SERVIZIO SANITARIO REGIONALE
EMILIA - ROMAGNA
Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

Dipartimento RIZZOLI Research Innovation Technology

Cellule e fattori di crescita nelle patologie cartilaginee

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XVII Corso Nazionale di Aggiornamento SIdEM,
Palermo-Mondello 18-20 Ottobre 2012

CLINICAL PROBLEM

John Hunter (1728-1793)

" Ulcerated cartilage is a troublesome thing, once destroyed, it is not repaired…."

Cartilage damage (acute/chronic)

↓

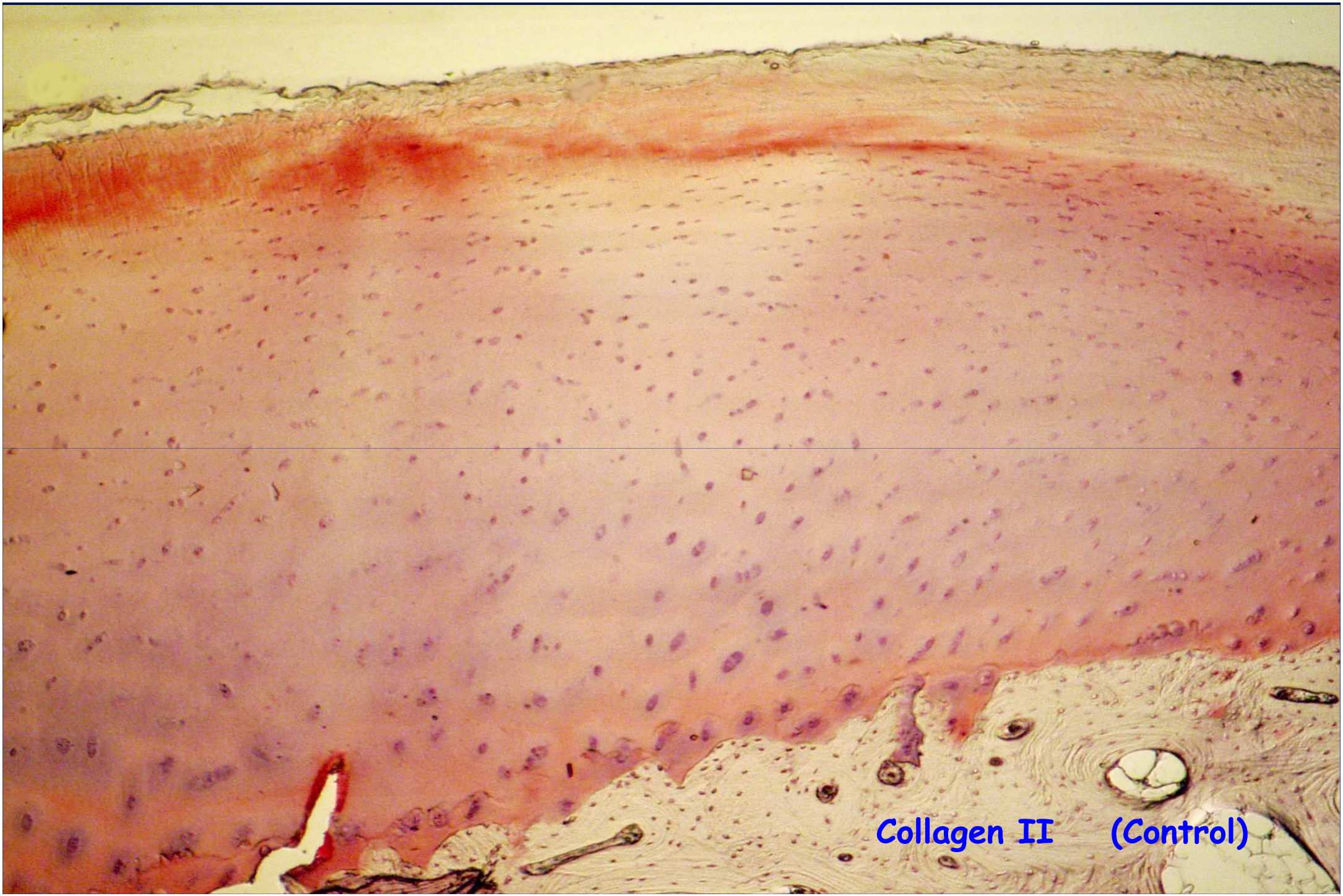
Loss of tissue

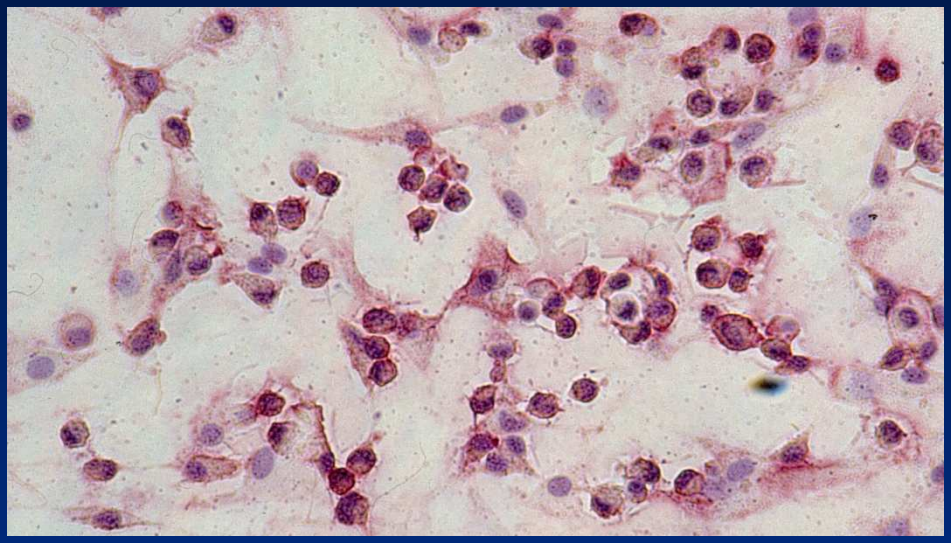
↓

Fibrous repair

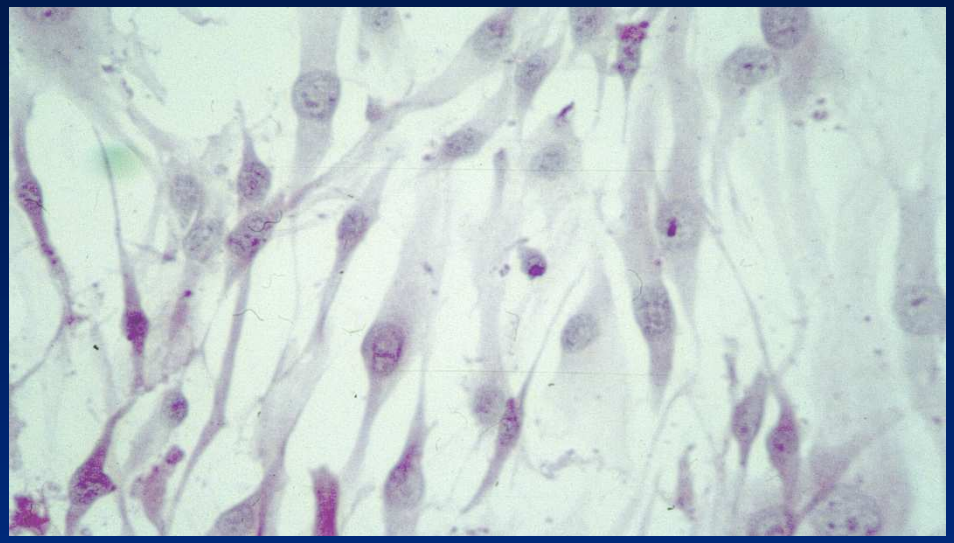
↓

Fibrocartilage → osteoarthritis


Collagen II (Control)



Chondrocytes p=0



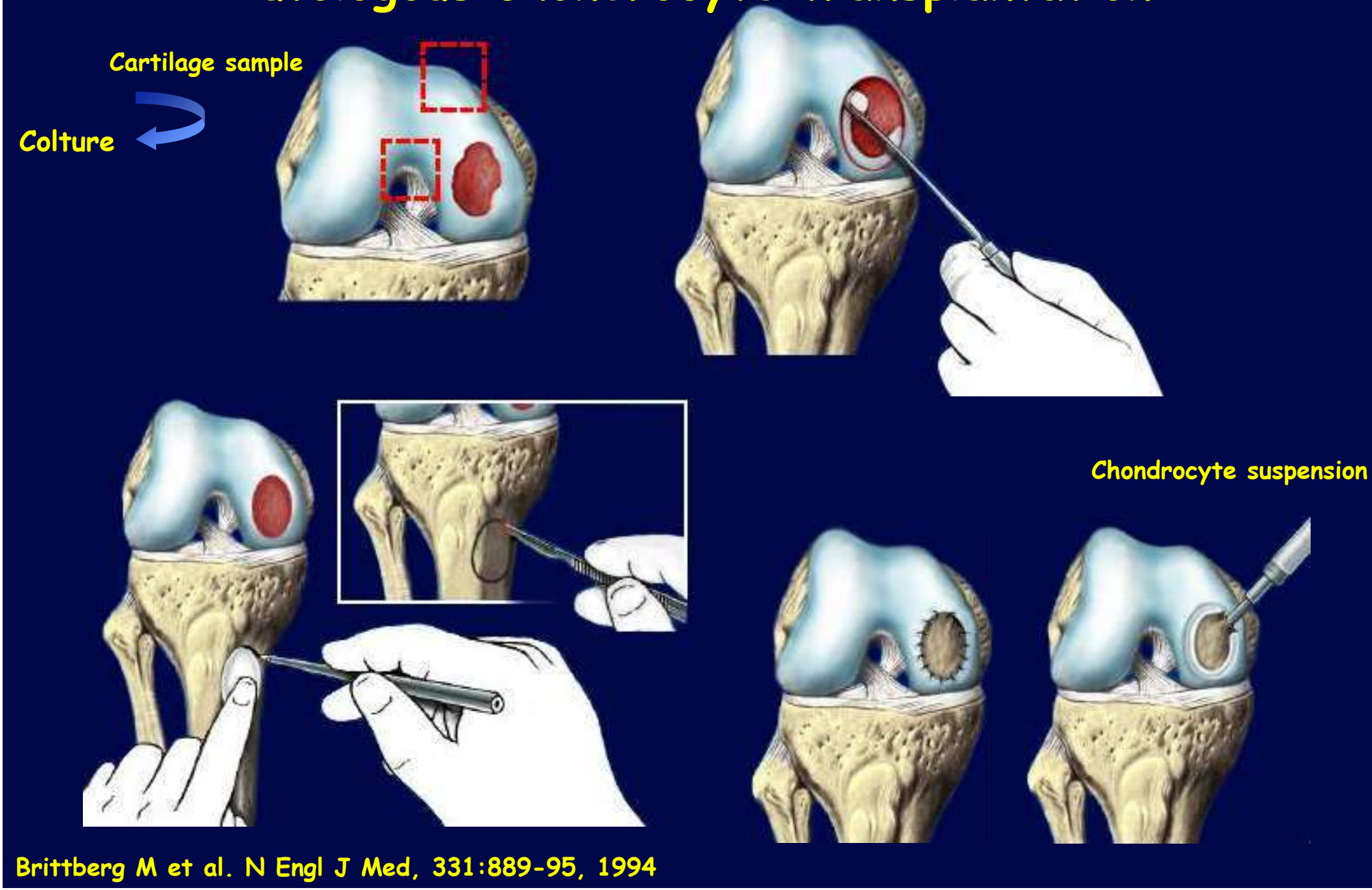
Chondrocytes p=3/4/5/6……..

↑ Expression of Collagen II
↑ Proteoglycans (aggrecan)
↓ Expression of Collagen I
↑ FGF R3
↑ BMP-2

↓ Expression of collagen II
↑ Expression of proteoglycans (versican)
↑ Expression of Collagen I
↓ Activin receptor-like kinase 1

De-differentiation process

Autologous chondrocyte transplantation



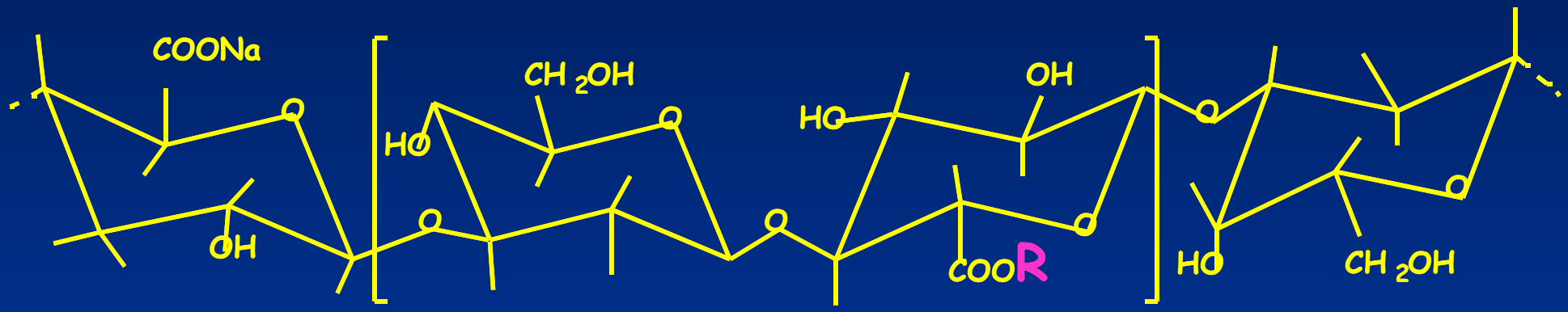
Brittberg M et al. N Engl J Med, 331:889-95, 1994

2nd generation ACT
Cells seeded on three-dimensional scaffolds

Main advantages

- ✓ Good handling properties (growth and differentia.)
- ✓ No periosteal flap required
- ✓ Suitable for large defects
- ✓ Can be applied by mini-arthrotomy or arthroscopy (reduced surgical and recovery time)

HYAFF® (FAB)



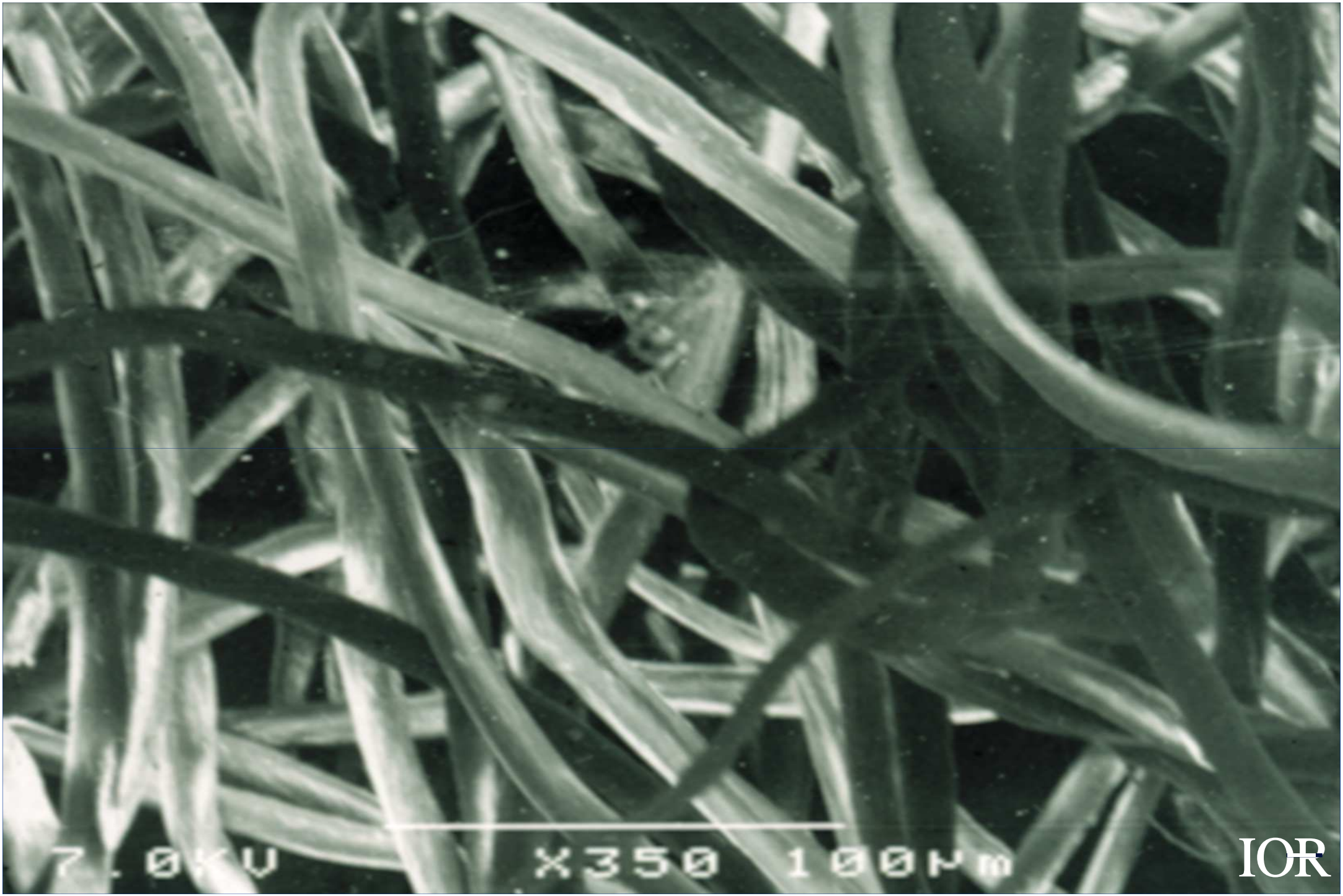
Derived from total esterification of the carboxyl groups (R) of hyaluronic acid with benzyl alcohol residue

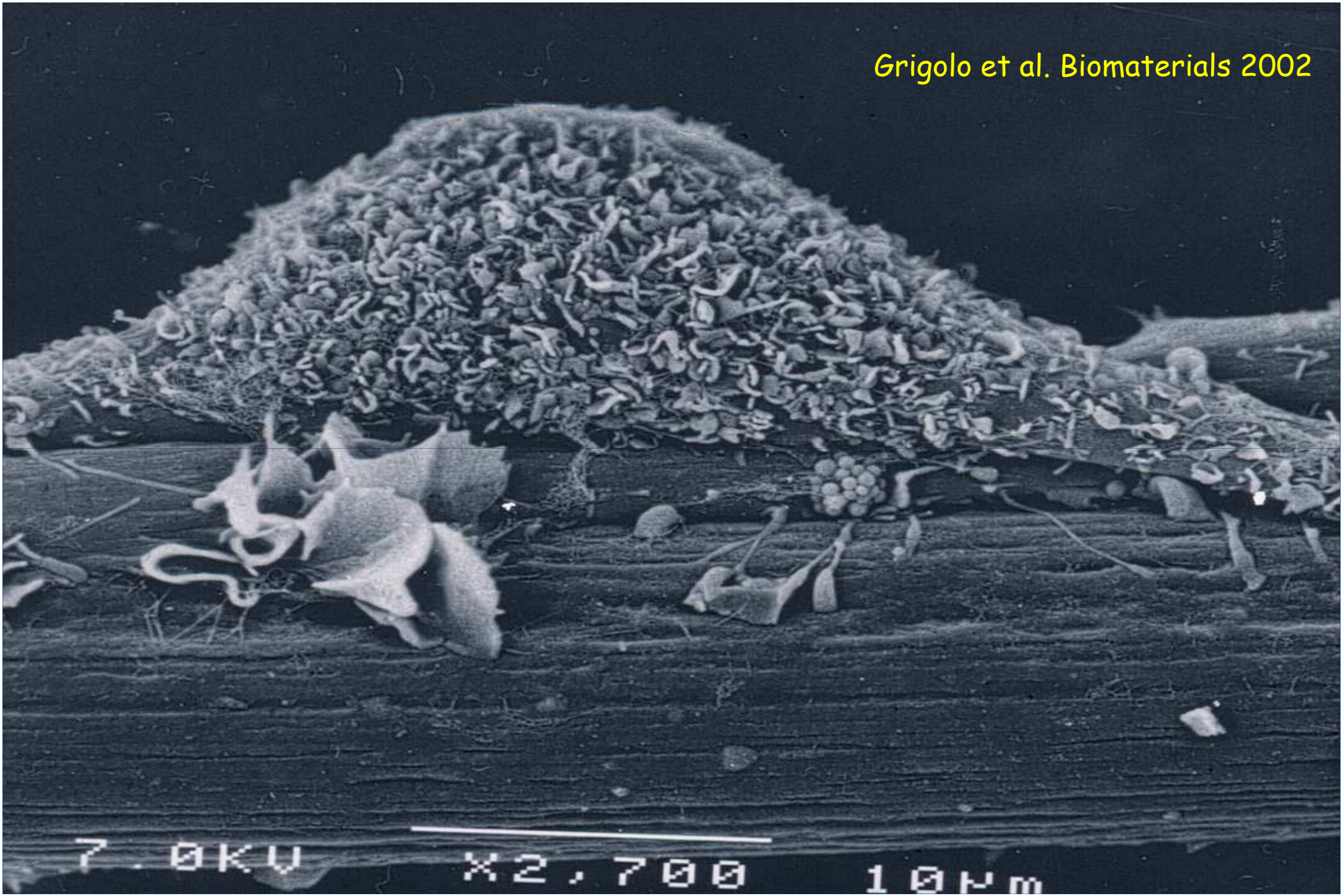
IOR

Development of a 3D scaffold for cartilage engineering

Hyaff-11 non-woven pad

- Fibre diameter: 10 μm
- Weight: 120 g/m^2
- Thickness: 2 mm


7.0kV X350 100µm


Grigolo et al. Biomaterials 2002
7.0kV X2,700 10µm

ELSEVIER

Biomaterials 23 (2002) 1187–1195

Biomaterials

www.elsevier.com/locate/biomaterials

Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAFF®11): molecular, immunohistochemical and ultrastructural analysis

Brunella Grigolo[a], Gina Lisignoli[a], Anna Piacentini[a], Mauro Fiorini[a], Pietro Gobbi[b], Giovanni Mazzotti[b], Manuela Duca[c], Alessandra Pavesio[c], Andrea Facchini[a,d,*]

[a] *Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti, Istituti Ortopedici Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy*
[b] *Unità Complessa di Scienze Anatomiche Umane e Fisiopatologia dell'Apparato Locomotore, Università di Bologna Via Irnerio 48, 40126 Bologna, Italy*
[c] *Fidia Advanced Biopolymers, Via Ponte della Fabbrica 3/A, 35031 Abano Terme, Padova, Italy*
[d] *Dipartimento di Medicina Interna e Gastroenterologia, Università di Bologna, Via Massarenti 9, 40138 Bologna, Italy*

Received 2 January 2001; accepted 22 June 2001

ELSEVIER

Biomaterials 22 (2001) 2417–2424

Biomaterials

www.elsevier.com/locate/biomaterials

Transplantation of chondrocytes seeded on a hyaluronan derivative (Hyaff®-11) into cartilage defects in rabbits

Brunella Grigolo[a], Livia Roseti[a], Mauro Fiorini[a], Milena Fini[b], Gianluca Giavaresi[b], Nicolò Nicoli Aldini[b], Roberto Giardino[b], Andrea Facchini[a,c,*]

[a] *Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla-Putti, Istituti Ortopedici Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy*
[b] *Servizio di Chirurgia Sperimentale, Istituto di Ricerca Codivilla-Putti, Istituti Ortopedici Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy*
[c] *Dipartimento di Medicina Interna e Gastroenterologia, Università degli Studi di Bologna, Via Massarenti 9, 40138 Bologna, Italy*

Received 10 July 2000; accepted 7 December 2000

Autologous chondrocyte transplantation by the use of Hyaff-11 scaffold (Hyalograft C)

Transplantation

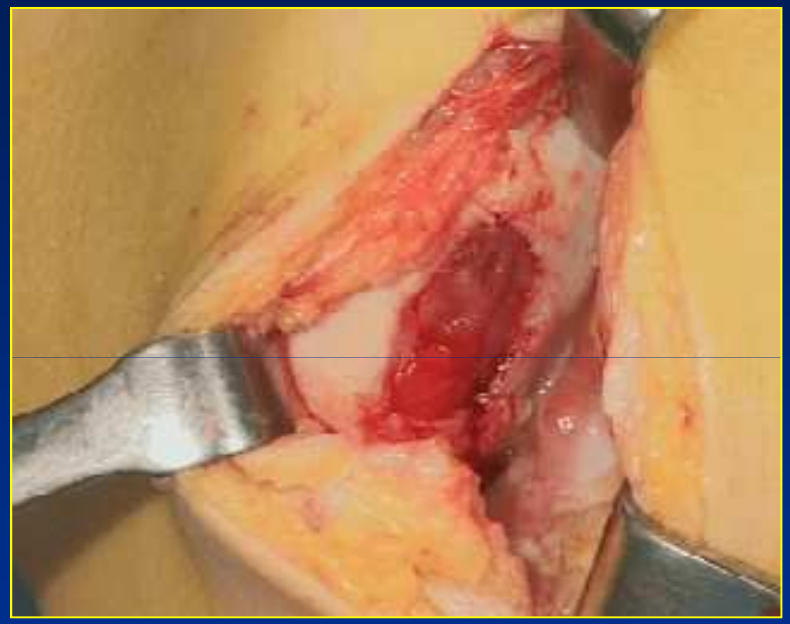
Cartilage biopsy

In vitro chondrocyte culture

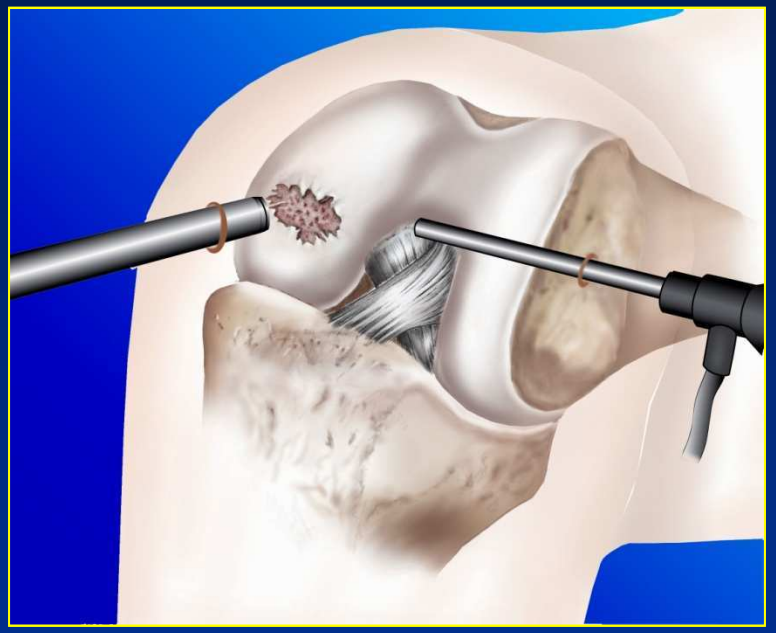
3-4 weeks

Seeding onto the biomaterial

Clinical application of a biocompatible scaffold (Hyalograft C) for cartilage defects of the knee



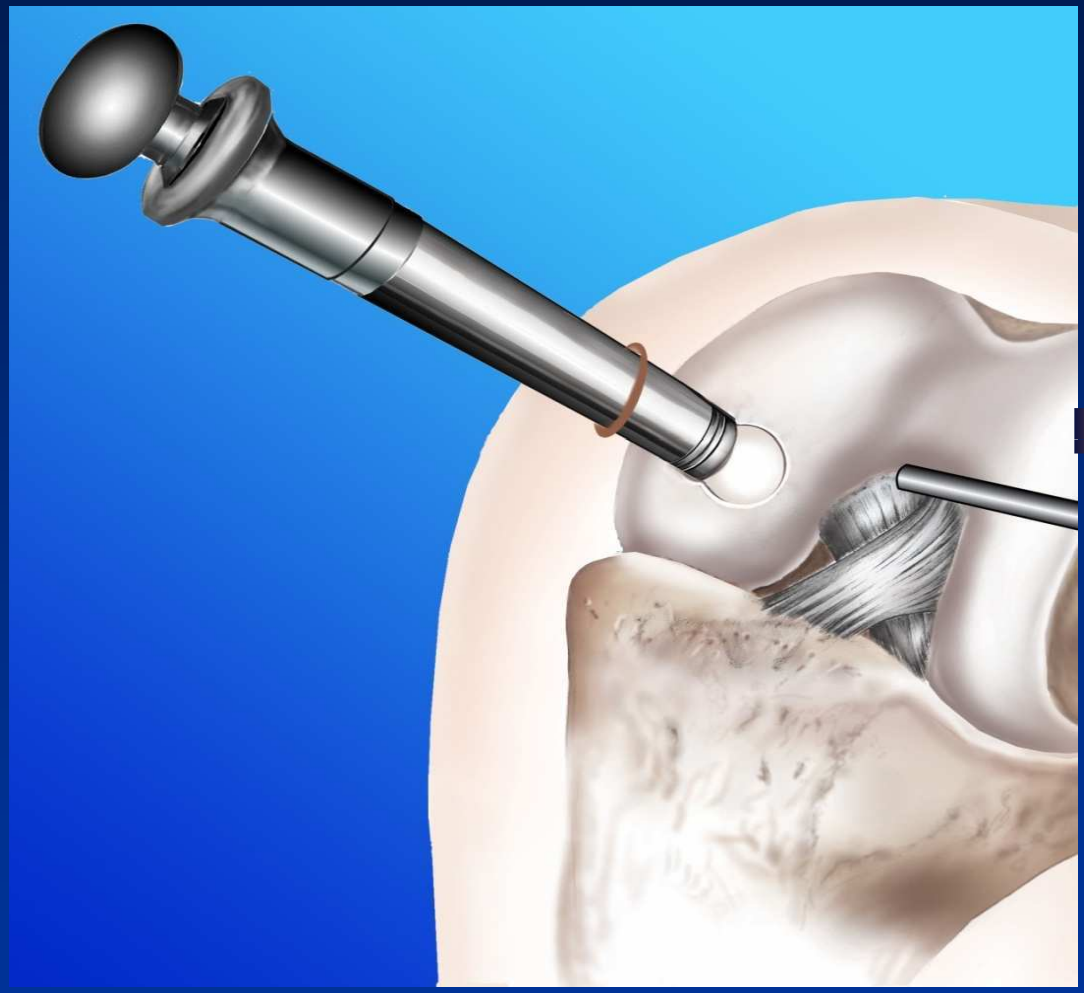
HYALOGRAFT C TRANSPLANT THROUGH MINI-ARTHROTOMY

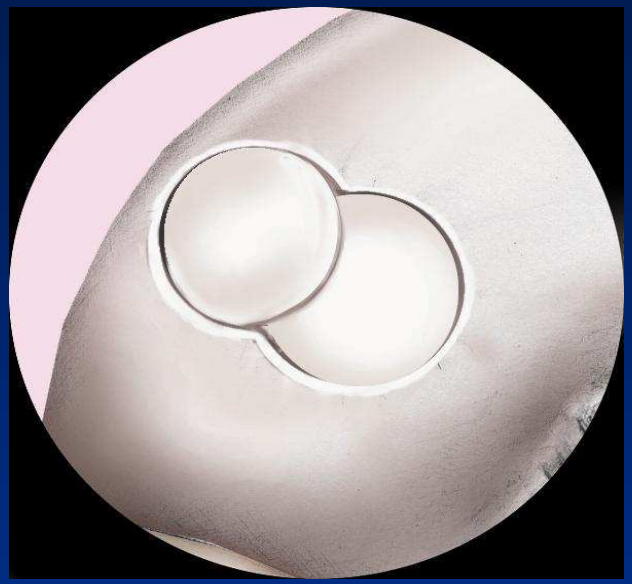


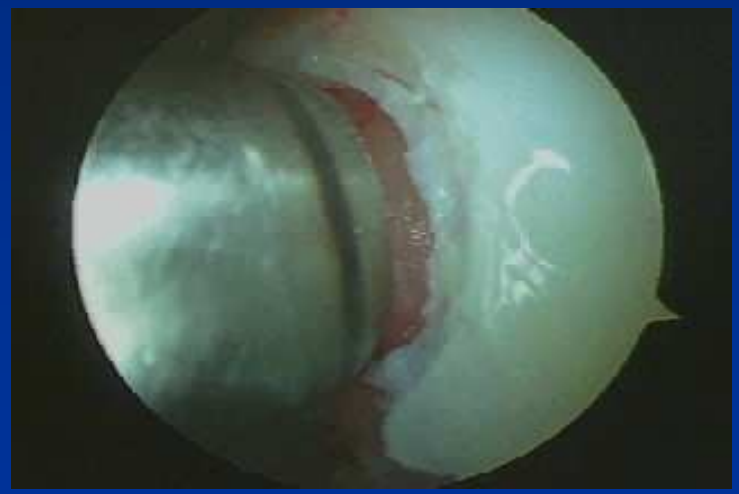
DEVELOPMENT OF ARTHROSCOPIC IMPLANT TECHNIQUE

Marcacci M et al. Knee Surg Sport Art, 2002, 2007

LESS INVASIVE NEW TECHNIQUE FOR CARTILAGE REGENERATION

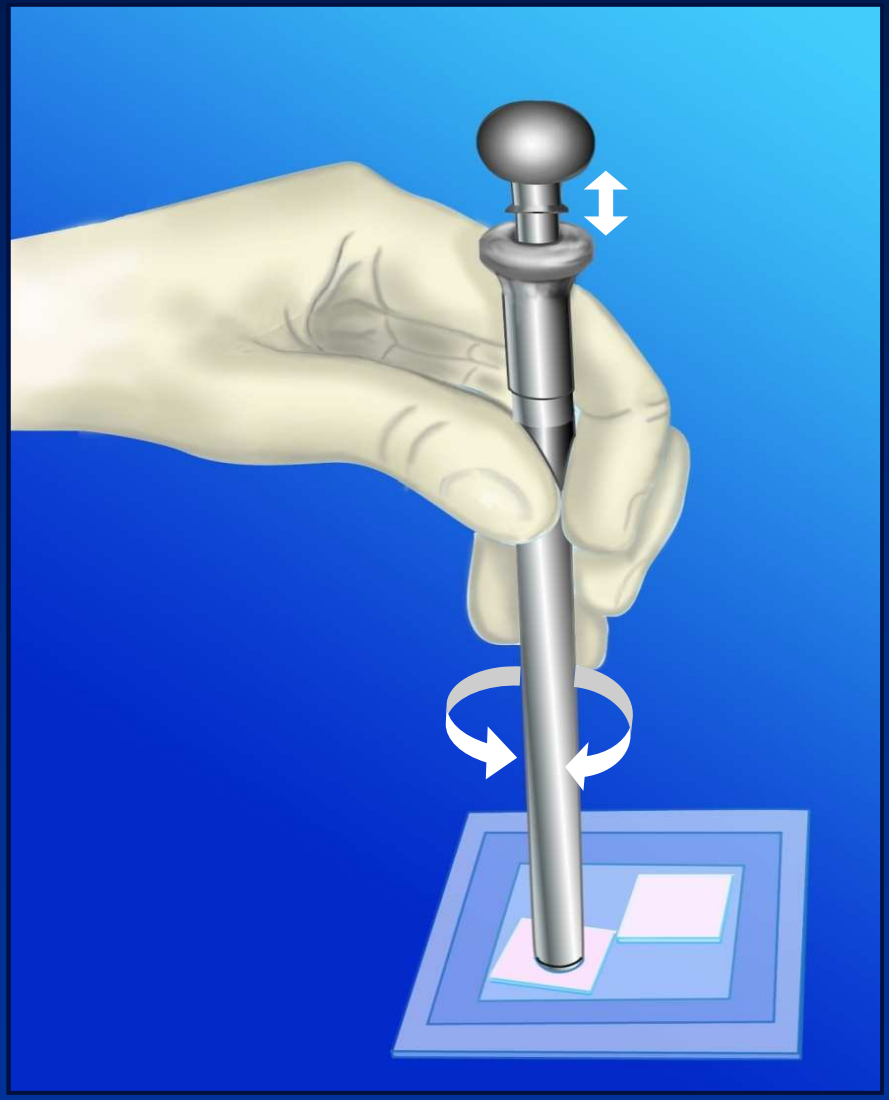


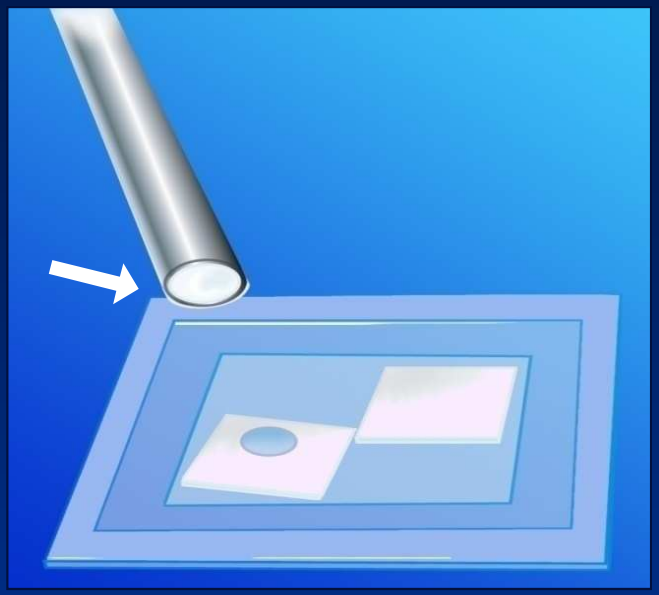




Marcacci M et al. Knee Surg Sport Art, 2002; 2007

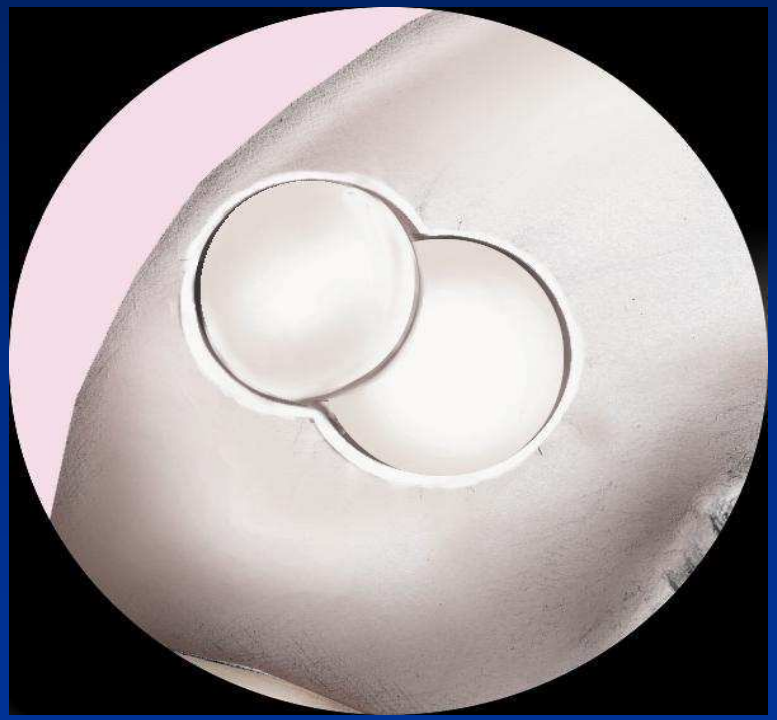
LESS INVASIVE NEW TECHNIQUE FOR CARTILAGE REGENERATION

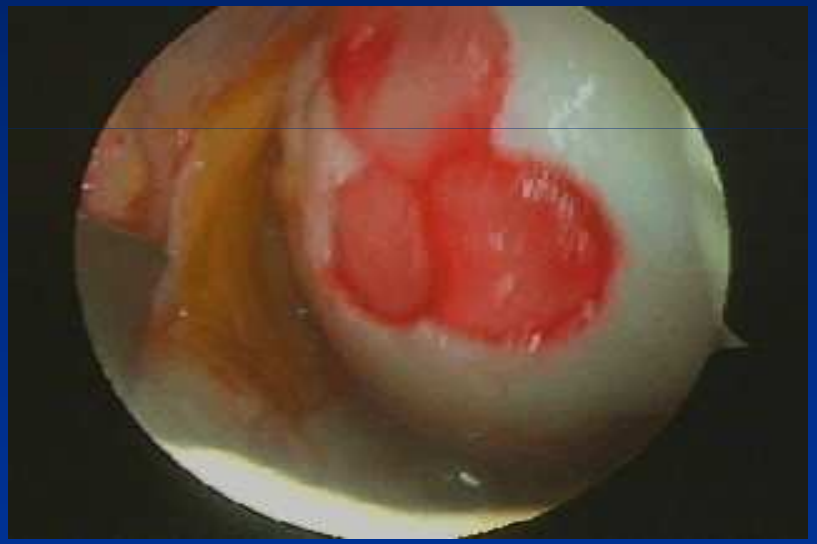




Marcacci M et al. Knee Surg Sport Art, 2002; 2007

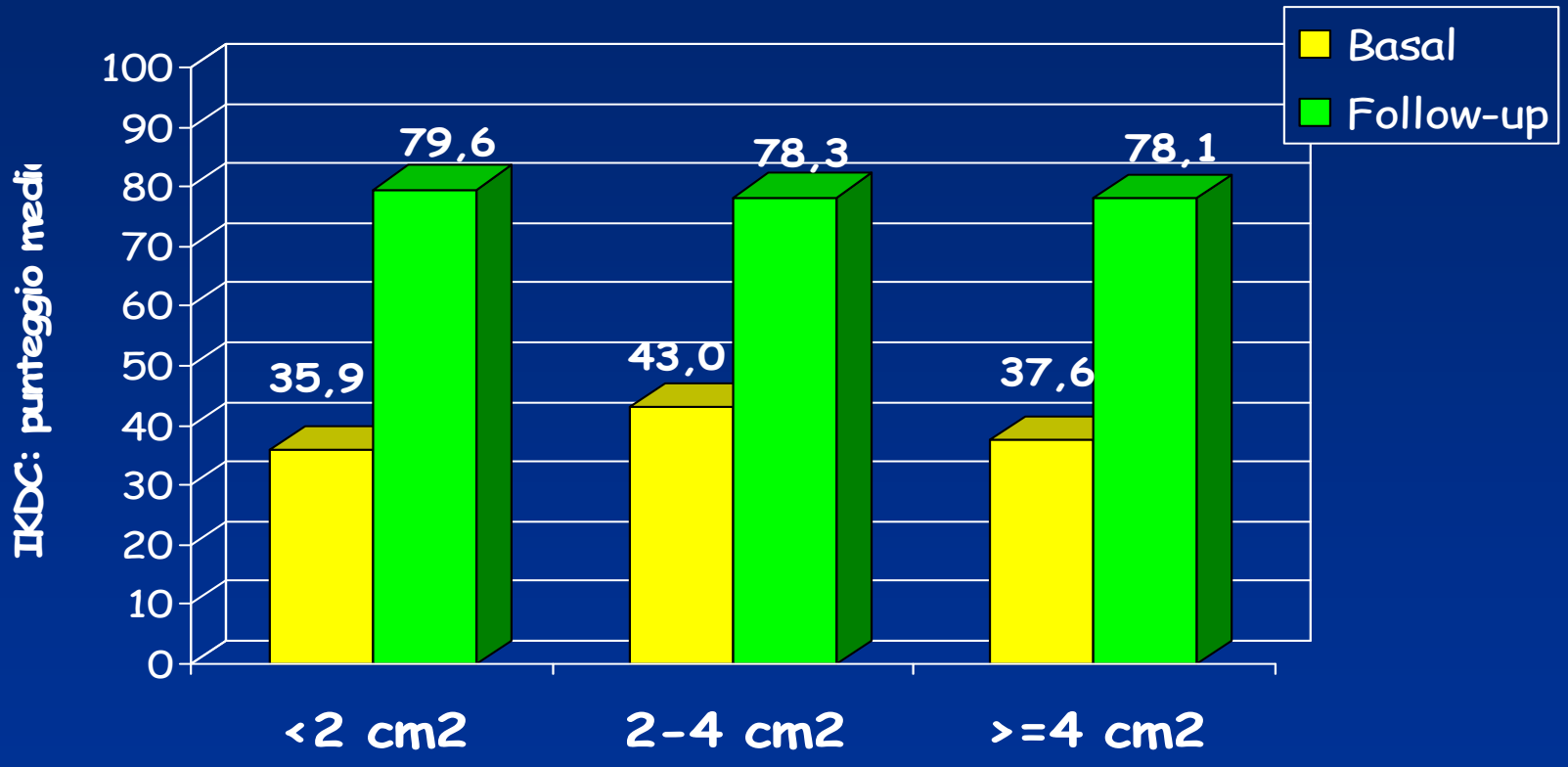
LESS INVASIVE NEW TECHNIQUE FOR CARTILAGE REGENERATION





Marcacci M et al. Knee Surg Sport Art, 2002; 2007

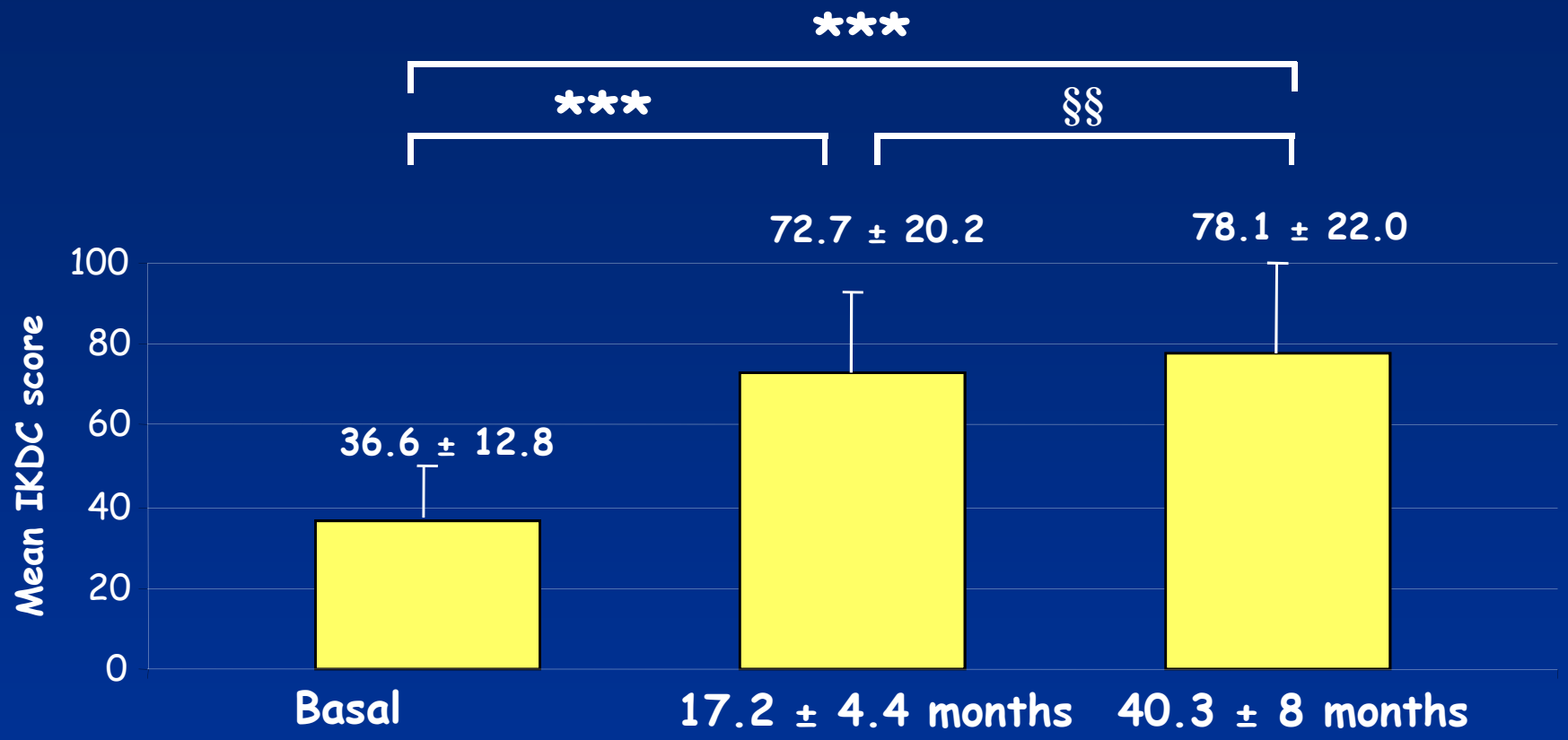
IKDC patient evaluation:
Basal vs follow-up related to lesion size



	<2 cm2	2-4 cm2	>=4 cm2
	n=42	*n=61*	*n=34*
Improved patients:	**92,9%**	**90,2%**	**91,2%**

Marcacci M .et al , CORR, 435, 96-105, 2005

Mean IKDC score vs time (n=109)

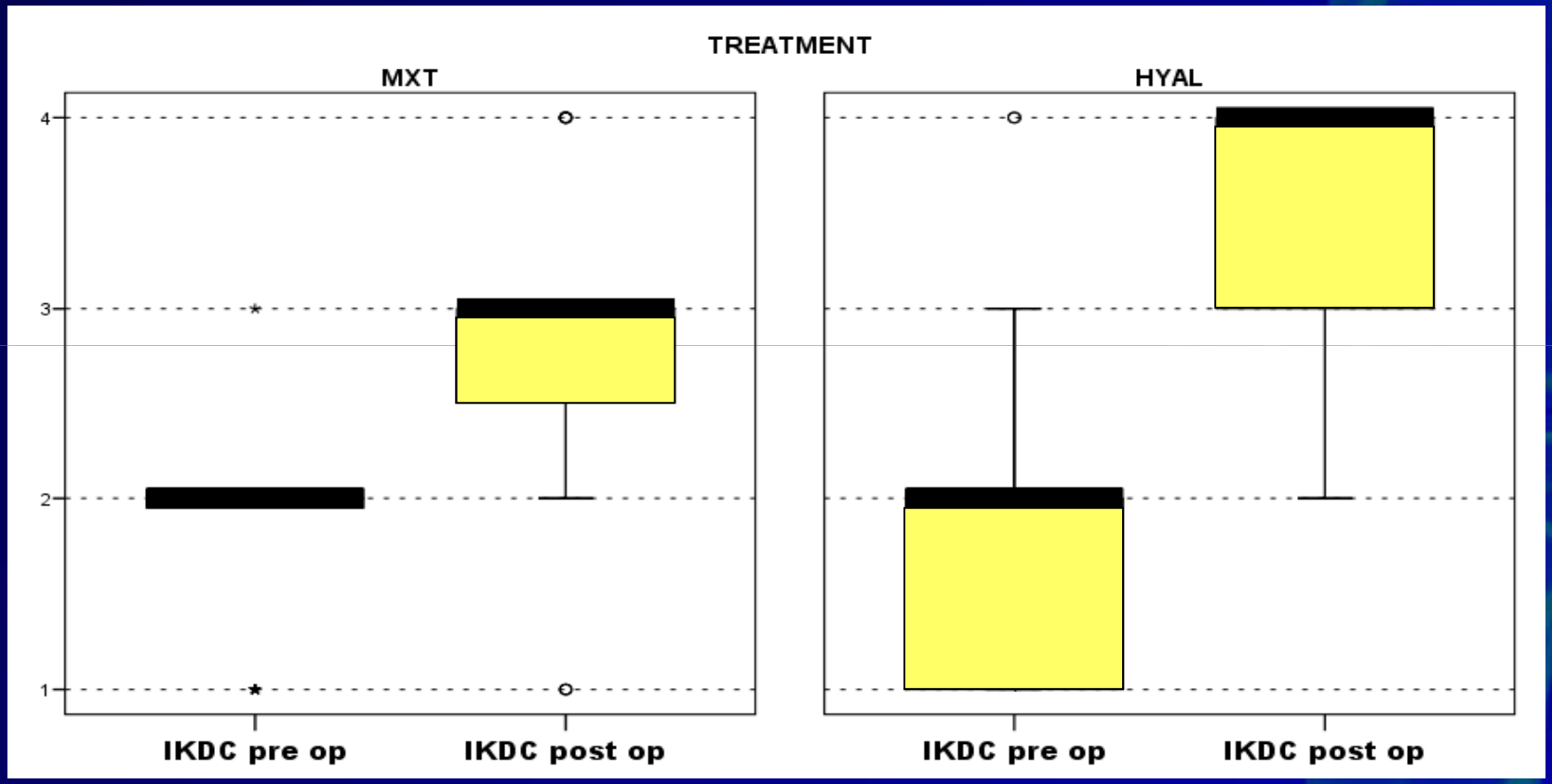


Significant diference among groups
(*** p<0.0001, §§ p< 0.005 Paired t-test)

Marcacci M. et al , CORR 435, 96-105, 2005

VALUTAZIONE OGGETTIVA IKDC a 5 ANNI

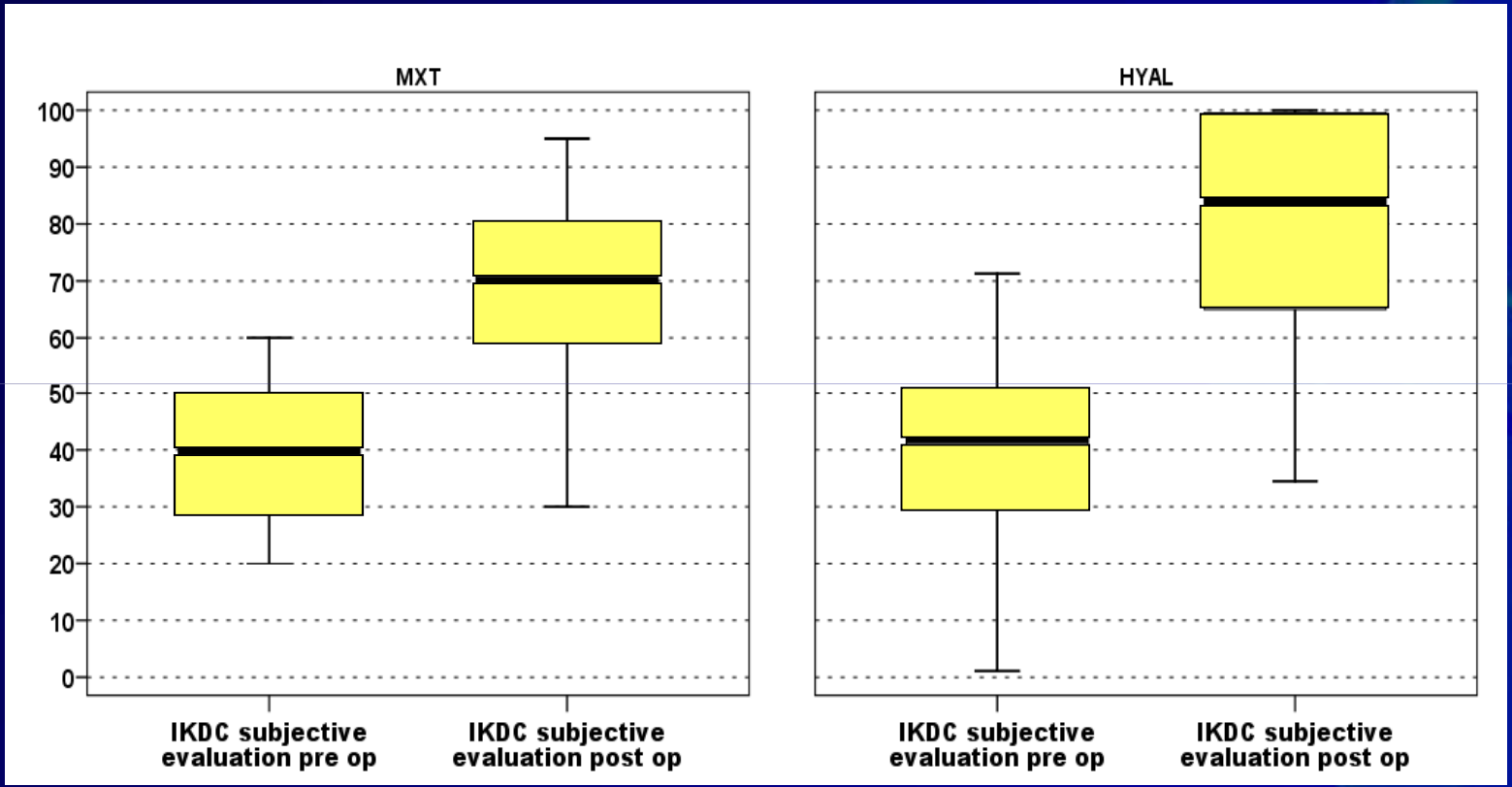
MICROFRATTURE vs HYALOGRAFT C



p<0,0005

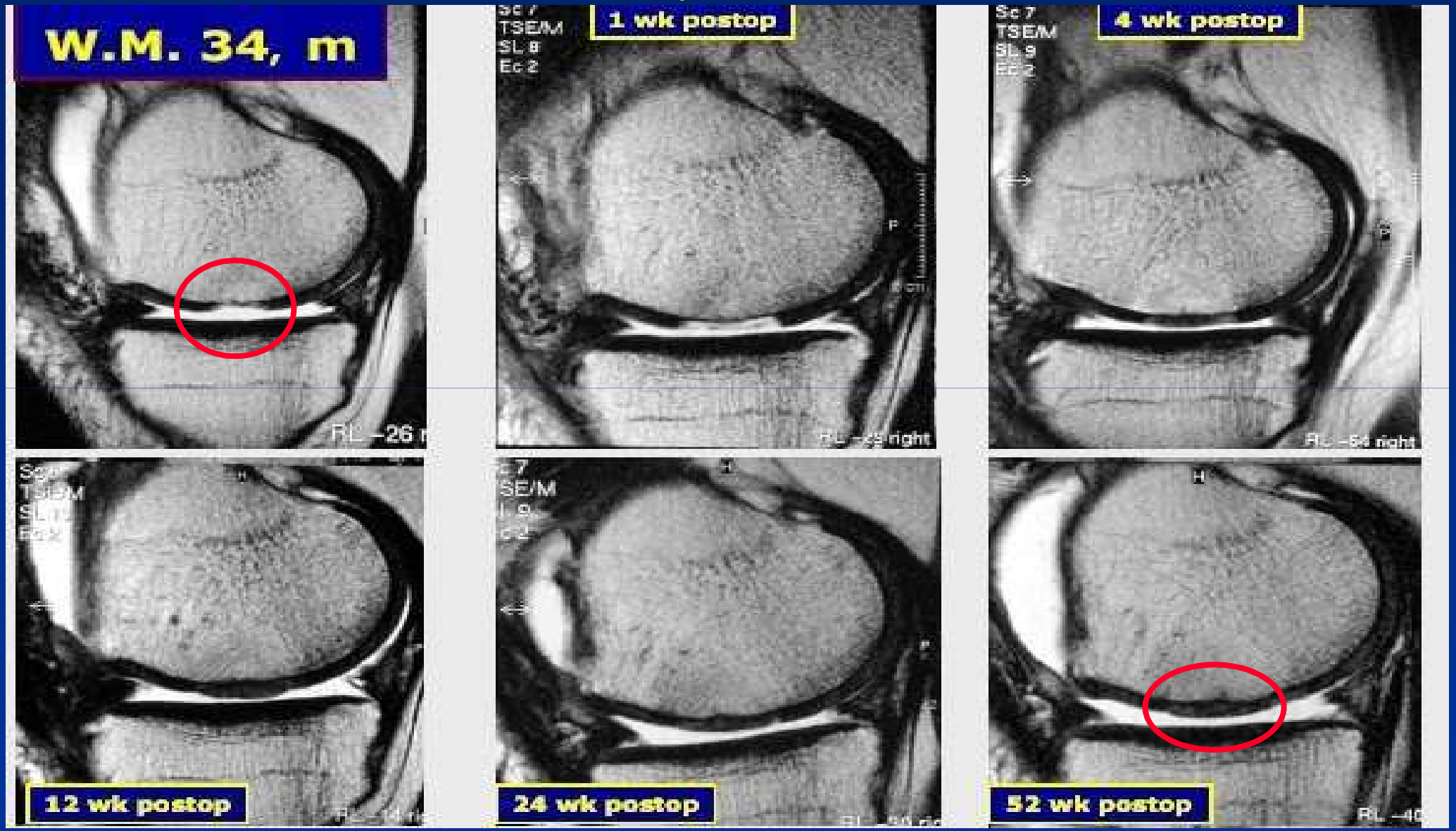
VALUTAZIONE SOGGETTIVA IKDC a 5 ANNI

MICROFRATTURE vs HYALOGRAFT C



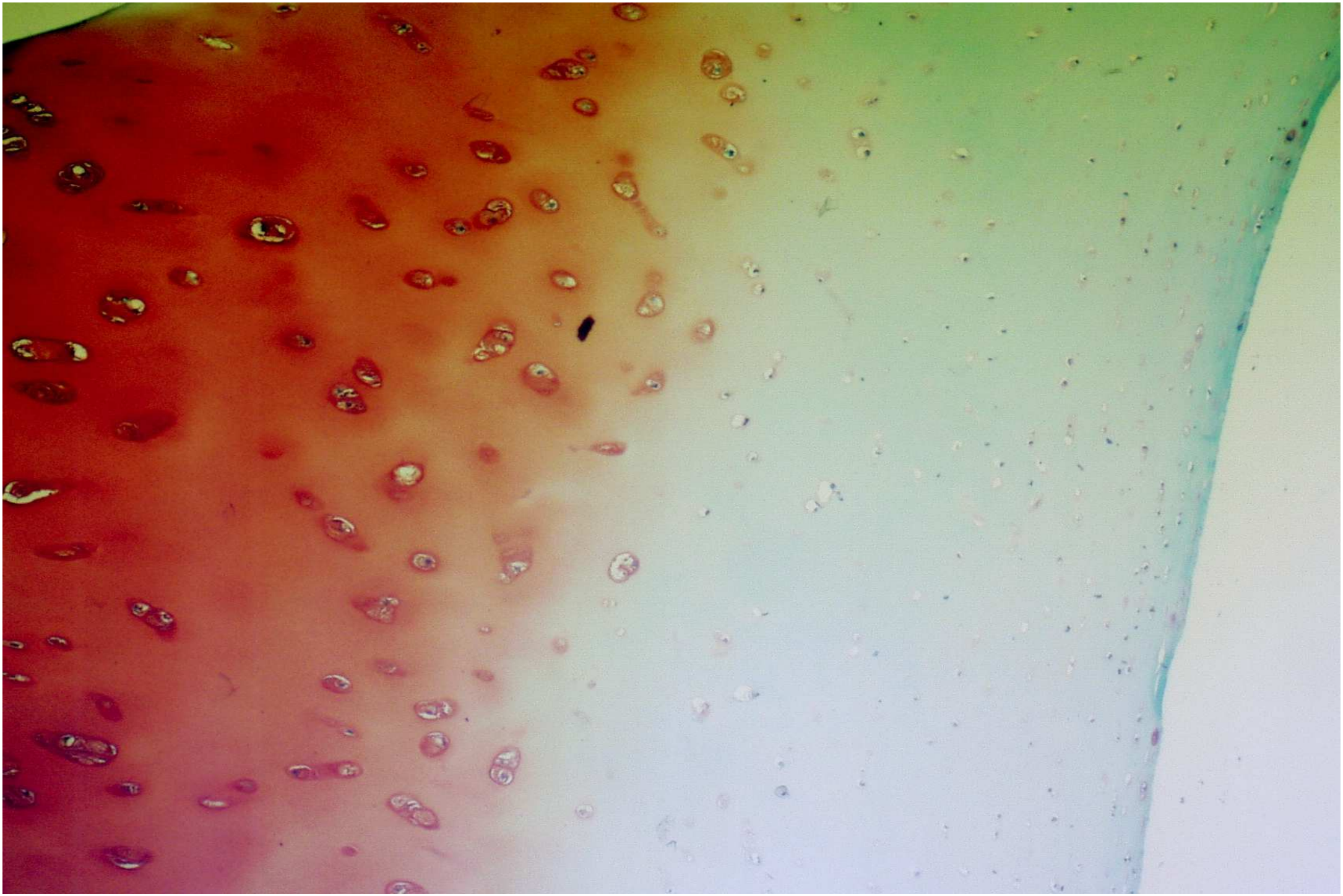
p=0,0003

Graft stability - MRI results



Cartilage maturation: columnar re-organization

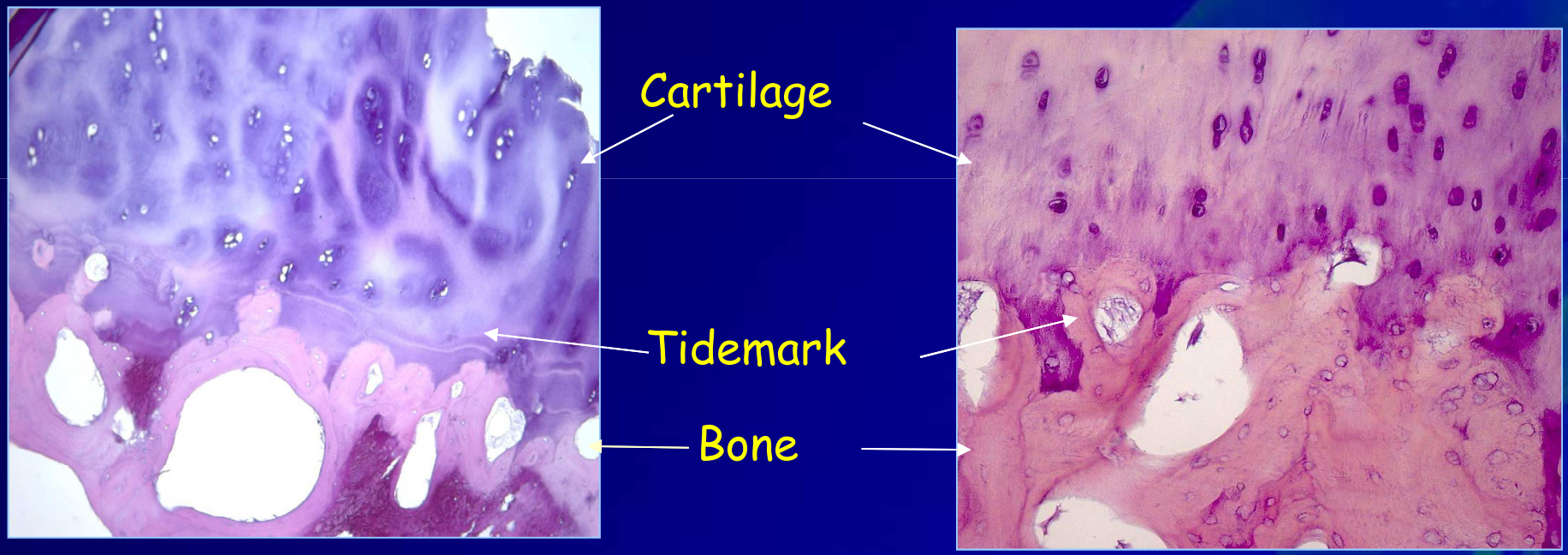
3) Columnar Organization



Integration with subchondral bone

Normal Cartilage

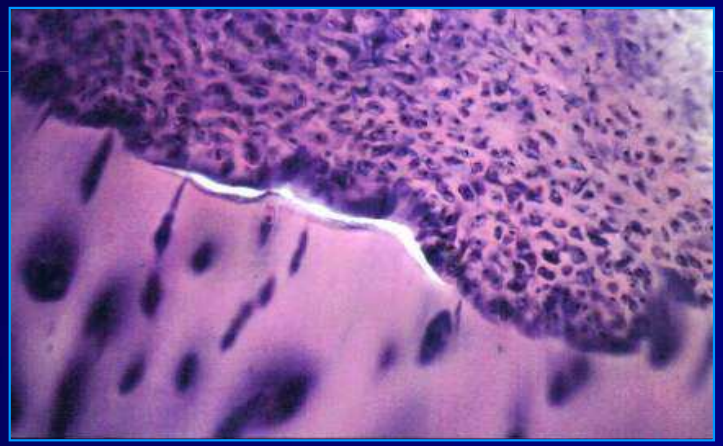
II° look Biopsy

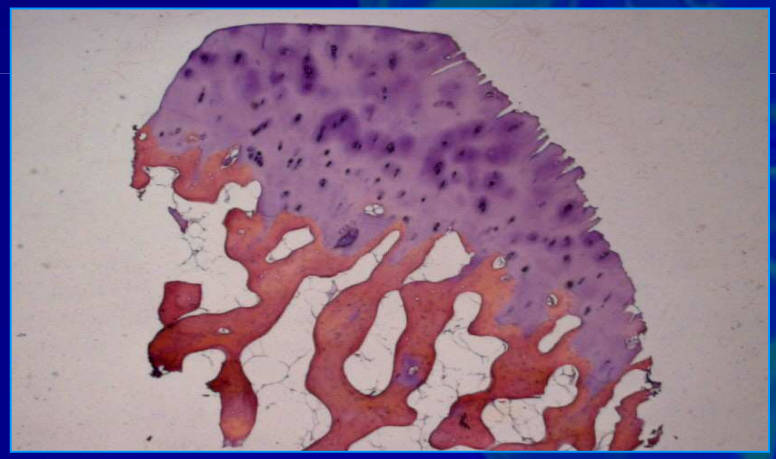


STUDIO COMPARATIVO

MICROFRATTURE vs HYALOGRAFT C

40 PAZIENTI VS 40 PAZIENTI

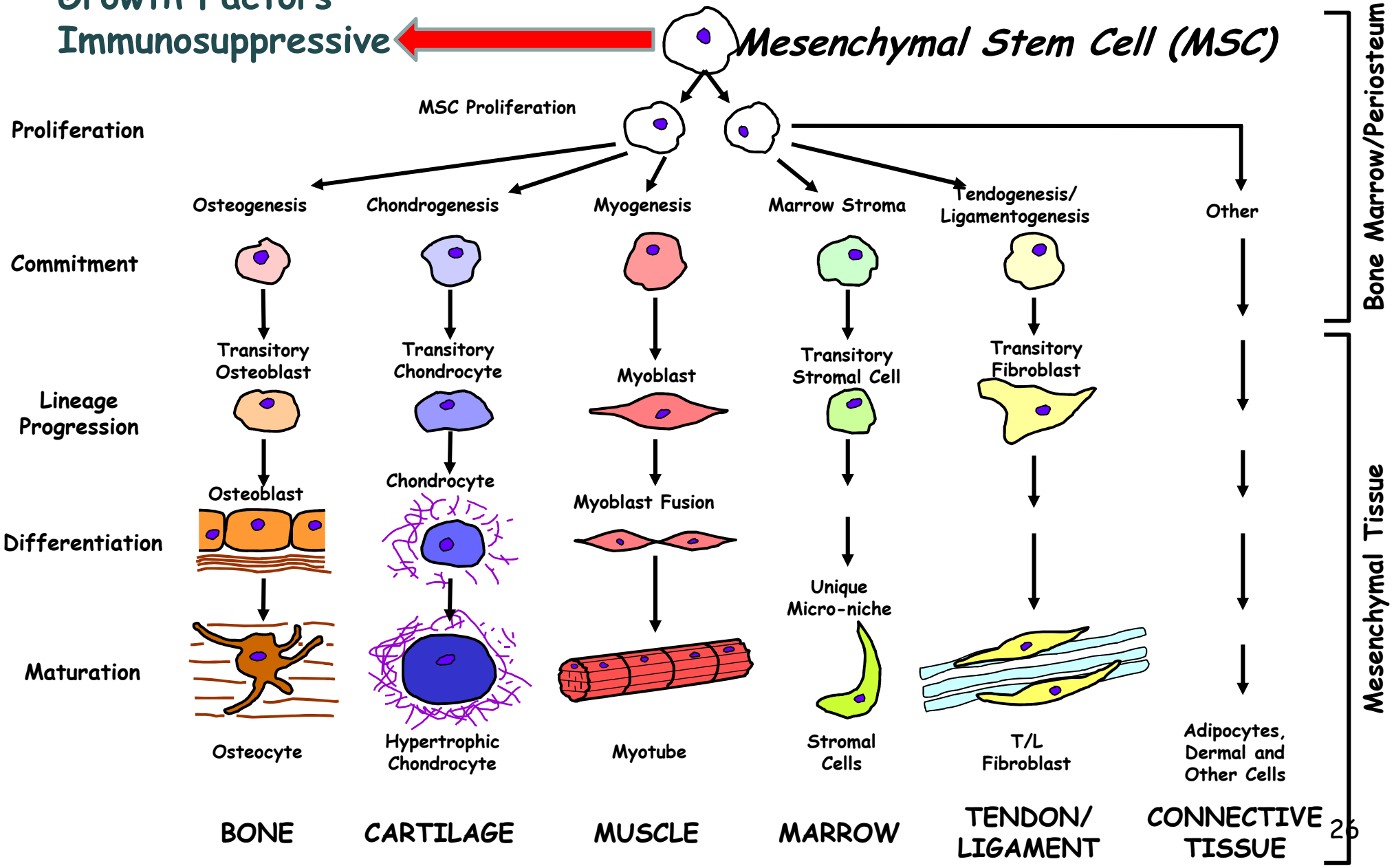




Kon E, Gobbi A, Filardo G, Delcogliano M, Zaffagnini S and Marcacci M
Arthroscopic Second Generation Autologous Chondrocyte Implantation Compared with Microfracture treatment for chondral lesions of the Knee.
Am J Sport Med, 2009

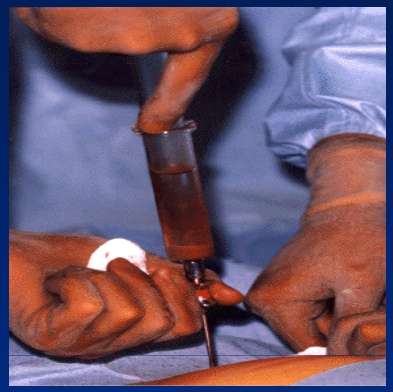
The American Journal of Sports Medicine
www.ajsm.org

THE MESENGENIC PROCESS



Main sources for human MSC, e.g.:

Bone Marrow



Pittenger M, et al.
Science 1999

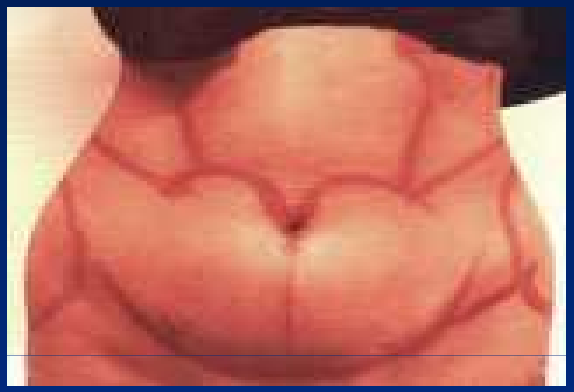
\+

Best characterised
(Pre-) Clinically used

\-

Invasive procurement
Age related detriments

Adipose Tissue



Zuk P, et al.
Tissue Eng. 2001

\+

High MSC Frequency
(Pre-) Clinically used

\-

Invasive procurement
Age related detriments ?

Umbilical Cord Blood/ Amnios/Placenta

Erices AA et al.
Brit. J. Haematol. 2000

\+

Youngest Adult Stem Cells
Easily Accesible

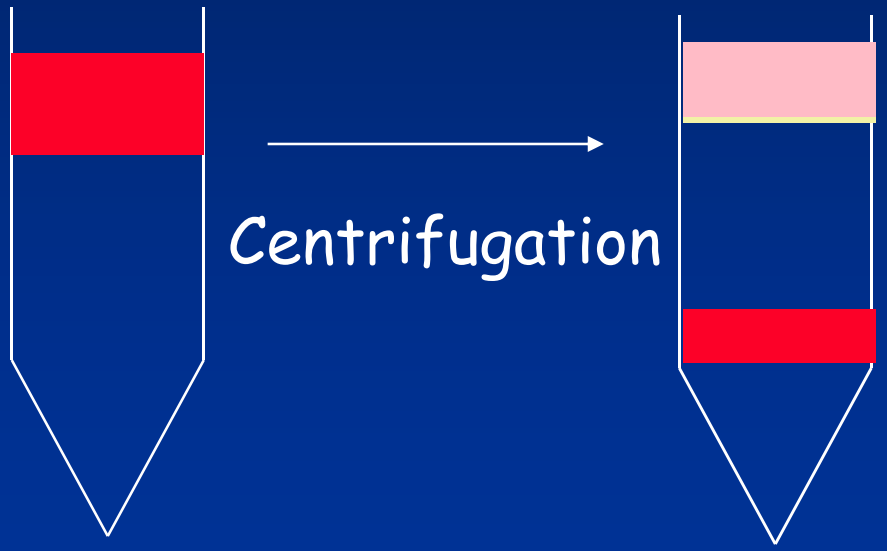
\-

MSC present in term CB?
Low frequency
Fresh UCB units needed

From: K.Bieback ,2007

H-MSC s isolation and culture expansion

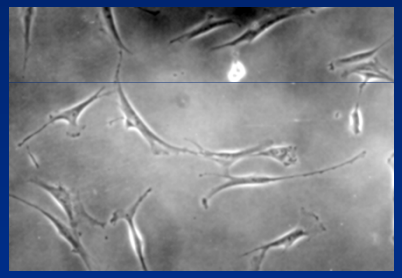
Bone marrow aspirate



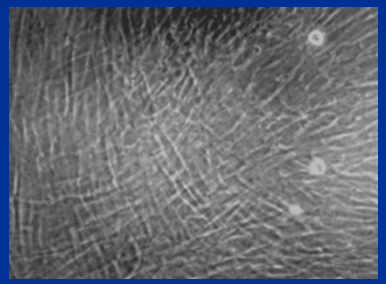
1.073g/ml Percoll

Centrifugation

Plate cells at interface → Colony formation



Primary Culture



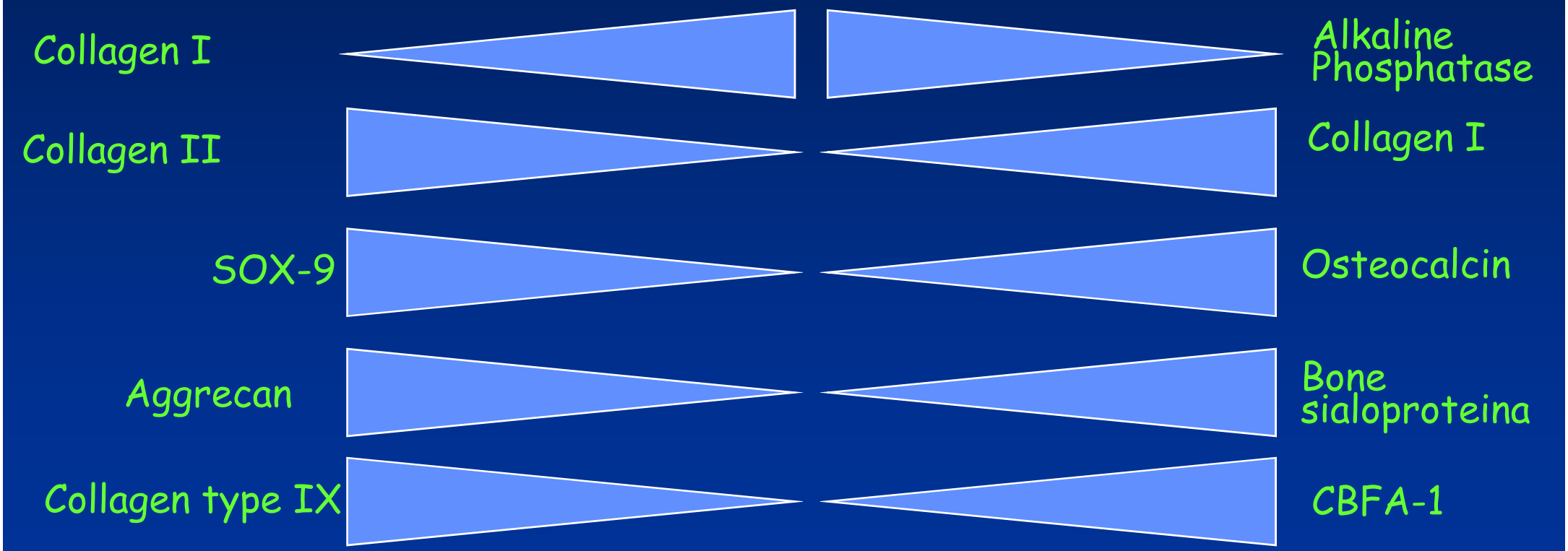
hMSCs markers

- There is not a single marker for their identification
- They are generally positive for: CD29 (fibronectin R), CD44 (hyaluronic acid R), CD73/SH3 (Ecto-5-endonuclease), CD90 (Thy-1,HSC) CD105/SH2 (endoglin ,TGFβ1-3R),; CD147 (neurotelin), CD166, CD271 (NGFR) and STRO-1.
- They are generally negative for: CD14 (MØ), CD31 (endotelials cells), CD34 (HSC), CD45 (CLA), CD117(SCFR).

Minimal ITSC criteria for MSC identification

Mariani E ,Facchini A Curr.Pharm. Des. 2012

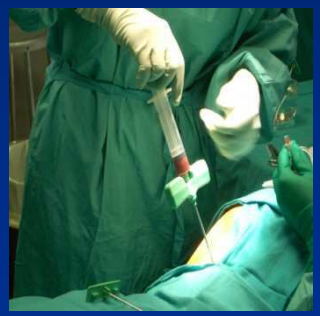
Gene expression during MSC differentiation

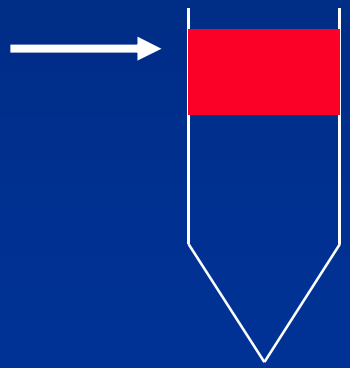


Procedure "one step": BMMSCs

All procedures are performed during surgery

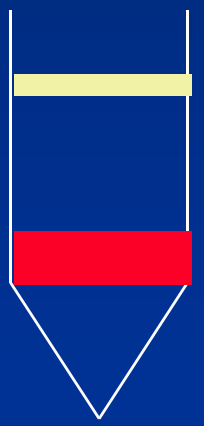
Eg: BM /Fat

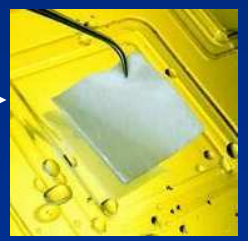




Density gradient

Enriched Mononuclear cell fraction





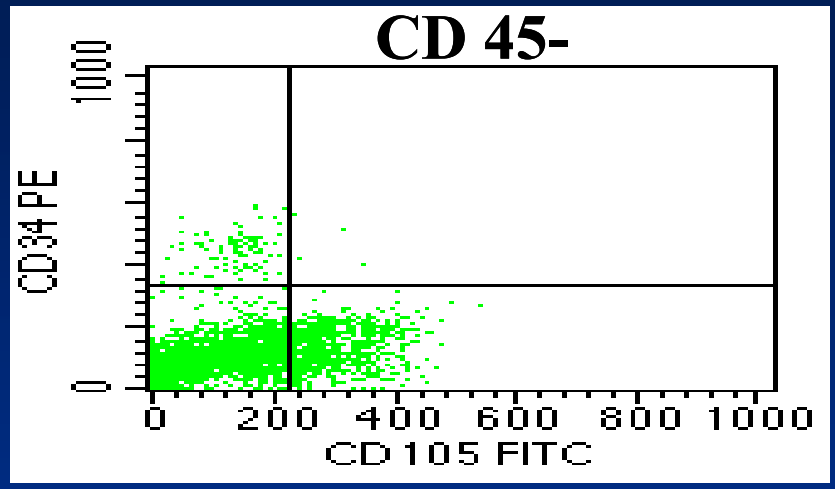
Loading on biomaterial

Implantation

CELL PHENOTYPE AND DIFFERENTIATION

FACS Analysis

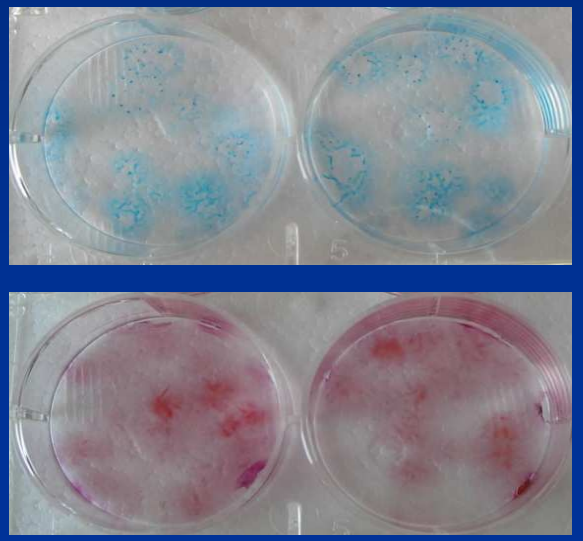
CD 34-/ CD45- /CD 105+



In vitro differentiation potential

Chondrogenic

Osteogenic

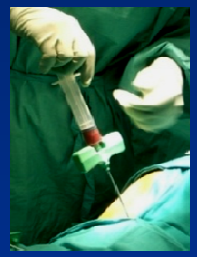


IOR

"One step" procedure

Bone marrow concentrated stem cells + scaffold (Hyaff 11)

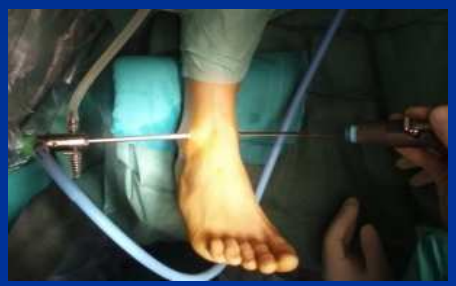
1) Platelet gel production (growth factors)



2) Marrow concentration

(dedicated centifuge)

3) Concentrated BM Stem cells loaded on biomaterial

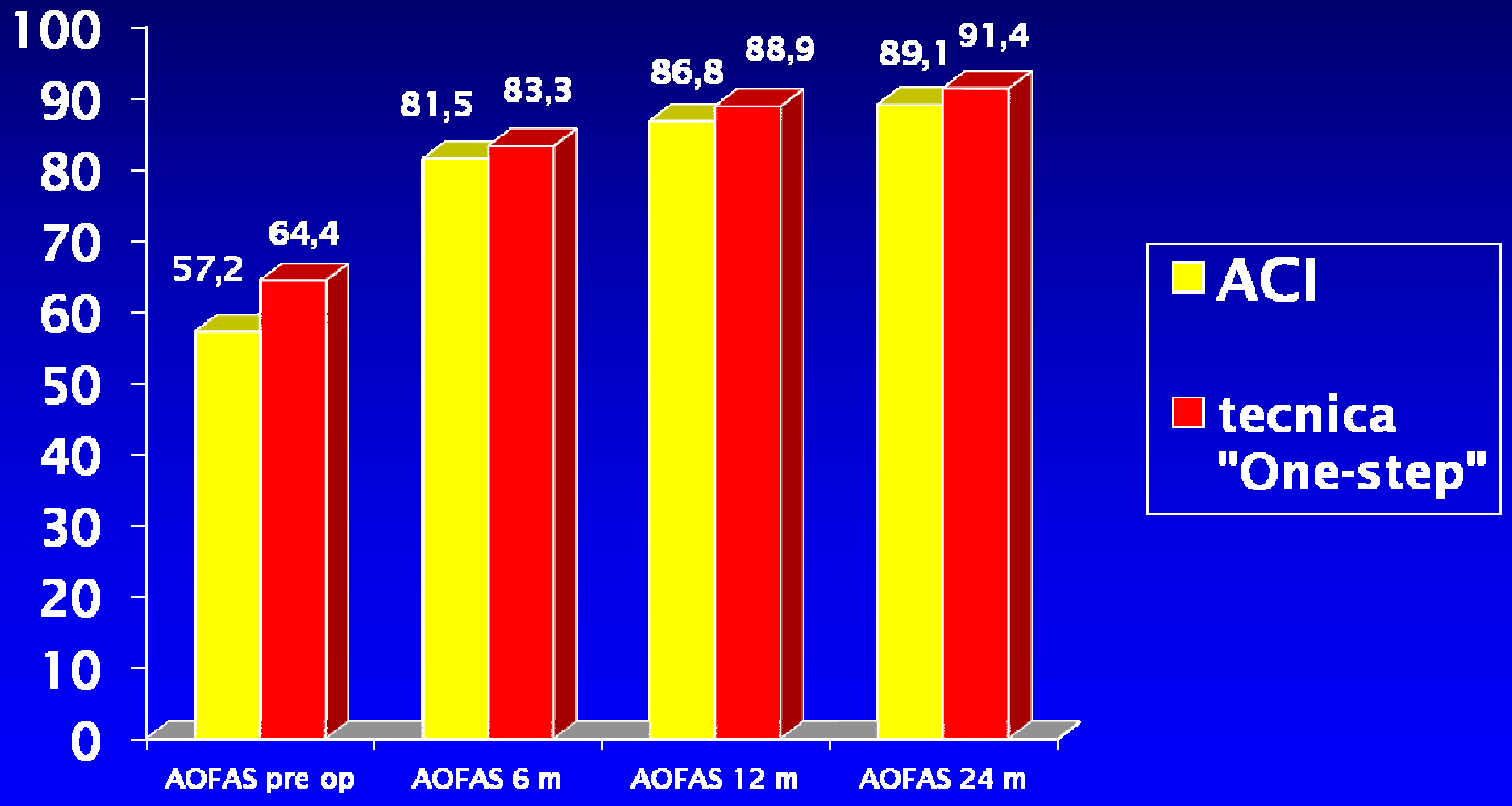


4) Arthroscopic implant

IOR

ACI vs "One step" procedure

Similar trend of improvements



93 patients with 6 months minimum FU (all cases 112)

Buda R. JBJS 2010;
Giannini S. Injury 2010

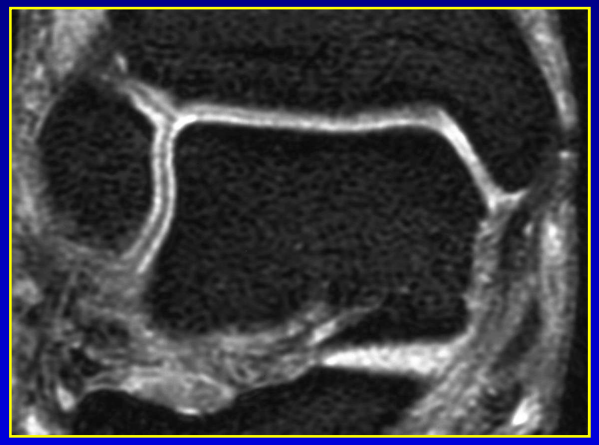
IOR

FU evaluation in "One step"

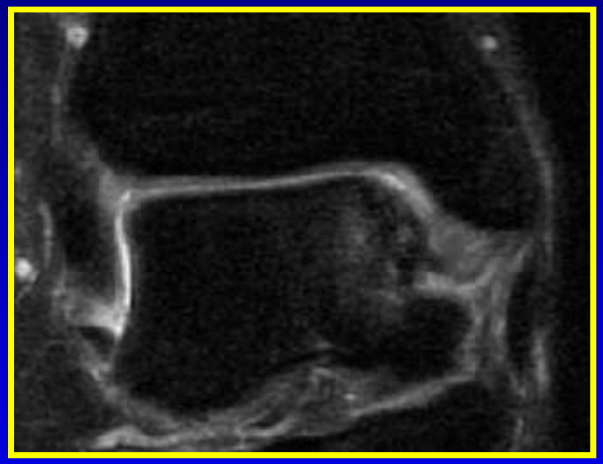
MRI T2 mapping

Multiecho sequence to evaluate the presence of water in cartilage according to colour scale

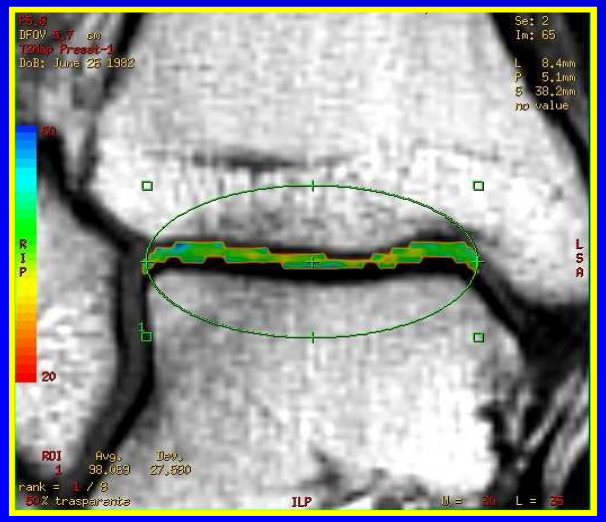
normal cartilage (3D SPGR)



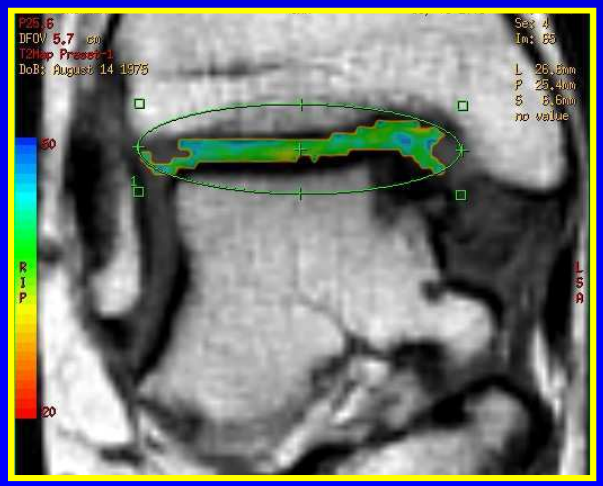
ONE-STEP, 18 Months FU



T2-mapping: 45 msec mean



HighT2 (45 msec) areas hyline cartilage expression



Battaglia E J.Radiol.2011

Histology

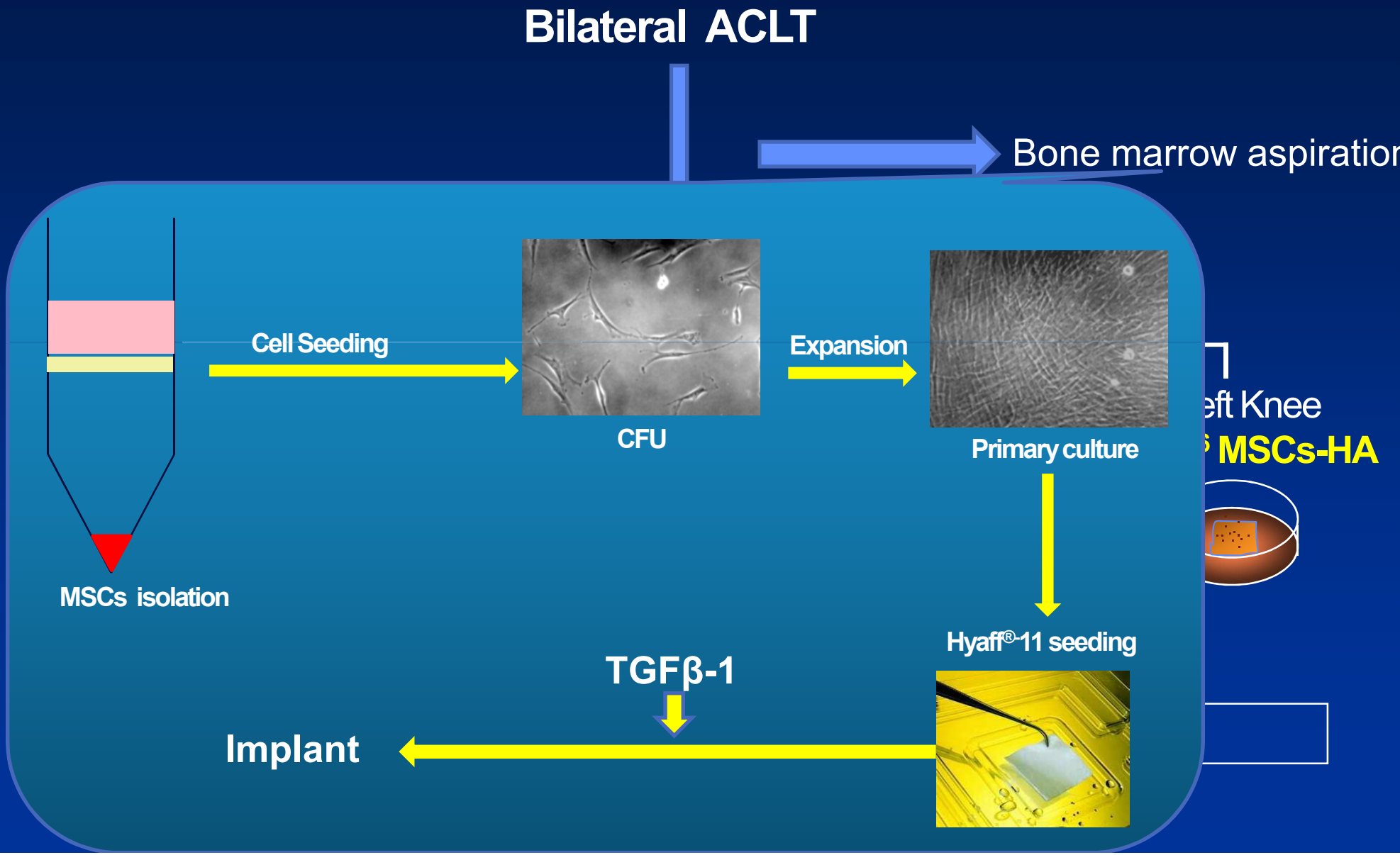
Safranin O staining

Hyaline like cartilage in remodelling
High proteoglycan expression (red)
Low evidence of Col I
Good osteochondral integration

Buda R. JBJS 2010

Can MSC treatment be useful in osteoarthritis ?

EXPERIMENTAL MODEL



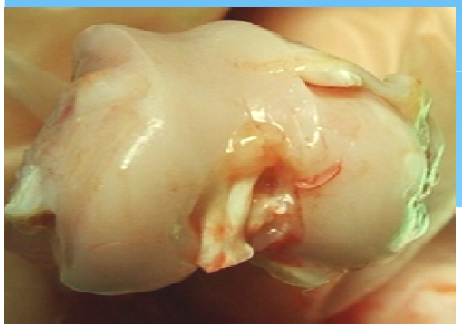
Experimental rabbit model

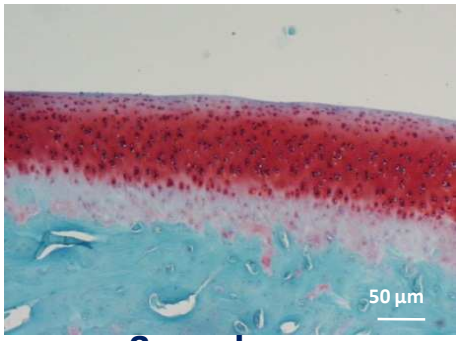
ANTERIOR CRUCIATE LIGAMENT TRANSECTION (ACLT)

New Zealand male adult rabbits

HYCR specific pathogen free

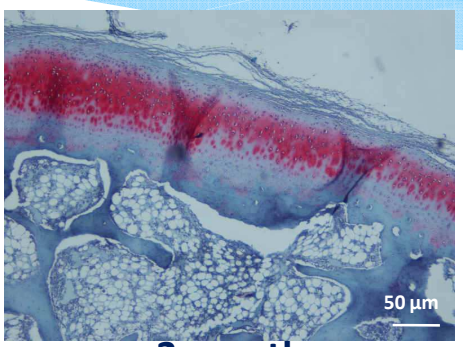
SHAM



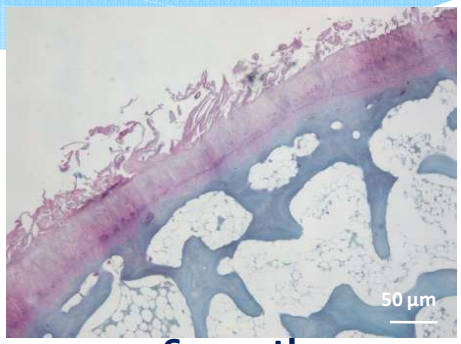


8 weeks

8 weeks



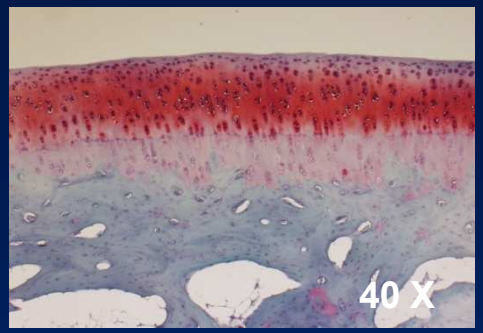
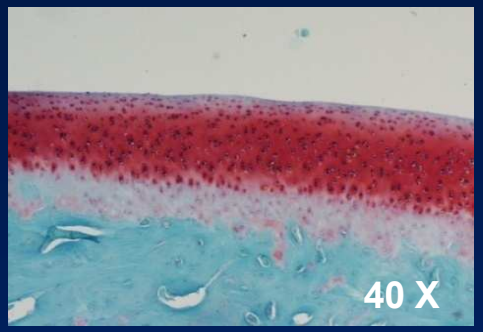
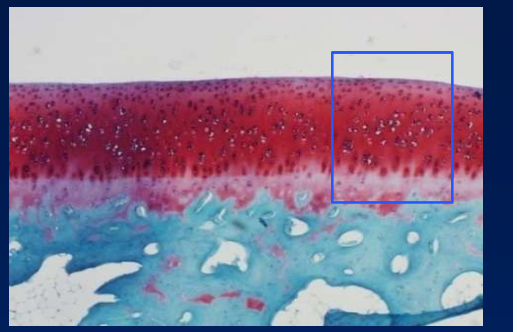
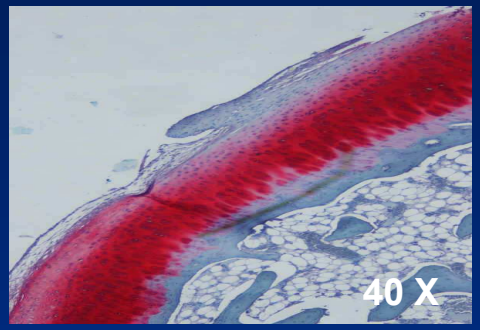
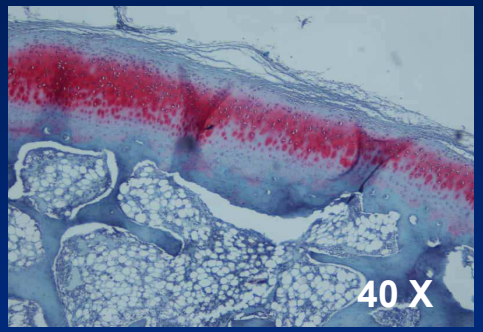
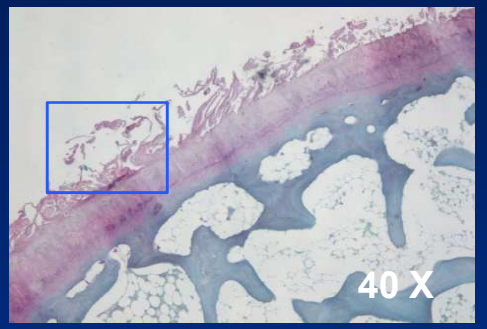
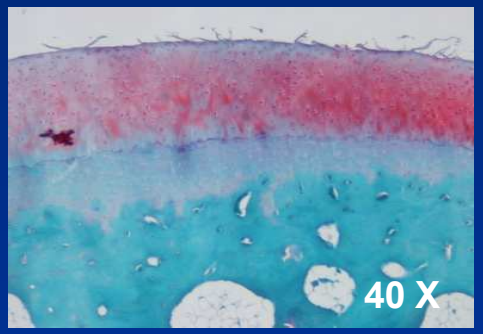
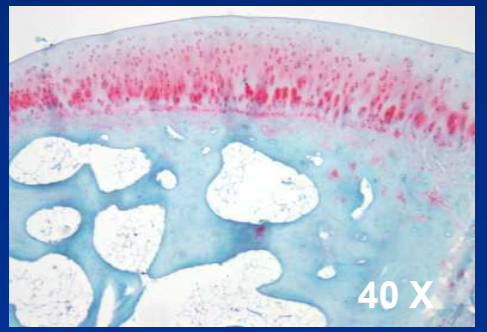
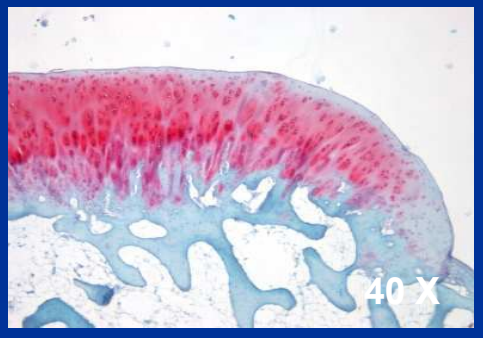
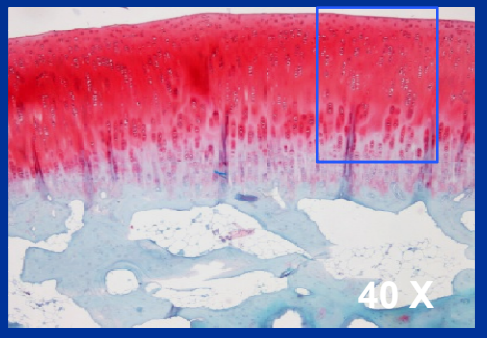
3 months



6 months

(Grigolo B. et al. Tissue Engineering, 2009)

Safranin-O

	8 weeks	3 months	6 months
Sham operation group (Control)			
ACLT (OA) group			
HA treated group			
MSCs-HA treated group			

Grigolo B. et al. Tissue Eng. 2009

Adipose derived stromal cells for osteoarthritis treatment (ADIPOA)

Collaborative Project Health-2009-1.4-3 Grant 241719
Open multi dose Phase I Clinical trial / Phase I-II
for therapeutic effect of intraarticular ASC in human OA

Adipose Stem Cells (ASC)

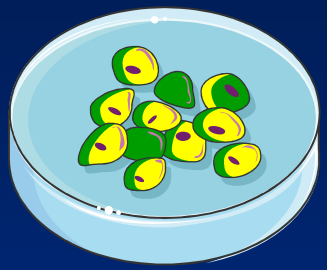
1. ASC can be easily isolated by liposuction aspiration (less painfull than BM aspiration)
2. ASC concentration in adipose tissue is 500 folds of that found in BM (approx.300.000ASC /g)
3. ASC can be expanded 7 times more than BMSC.
4. ASC present similar differantiation capacity, anti-fibrotic, anti apoptotic and anti-inflammatory features of BMSC.

ADIPOA

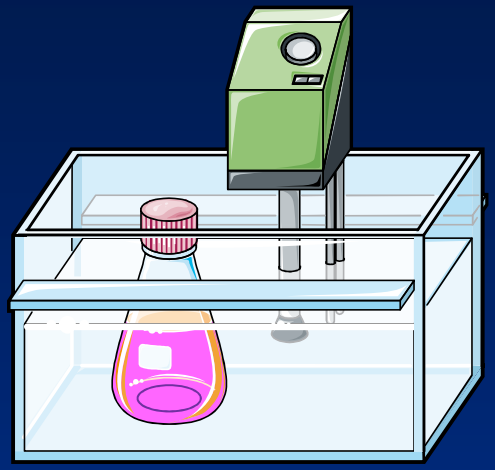
ASC isolation

Adipose tissue

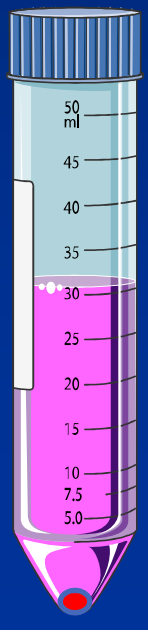
Fragmentation



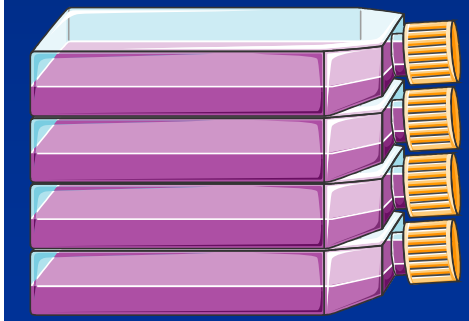
Enzymatic treatment



Centrifugation

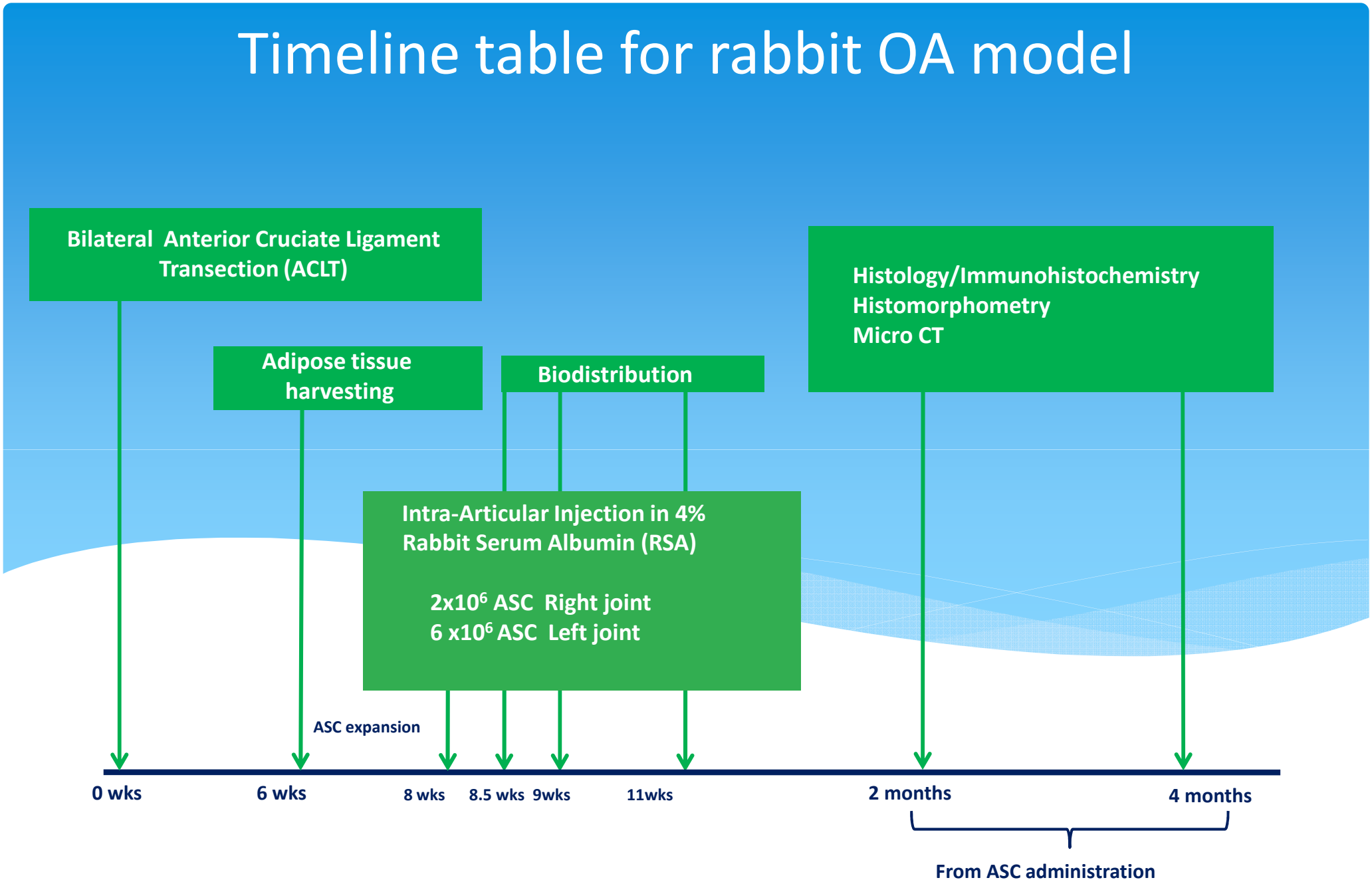


Pellet seeding



ASC

Timeline table for rabbit OA model

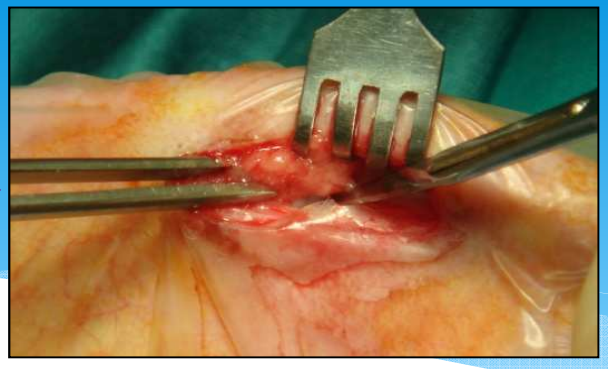


WP2 PRECLINICAL DEVELOPMENT IN RABBIT MODEL

* **To assess safety and bio-distribution of ASCs intra-articular injections in an experimental osteoarthritis rabbit model at two different experimental times: 2 and 4 months follow-up.**

* **To determine the most effective dose by injecting two different ASC concentrations ($2.0x10^6$ and $6.0x10^6$) in rabbit knee joints. To test capacity of cells to form ectopic cartilage**

Skeletally mature male New Zealand (12 months old)

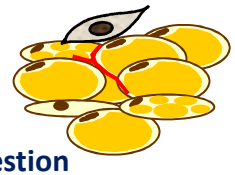


Bilateral Anterior Cruciate Ligament Transection (ACLT)

6 wks

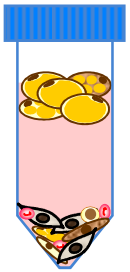
Adipose tissue harvesting

ASC isolation



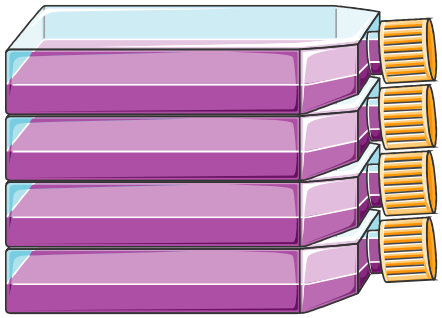
Enzymatic Digestion

30 min
37°C

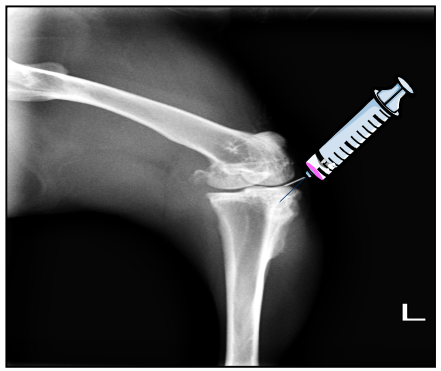


Culture with Plastic Adhesion

2 weeks expansion



Intra-articular injection



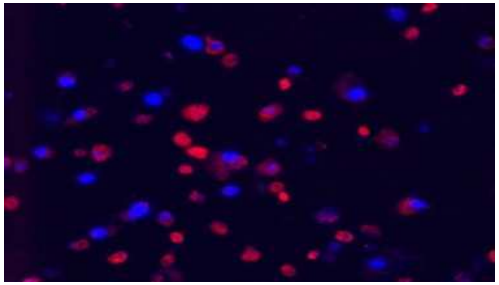
LOCAL BIODISTRIBUTION OF RABBIT ASC

Rabbit ASC were labelled with CM Dil and injected i.a.

CM Dil: is a lipophilic carbocyanine which reacts with proteins and molecules containing tiolic groups.

Emission spetrum: 570 nm

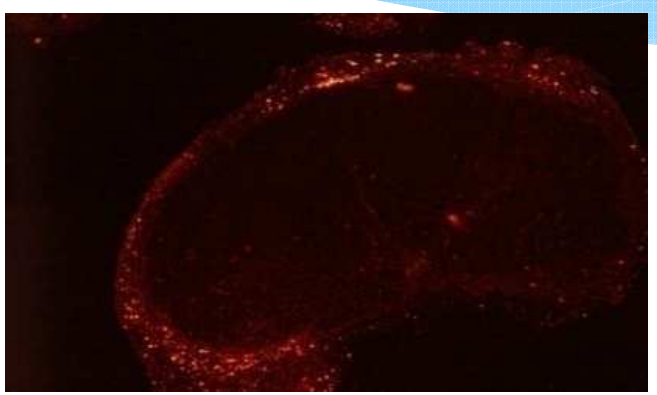
In vitro

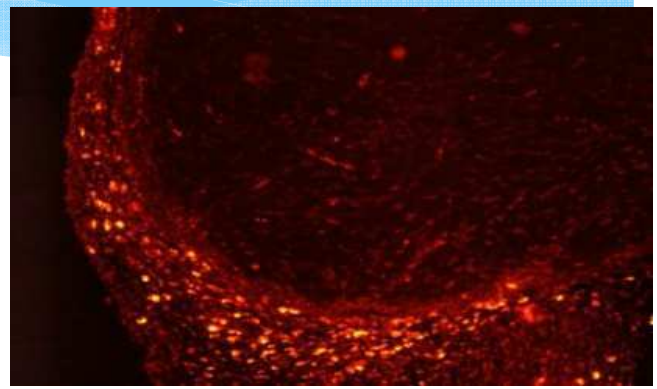


6 uM

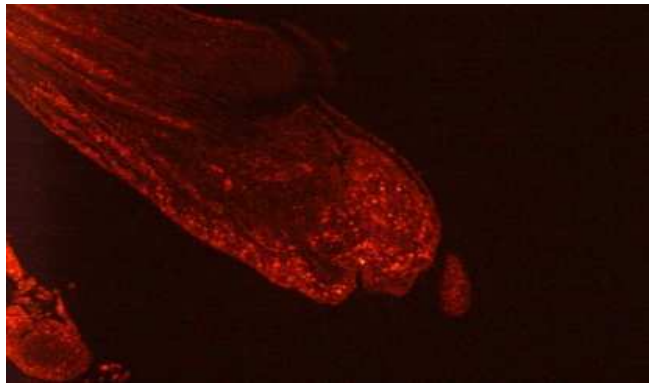
In vivo

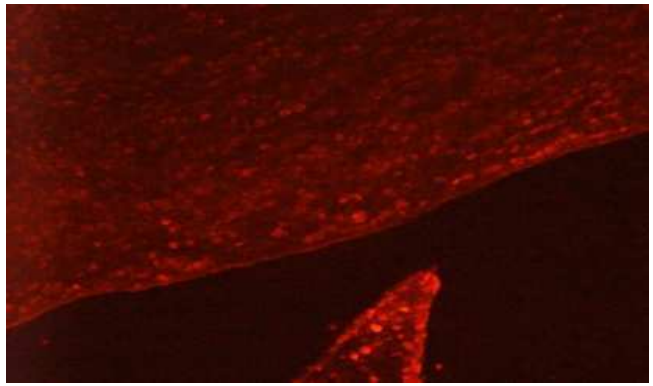
synovium





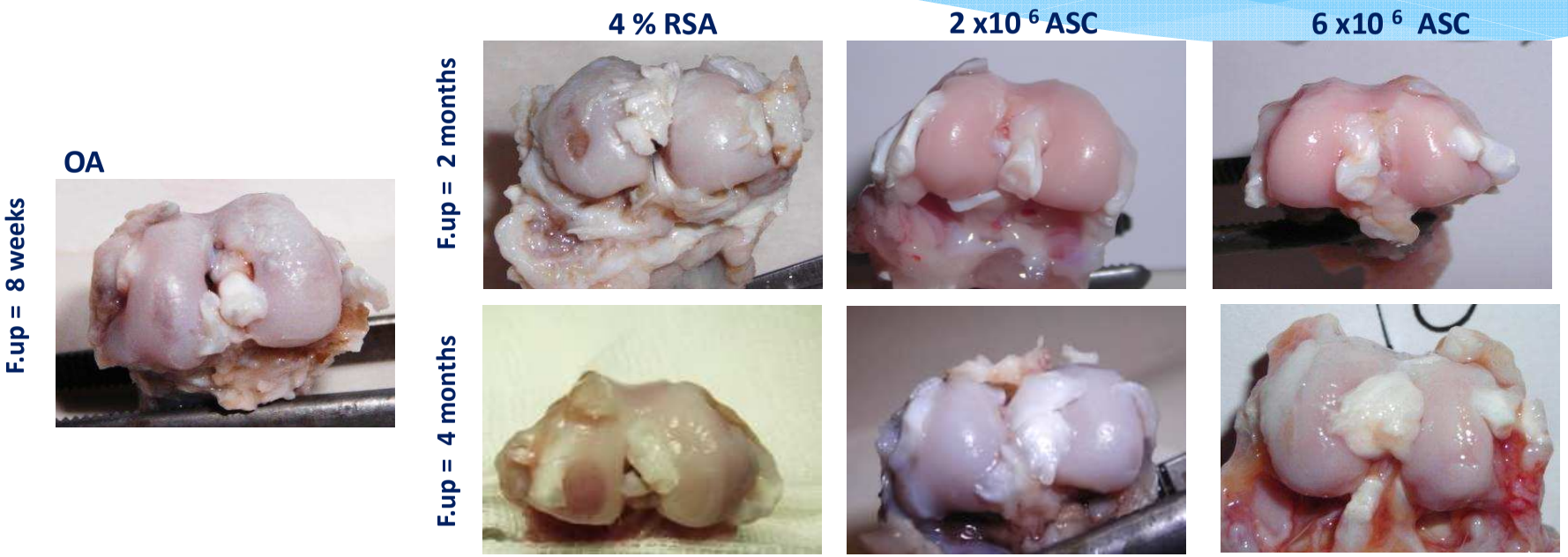
Medial meniscus





Incubation time = 7 days after an injection of 6.10^6 ASC

Macroscopic analyses



Laverty's score (0-24)

(Laverty S et al. Osteoarthritis & Cartilage, 2010)

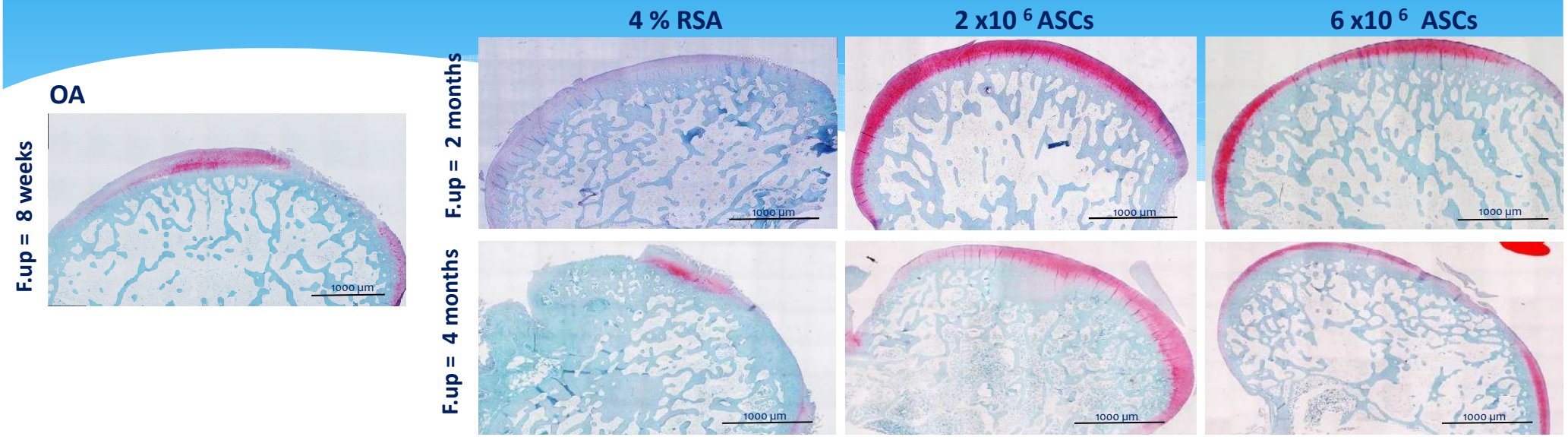
I. Safranin-O/Fast Green staining	0-6
II. Structure	0-11
III. Chondrocyte density	0-4
IV. Cluster formation	0-3

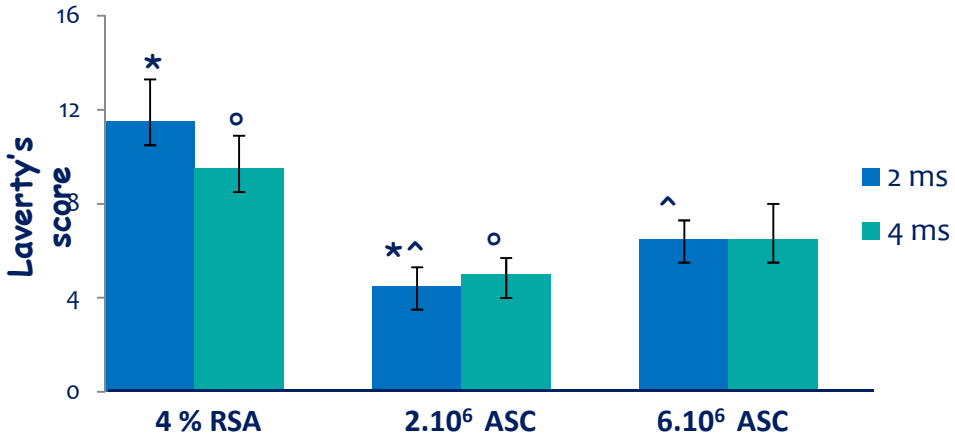
Where

0 is normal healthy cartilage

24 severe cartilage lesions

Histological evaluation: Safranin-O/Fast Green staining





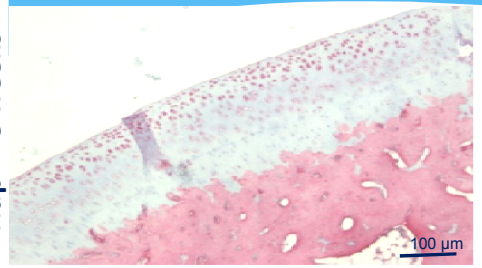
*p<0.0005; ^p=0.016; °p<0.002

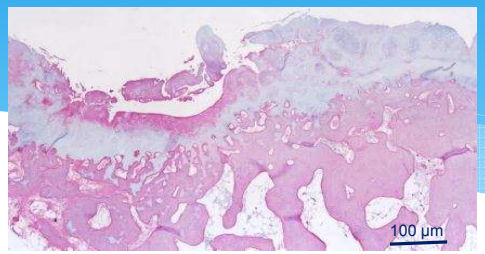
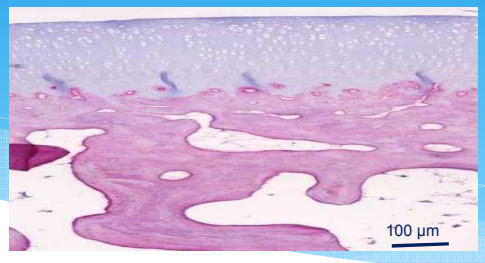
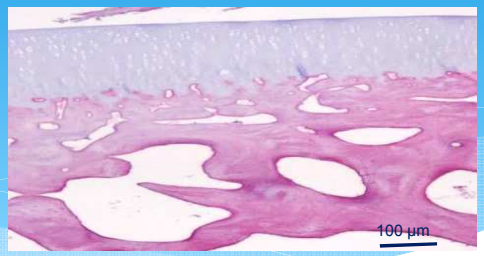
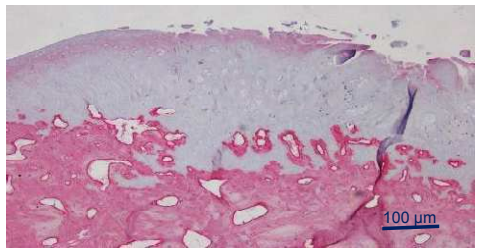
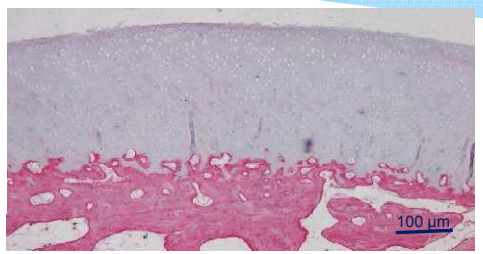
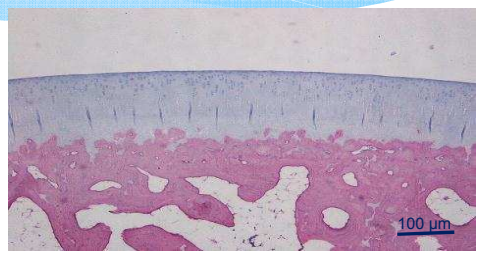
Immunohistochemical evaluations

Collagen Type I

OA

F.up = 8 weeks

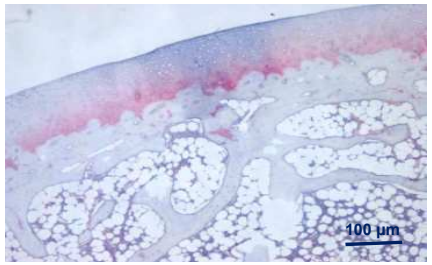


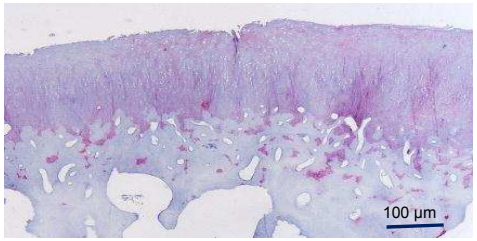
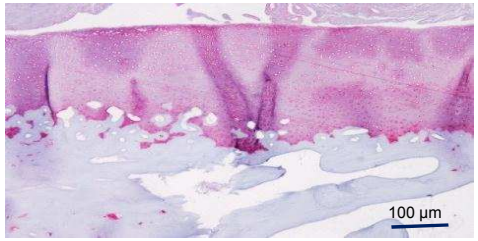
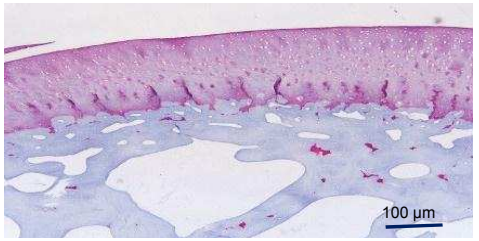
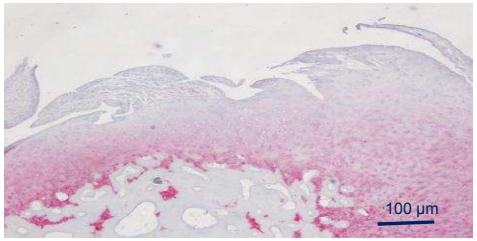
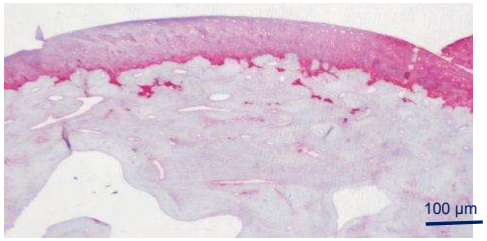
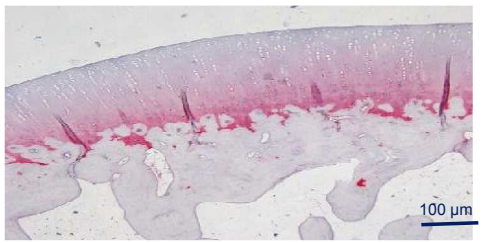
	4 % RSA	2 x10 ^{6} ASCs	6 x10 ^{6} ASCs
F.up 2 months			
F.up 4 months			

Collagen Type II

OA

F.up = 8 weeks



	4 % RSA	2 x10 ^{6} ASCs	6 x10 ^{6} ASCs
F.up 2 months			
F.up 4 months			

Immunohistochemical evaluations

MMP-1

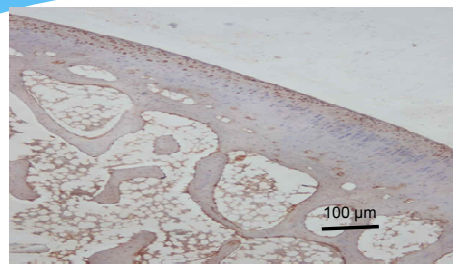
OA

4 % RSA

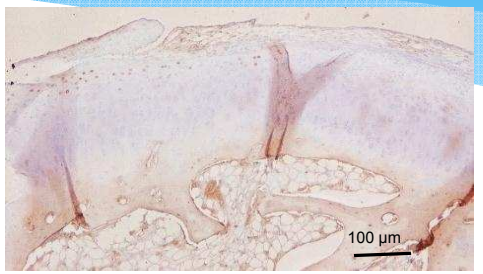
2 x10 ^{6} ASCs

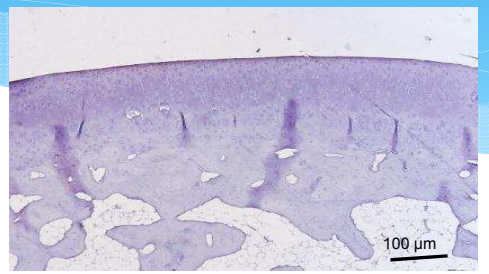
6 x10 ^{6} ASCs

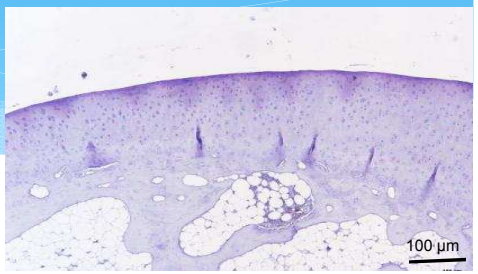
F.up 8 weeks



F.up 2 months







MMP-3

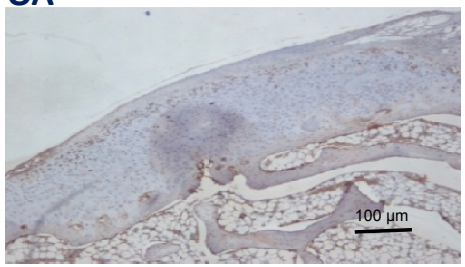
OA

4 % RSA

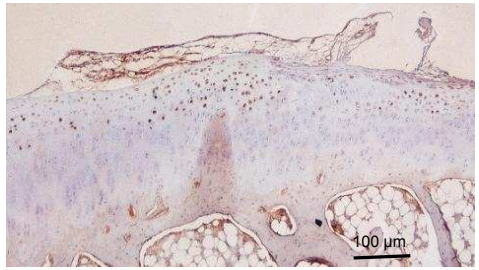
2 x10 ^{6} ASCs

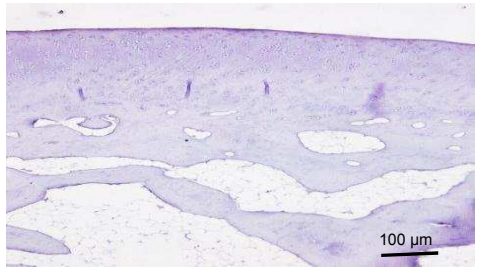
6 x10 ^{6} ASCs

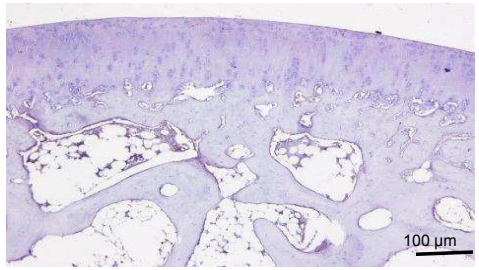
F.up 8 weeks



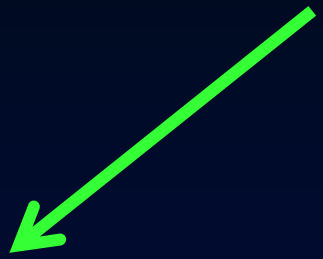
F.up 2 months







Which mechanisms are involved in tissue regeneration by ASC?



Differentiation of ASC into cartilage

Effects of trophic factors (paracrine)

Effects of MSC-released trophic factors

Adipose derived stromal cells (ASC) secrete molecules that can modulate the release of:

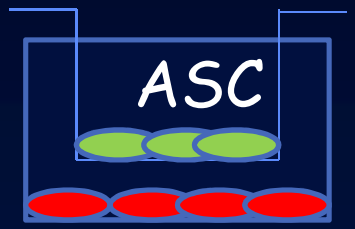
Inflammatory factors from OA chondrocytes and synoviocytes

Anti – fibrotic and anti- hypertrophic factors on chondrocytes

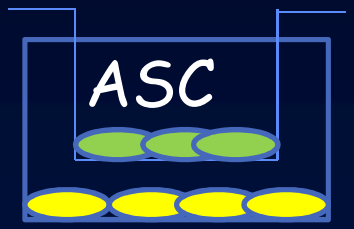
Experimental plan

- Isolation of OA chondrocytes and synoviocytes (n=10),
- Isolation of GMP-clinical grade ASC (n=5).

Co-Culture

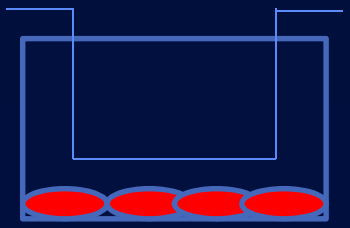


Chondrocytes

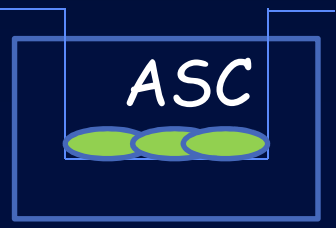


Synoviocytes

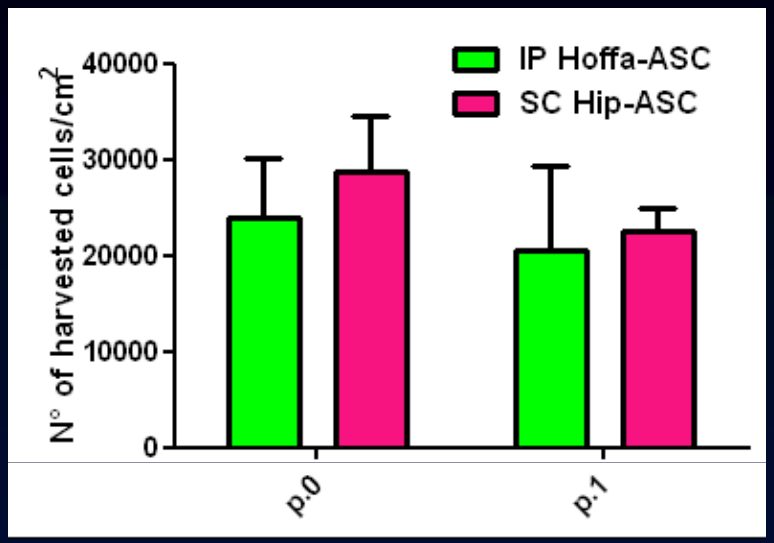
Control cells alone

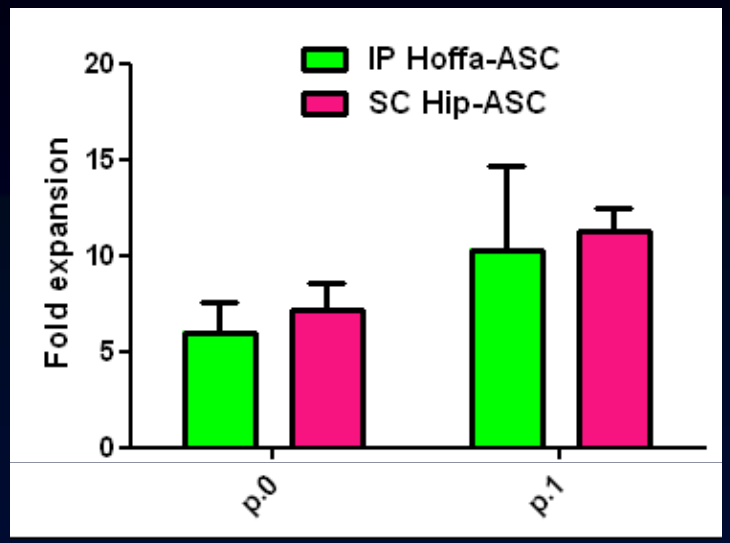


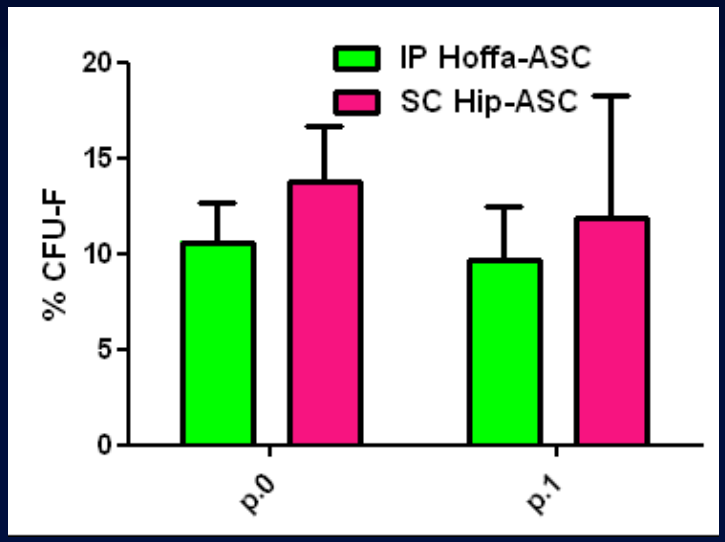
Cells

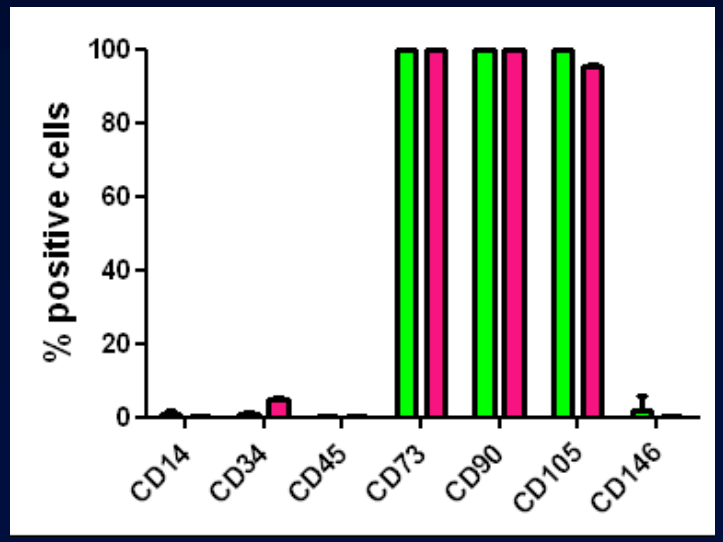


GMP-clinical grade ASC characterization









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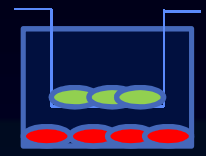
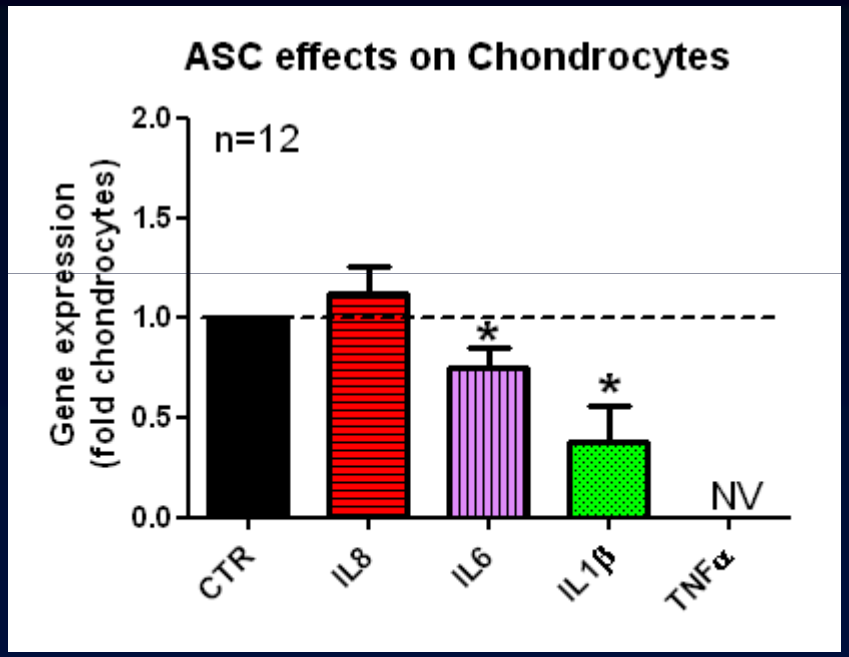
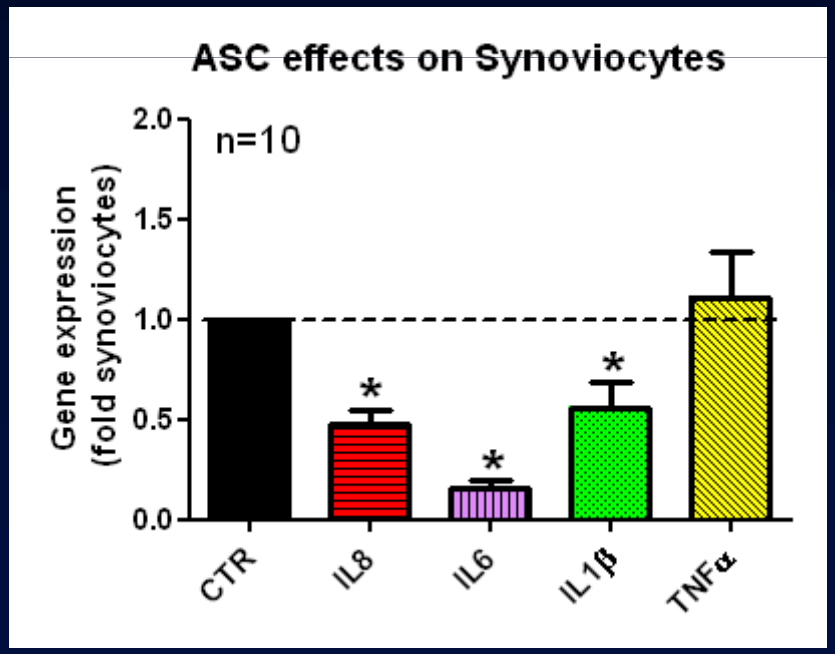
ASC in co-culture with chondrocytes or synoviocytes down-modulate the expresion of major OA inflammatory factors







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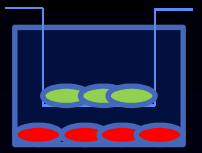
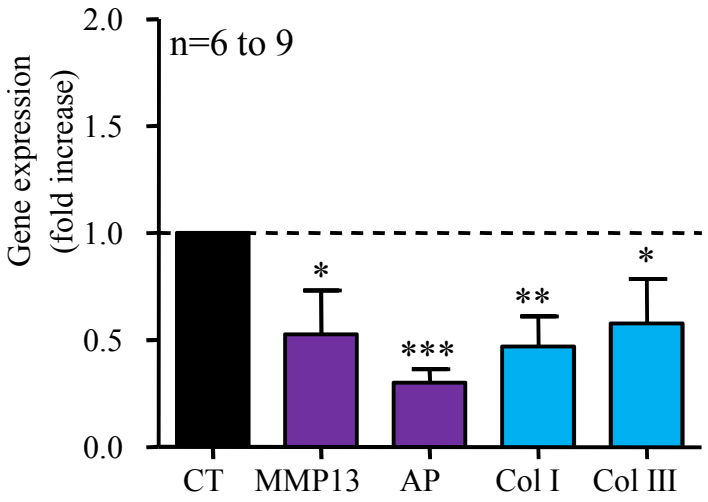
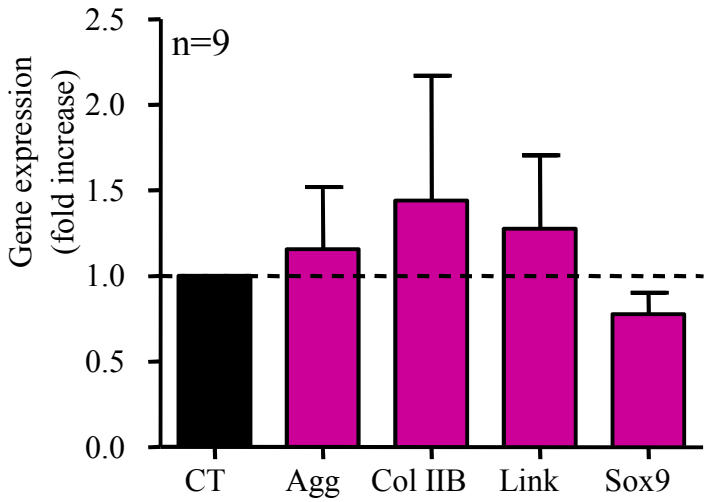


ASC co-culture inhibit fibrogenic and hypertrophic factors

Chondrocytes / ASC





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Are GMP grade ASCs a safe product ?

Conclusions

- No clonal alteration in karyotype analysis
- Progressive decrease of proliferation rates
- Low degree of random fluctuation in telomere dynamics
- Negligible telomerase activity
- No anchorage-independent colony formation
- Microsatellite stability and maintained allele patterns

Safe product

Grants from:


Presidenza del Consiglio dei Ministri
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IN BOLOGNA


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Univ.of Montpellier
Thank you