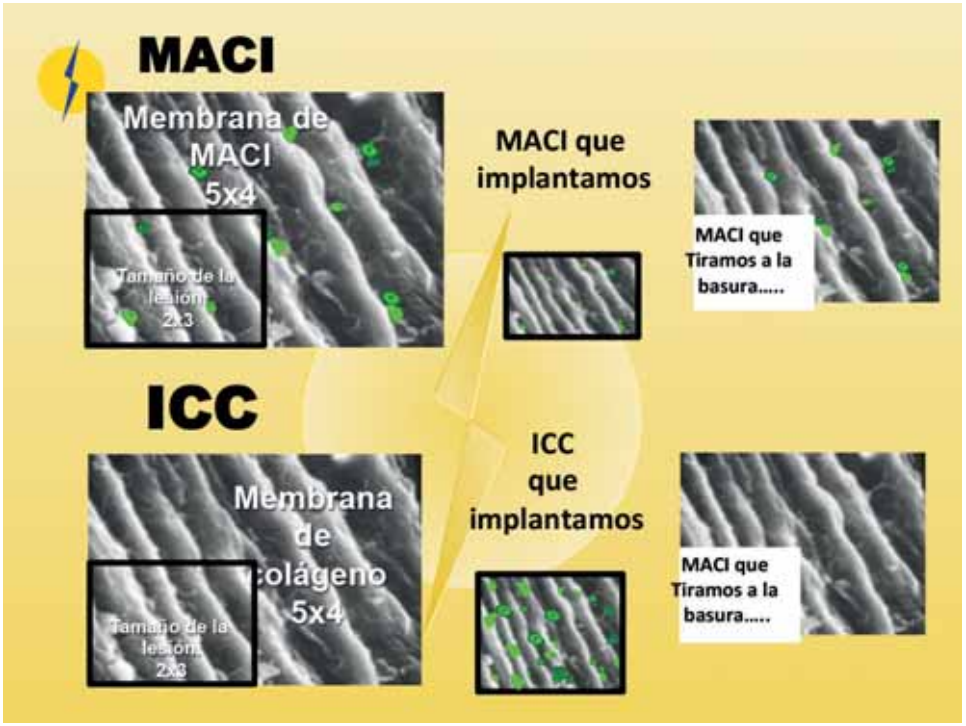


Figure 24

At Clinica CEMTRO / Universidad Catholic San Antonio de Murcia (UCAM) the concept of "Cell Density" concerning chondrocytes was investigated. In a recent article in Cartilage, Foldger, Gomol, Lind and colleagues reported "In the absence of systematic evaluations of the effects of cell density and clinical outcome, many clinicians continue to use one or two million chondrocytes per cm^2, which, despite its lack of evidence and the fact that most in vitro studies point toward benefits of high intensities, has been associated with favorable clinical outcomes and nearly approximates the densities found in native adult articular cartilage."[18]

In attempt to determine which type of cell (mesenchymal cell or chondrocyte) and the number of cells per square centimeter that are "optimal", at Clinica CEMTRO we studied 15 female merino sheep with articular cartilage lesions treated with autologous chondrocytes or mesenchymal cells seeded onto a porcine collagen I / III membrane. Experimental groups were 5 million chondrocytes per cm^2; 1 million chondrocytes per cm^2; 5 million mesenchymal cells per cm2; and microfracture. All samples were analyzed for cellular histology, type I collagen, type II collagen and aggrecan. The expression of aggrecans was seen in all samples. The expression profile of Col II (marker of hyaline cartilage) showed the control group was greater than 5 million chondrocytes, which was greater than 1 million chondrocytes, which was greater than 5 million mesenchymal cells, which was greater than microfracture. The expression profile of Col I was microfracture greater than 5 million mesenchymal cells greater than 1 million chondrocytes greater than 5 million chondrocytes. The results were statistically significant. The histology showed 5 million and 1 million chondrocytes to have a more hyaline-like cartilage structure than either the microfracture or implantation of 5 million mesenchymal cells. Increasing the density of chondrocytes improved the quality of the regenerated tissue.[4] (Fig 21,22,23)

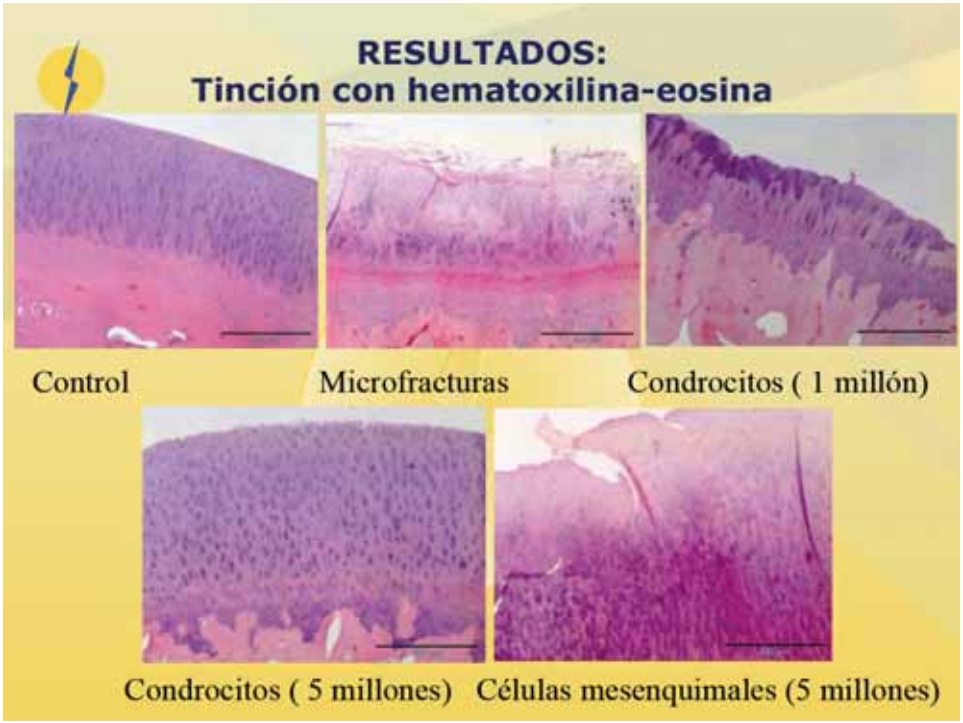


Figure 21

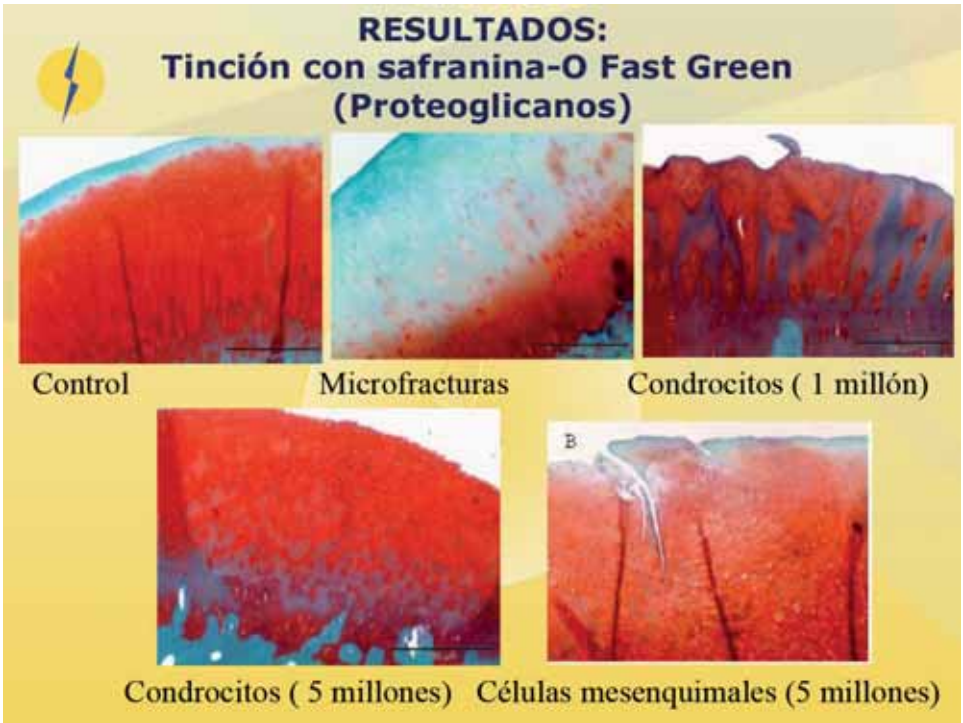


Figure 22

Figure 23

Based upon the fact that 5 million chondrocytes demonstrated a better regenerative cartilage tissue than 1 million chondrocytes or 5 million MSC, Clinica CEMTRO has developed a modification of the MACI procedure increasing the number of cells per cm^2 seeded on the collagen membrane. (Instant CEMTROCELL-ICC, Madrid, Spain). (Fig.24)

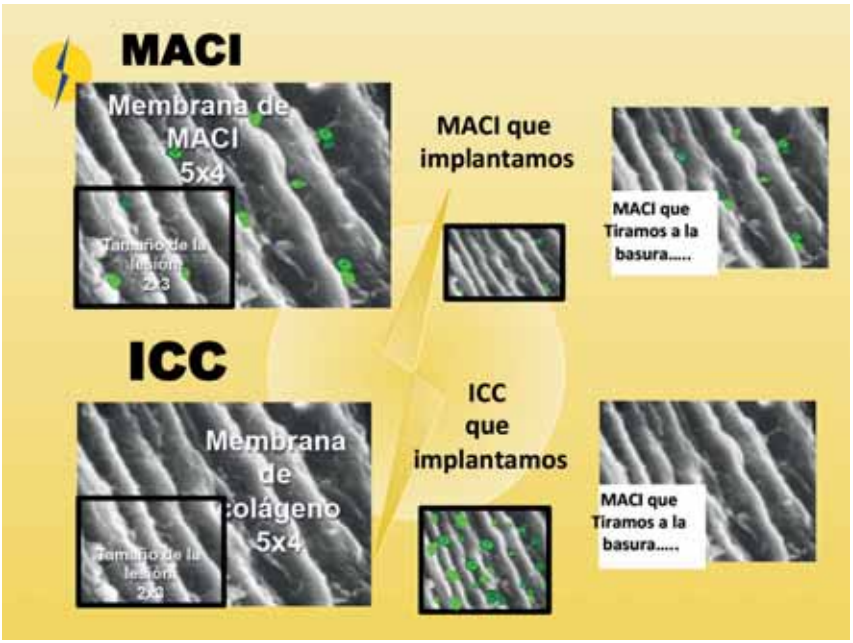


Figure 24

After biopsy by arthroscopy, isolation of chondrocytes, and cell culture to 20 million cells, the cell suspension is transferred to the operating room. The lesion is templated. (Fig. 14) The Chondro-Gide membrane is cut to the size of the lesion (Fig. 15,16) and all of the cell suspension is seeded on it. (Fig 17) The cells are seeded on the porcine collagen I / III membrane according to the method of Steinwachs.[11] the cultured chondrocytes are placed on the collagen membrane and after a 10-minute period of time to allow for the absorption of chondrocytes, the membrane is implanted on the articular cartilage defect. (e.g. a 2x3 cm^2 cartilage lesion would receive more than 3 million chondrocytes per cm^2 (Fig 17)

Figure 17: ICC cultured chondrocytes placed on Chondro-Gide membrane

Histological and genetic studies of ICC to date have shown a proliferation of collagen matrix, a population of viable mature chondrocytes, and immature population of chondrocytes with absence of expression of protein S-100, absence of atypical mitosis (absence of expression of P52), and a proliferative capacity.

Native Cartilage, Regenerated Cartilage, and MACI: A Comparative Study[16]

In an attempt to define the adequacy of the cultured chondrocytes we studied the cell distribution in tissue, cell morphology, collagen type II and X, and FGFR3 presence (Fibroblastic Growth Factor Receptor 3) in native cartilage, regenerated cartilage and MACI). (In achondroplasia there is a heterozygous mutation of the gene encoding fibroblastic growth factor 3).

Healthy cartilage had 117.6 +/- 6.2 cells / mm^2 compared to regenerated cartilage, which had 57.3 +/- 2.7 cells / mm^2. (Fig 27) A comparative analysis by Western Blot Electrophoresis demonstrated normal collagen type II in native and MACI cartilage and little type II cartilage in the regenerated tissue. (Fig 28) Looking at collagen type X, the reverse findings were present. There was abundant collagen type X in the regenerated cartilage and only minimal collagen type X in native cartilage and MACI cartilage. (Fig 29)

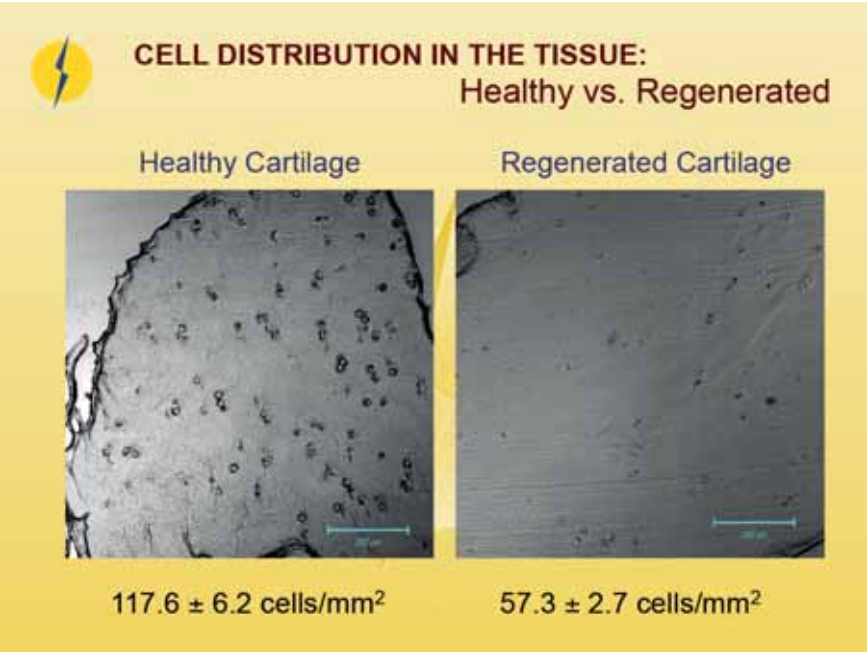


Figure 27

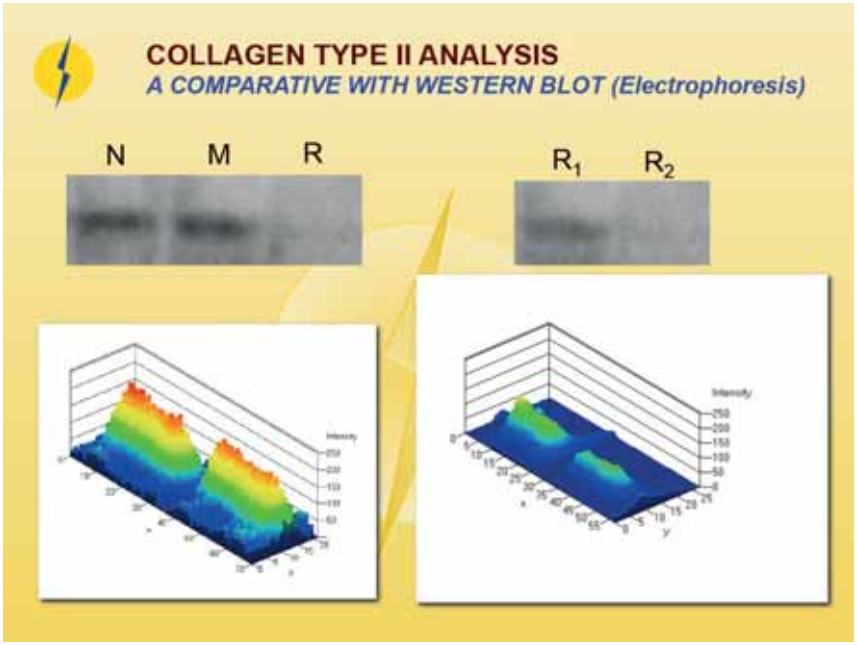


Figure 28

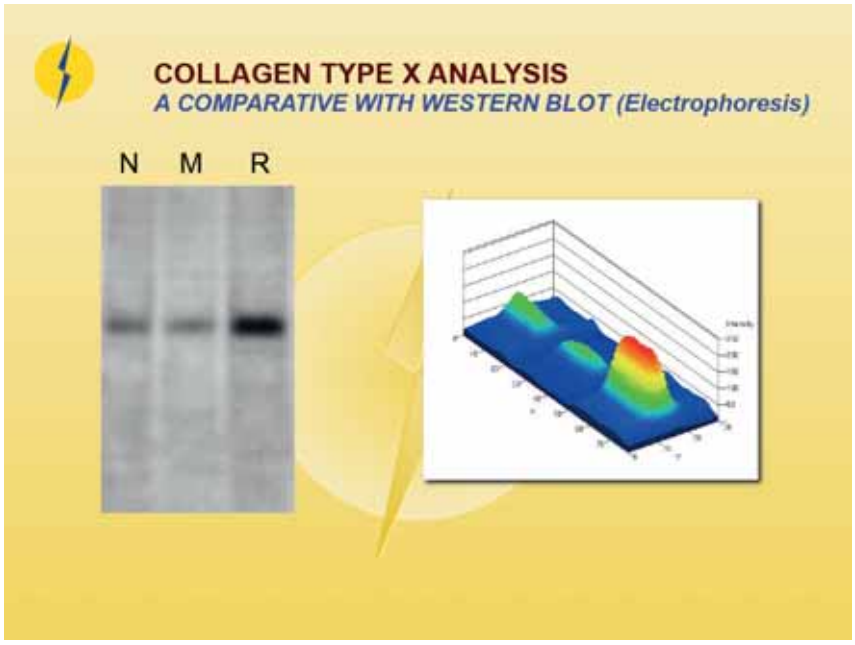


Figure 29

Analysis of the presence of fibroblastic growth factor receptor 3 demonstrated normal amounts of FGFR3 in healthy native cartilage chondrocytes and MACI cartilage chondrocytes. Regenerated chondrocytes showed only a small amount of FGFR3 (30%) (Fig 30,31)

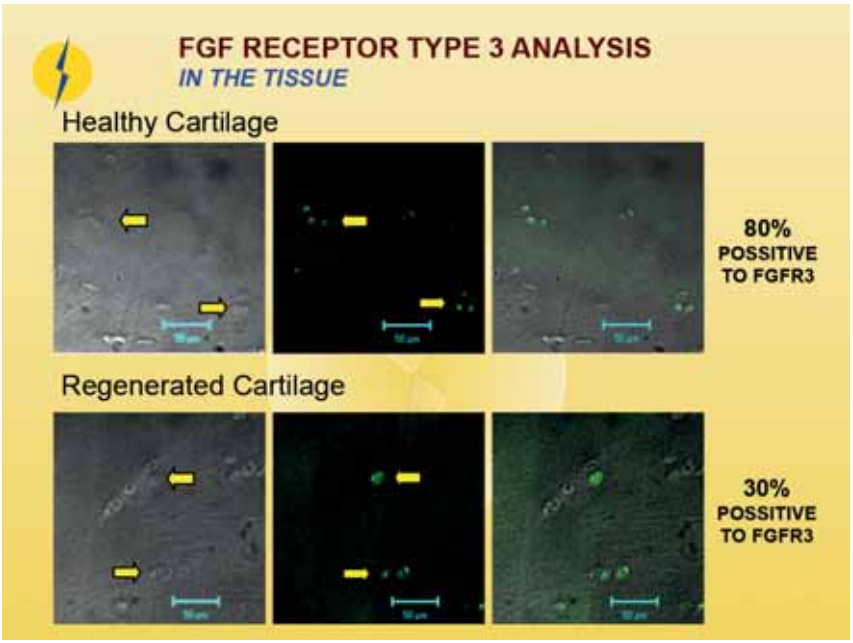


Figure 30

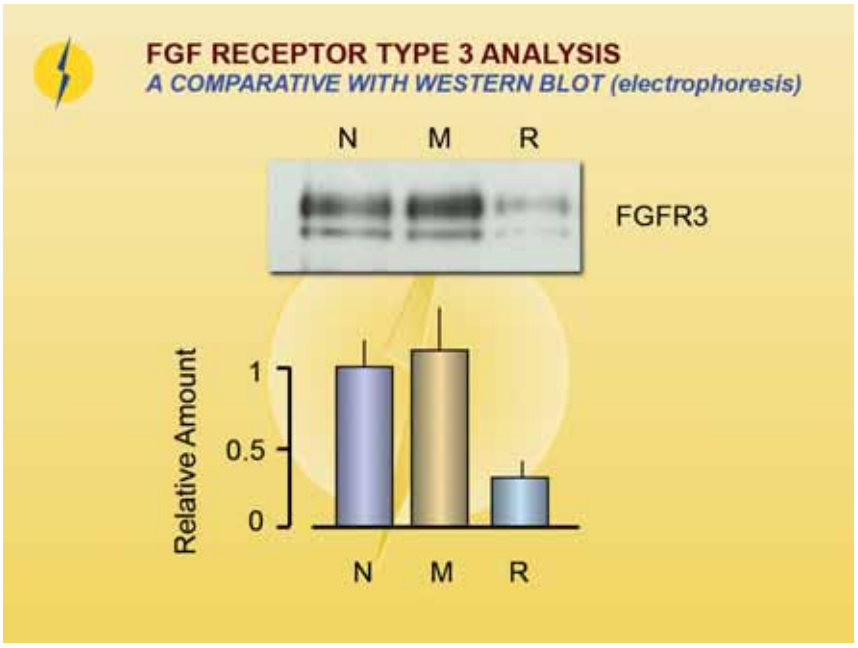


Figure 31

This study showed that regenerated cartilage compared to normal cartilage and MACI cartilage had 50% the number of cells. MACI chondrocytes compared to normal cartilage chondrocytes presents identical FGFR3 receptor amount, collagen type II levels, and collagen type X levels. Regenerated cartilage chondrocytes produce less collagen type II and more collagen type X than normal or MACI chondrocytes. Regenerated cartilage chondrocytes showed a lack of FGFR3 receptors compared to normal or MACI chondrocytes (only 30% of the cells had this receptor.

The clinical relevance of this study is that according to Arnold Caplan, PhD at the International Repair Society World Congress (Chicago, Il., USA May, 2015) the ratio of FGRF3 to FGRF 1 may be an important determining factor in the progession of chondrocyte progenitor cells into producing articular cartilage rather than enchondral ossification.

HOW CAN WE POTENTIATE THE CAPACITY OF THE CULTURED CHONDROCYTES?

In an attempt to potentiate the cultured chondrocytes many growth factors and other polypeptides and long-chain proteins have been utilized. Experimentation with the addition of a dinucleotide molecule (diadenosine tetraphosphate –Ap_4D_2) to the culture medium showed significant improvements in the growth characteristics of the cultured chondrocytes. (Fig. 32) Diadenosine tetraphosphate is a molecule that is found in human synovial fluid. (Fig. 33) The Ap_4D_2 favored the proliferation of chondrocytes by almost 30%. (Fig 35) The Ap_4D_2 favored the production of extracellular matrix by almost double.(Fig. 34) Treated tissue demonstrated more collagen type II and less collagen type X than the untreated tissue. The addition of Ap_4D_2 reduced the number of FGFR3 receptors in the chondrocyte membrane.

Animal experimentation is currently underway.

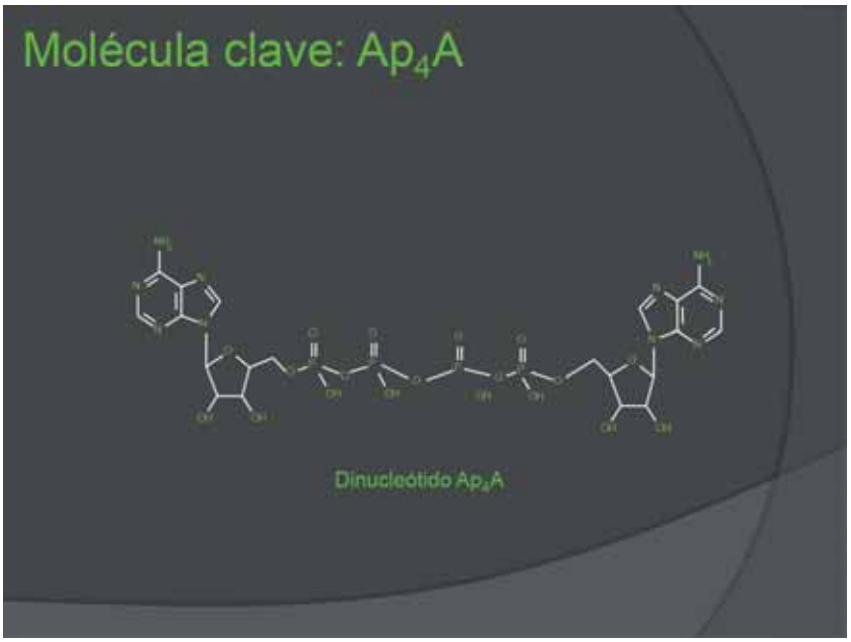


Figure 32

Figure 33

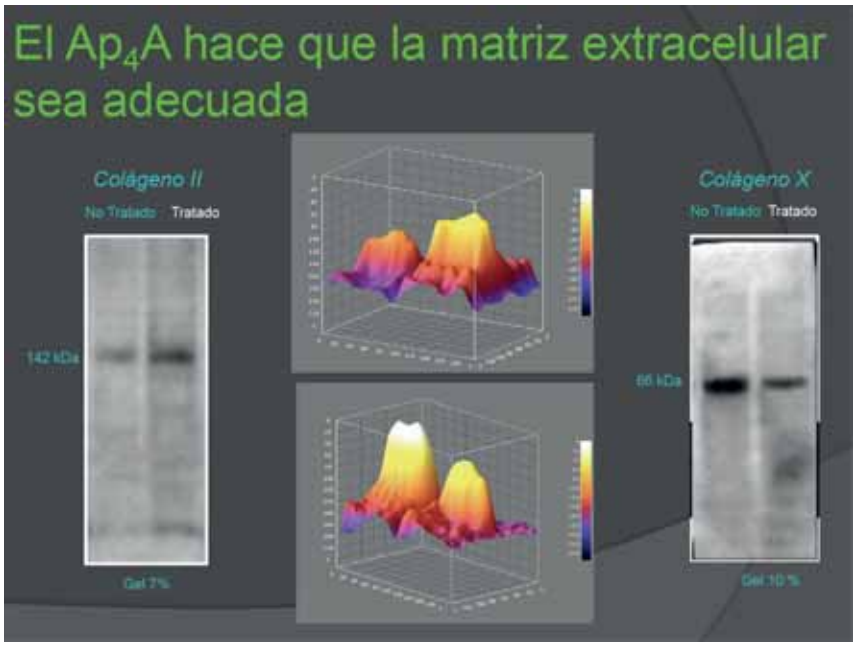


Figure 34

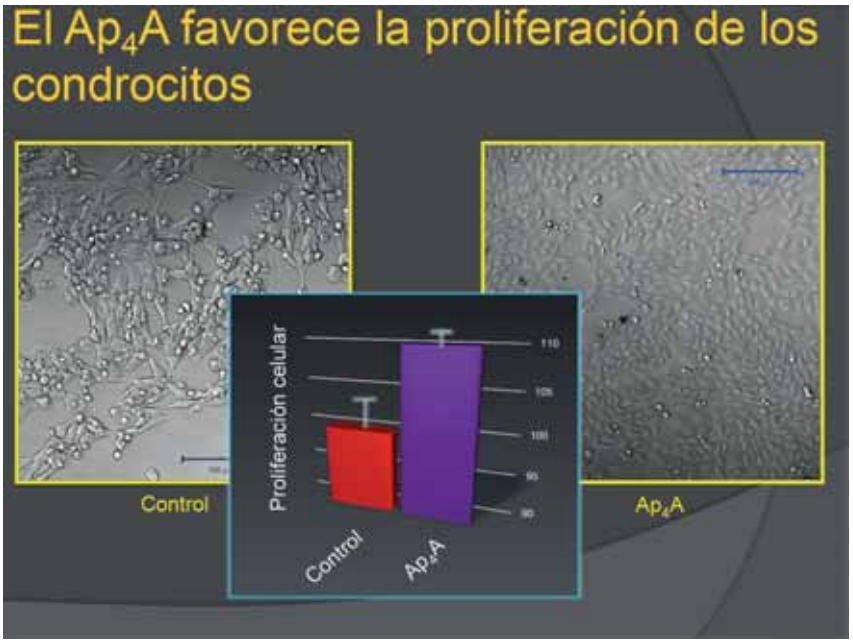


Figure 35

INDUCED PLURIPOTENT STEM CELLS (iPSC)

Induced Pluripotent Stem Cells (iPSC) are “embryonic-like” stem cells that are developed from a persons own cells such as skin, blood, MSCs, etc.)(Fig 36,37) and Re-engineer or Re-program these cells to differentiate into other tissue types including chondrocytes. These “embryonic-like” stem cells are developed by means of gene transduction using ESC-specific transcription factors.[17]

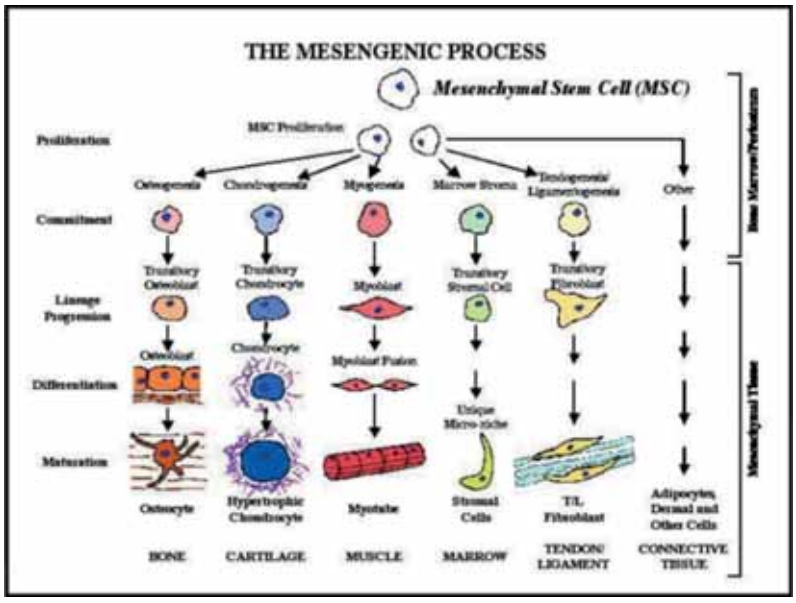


Figure 36

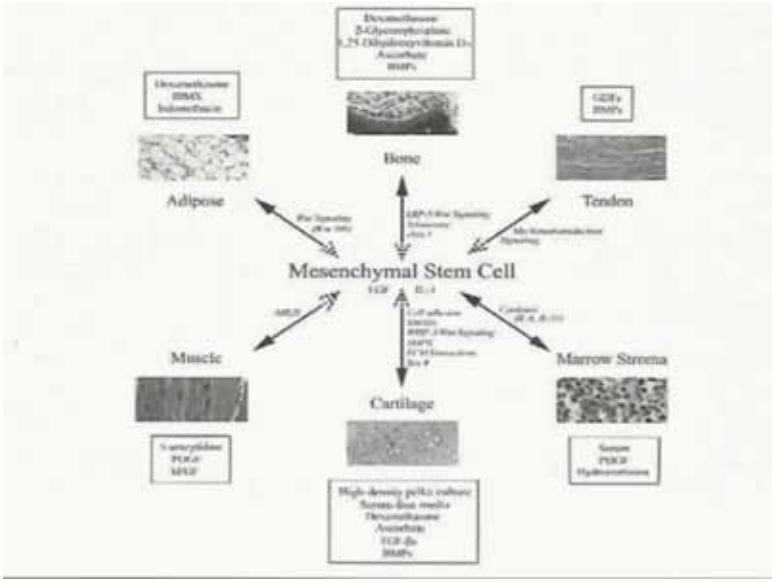


Figure 37

Yamanaka introduced the concept of Induced Pluripotent Stem Cells in 2006. Shinya Yamanaka and John Gurdon were Nobel Prize Winners in Medicine in 2012.(Fig. 38)

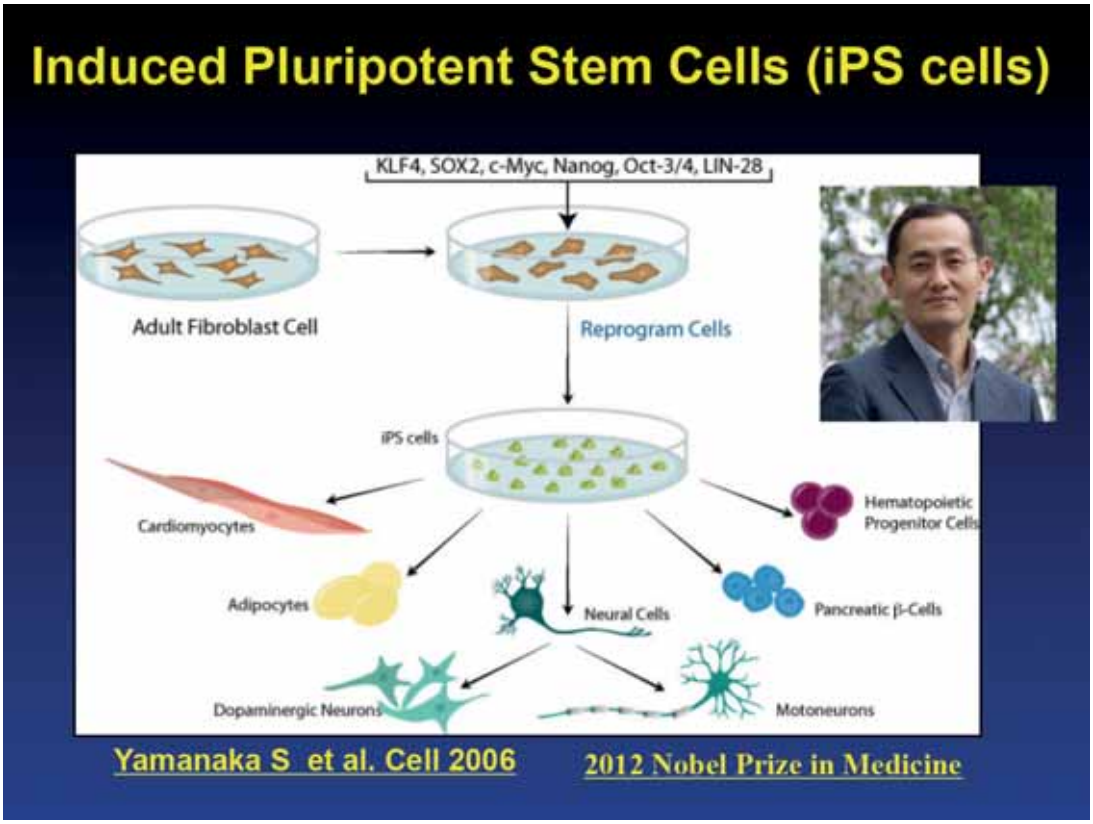


Figure 38

These iPSCs are pluripotent cells like ESCs and are autologous in origin. The iPSCs would theoretically be capable of indefinite self-renewal. There should be no ethical concerns. These iPSCs could be used in multiple tissue applications including cartilage regeneration.

Safety concerns with iPSCs would be that their undifferentiated nature and tendency to grow without restraint could lead to the development of tumor or teratoma formation.

A study performed by the University of Connecticut Stem Cell Laboratory compared normal chondrocytes, osteoarthritic chondrocytes, dermal fibroblasts (skin) and cord blood mononucleocytes in forming cartilage iPSCs.

Their study demonstrated that iPSCs derived from either normal or osteoarthritic articular chondrocytes possess a greater chondrocyte-forming potential compared to the iPSCs from skin or cord blood mononucleocytes. Their data showed that the tissue of origin affected the fate potential of iPSCs for differentiating into chondrocytes. They reported that the iPSCs derived from osteoarthritic articular chondrocytes displayed similar induction of early chondrogenic markers compared to iPSCs from normal chondrocytes.

Clinica CEMTRO (Madrid, Spain) / UCAM (Murcia, Spain) are in collaboration with the Salk Institute of San Diego, California, USA and have already started to culture mesenchymal stem cells transformed / reprogrammed into chondrocytes with the addition of TGF-beta.[19] (Figs. 39-44)

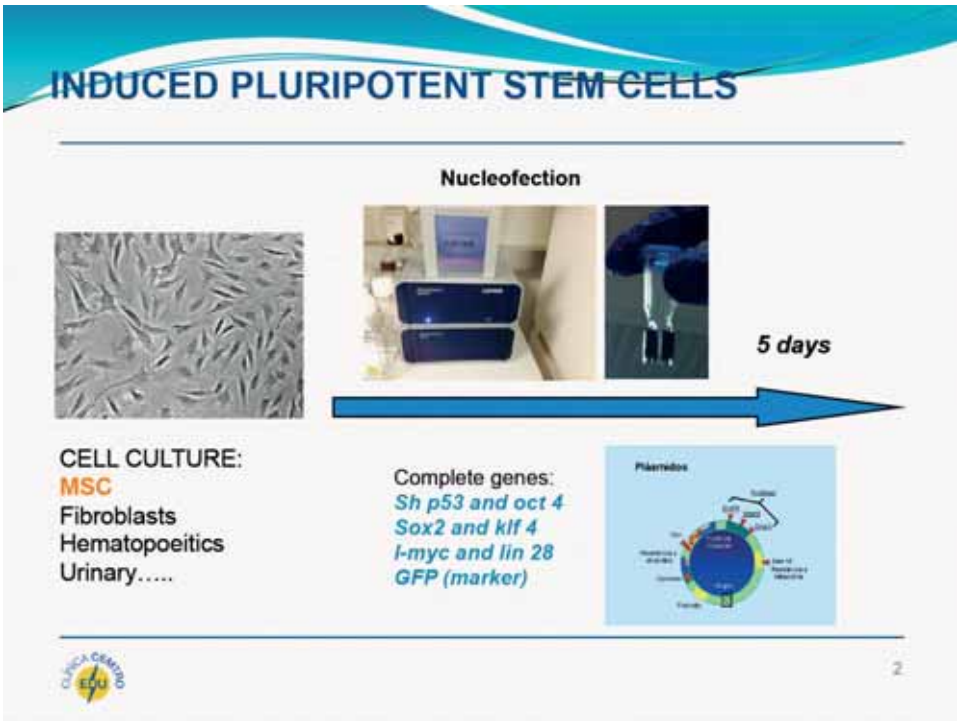


Figure 39

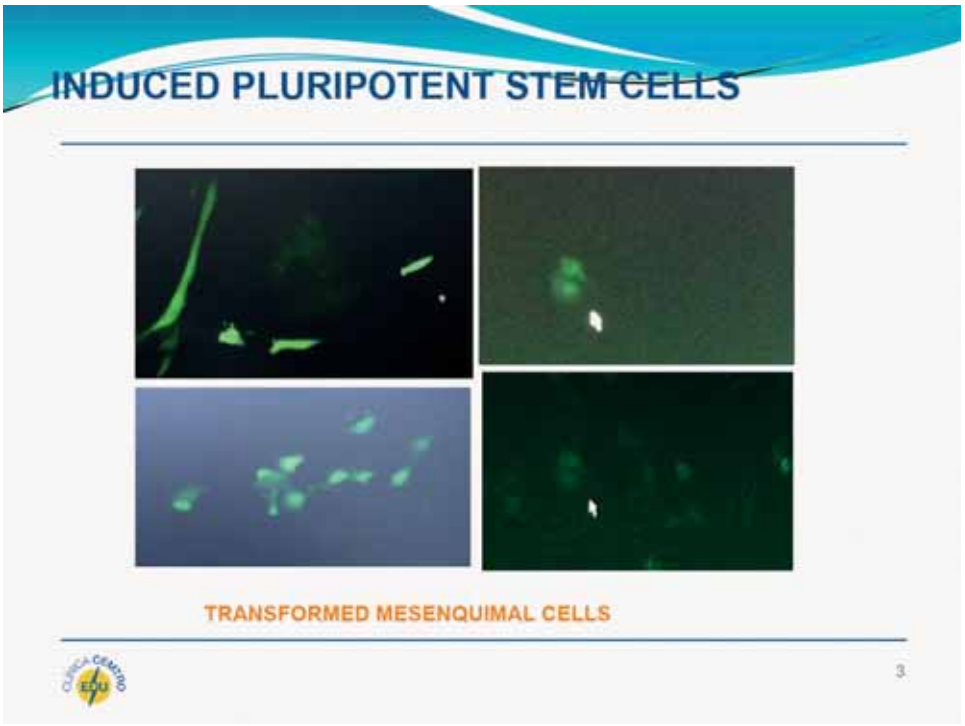


Figure 40

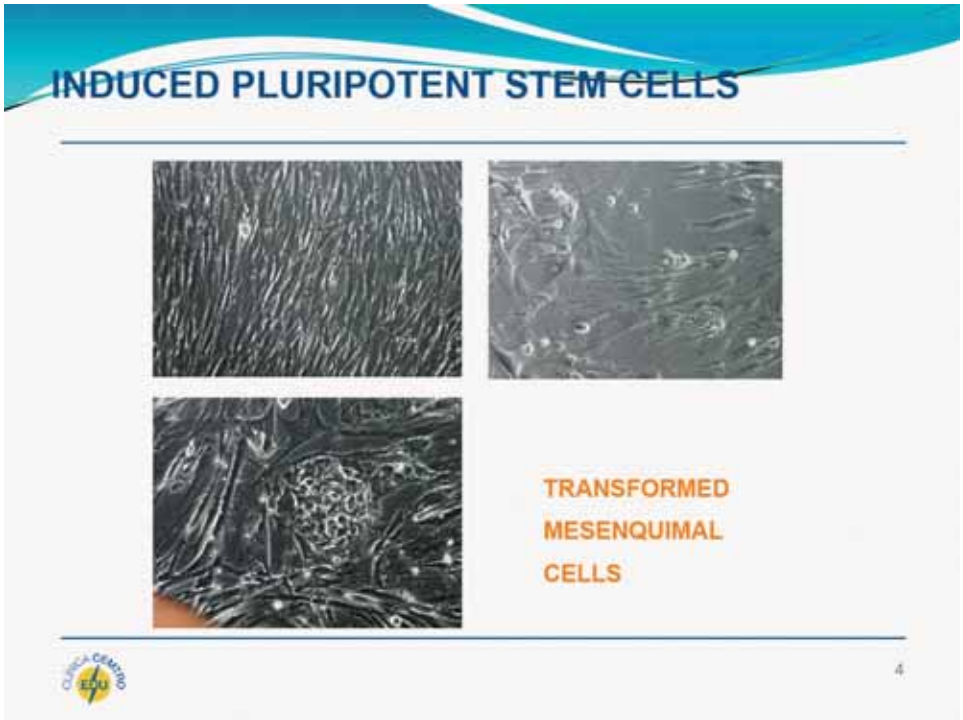


Figure 41

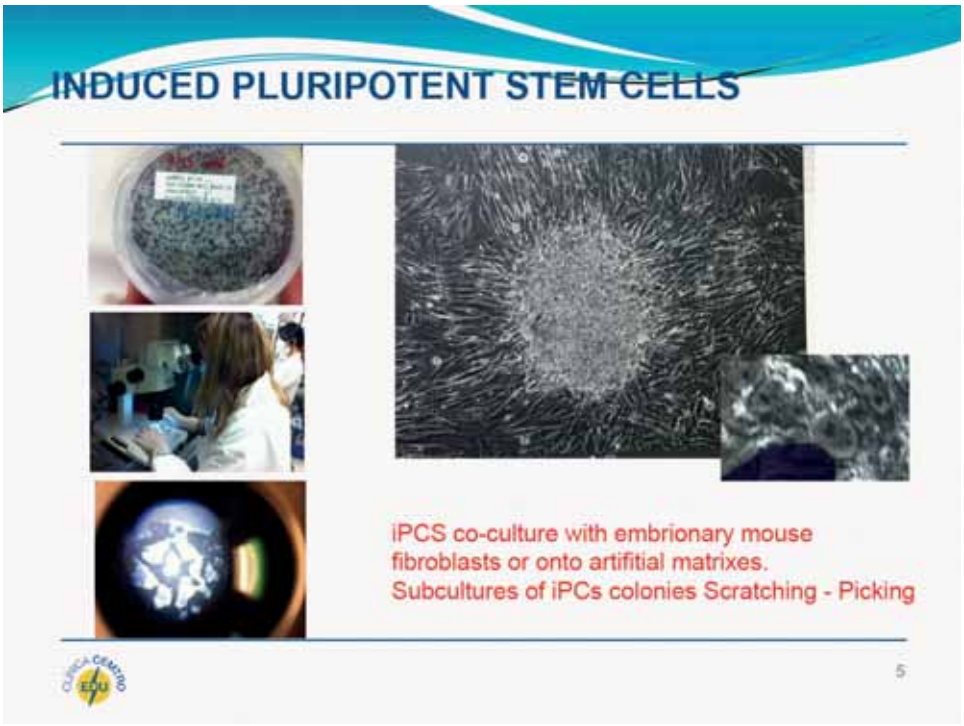
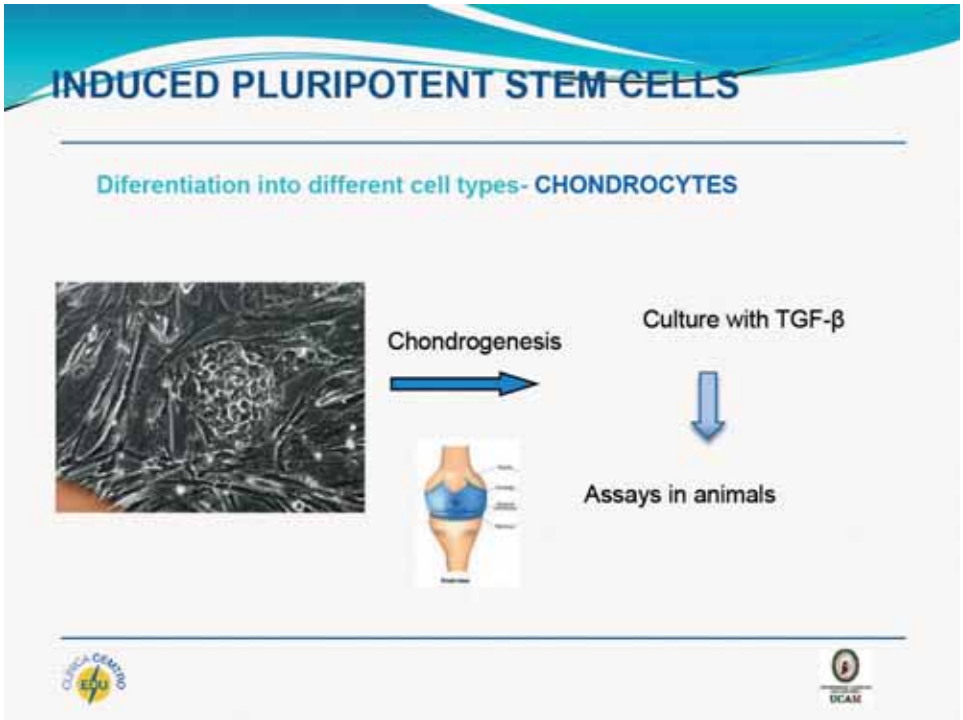


Figure 42



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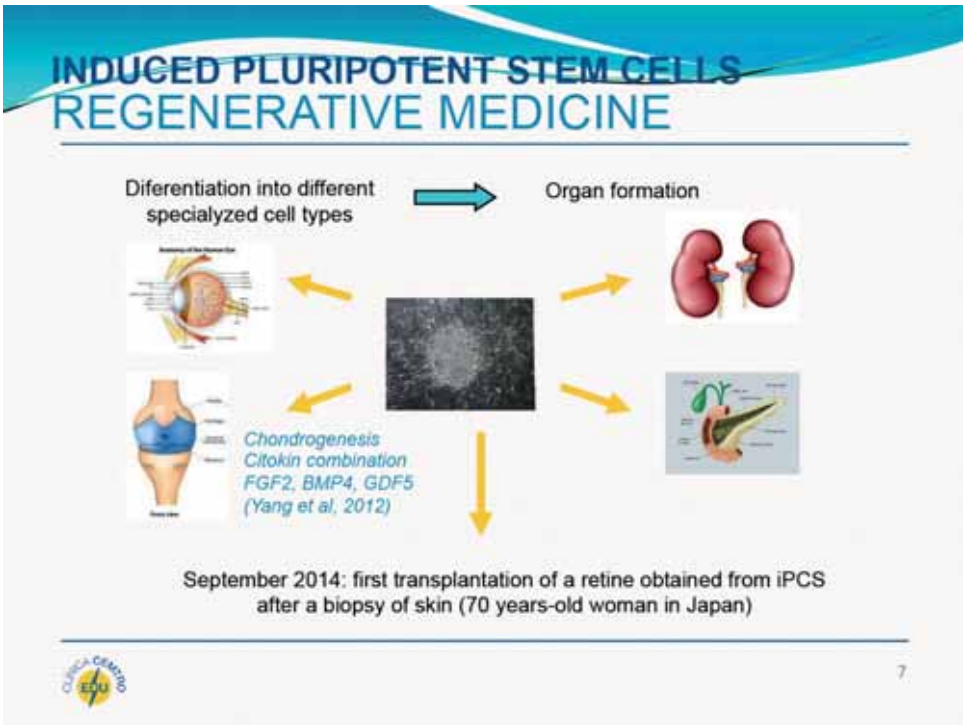

Figure 44

He dicho,
Gracias.

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