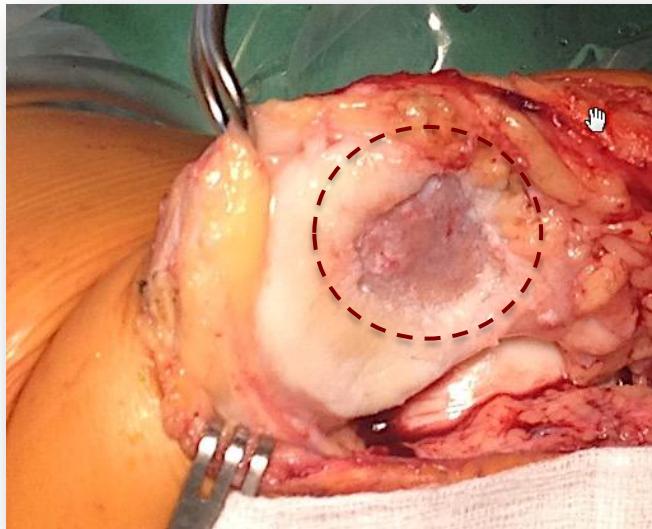


# CARTILAGE DEFECT: therapeutic challenge

- From **traumas** (professional/sport) or **spontaneous** defect
- Responsible for **chronic pain**
- Potential **arthritis** progression
- Young population affected



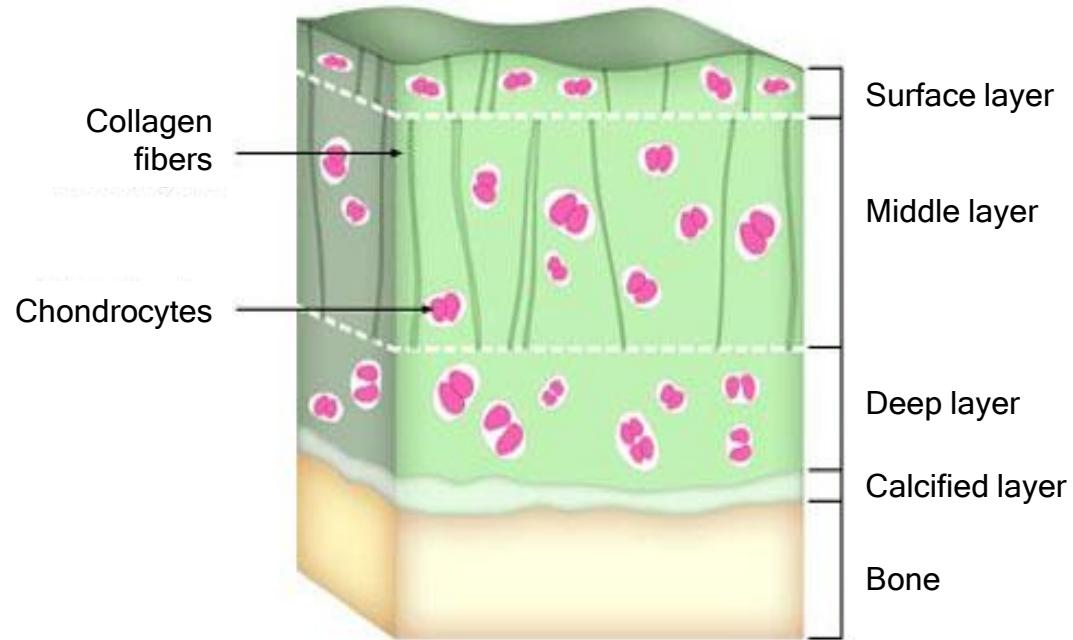
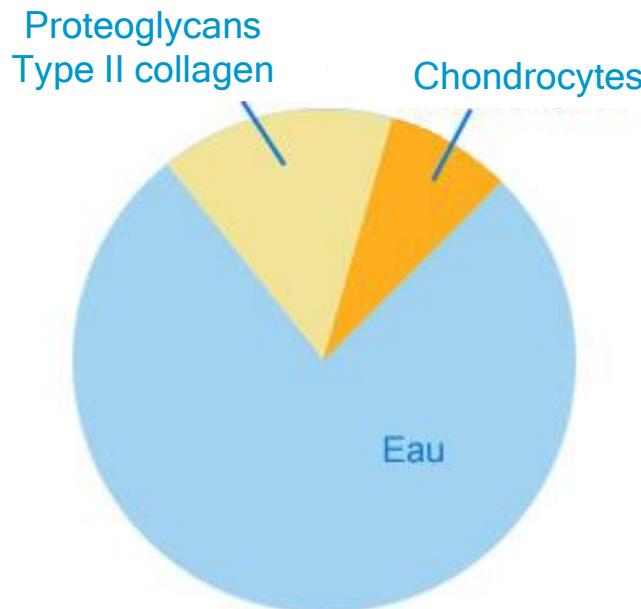
## Clinical case:

- 34 yo
- Patellar luxation
- grade IV lesion from IRCS classification

# ARTICULAR CARTILAGE

## Hyaline cartilage :

- non-innervated
- non-vascularized
- Composition : 95 % of ECM  
2-5 % of chondrocytes
- Low proliferation rate



# AIM OF CARTILAGE THERAPY

Pain reduction

Closure of the defect

Restoration of congruency



## **Aim:**

- Functional improvement
- Delay defect progression

# THERAPEUTIC OPTIONS

## REPAIR:

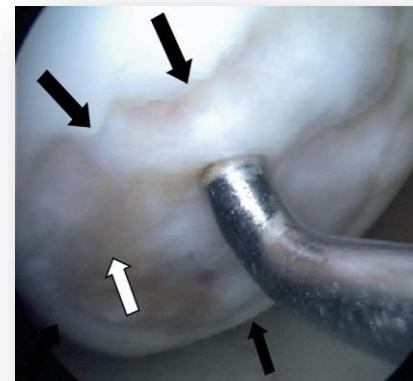
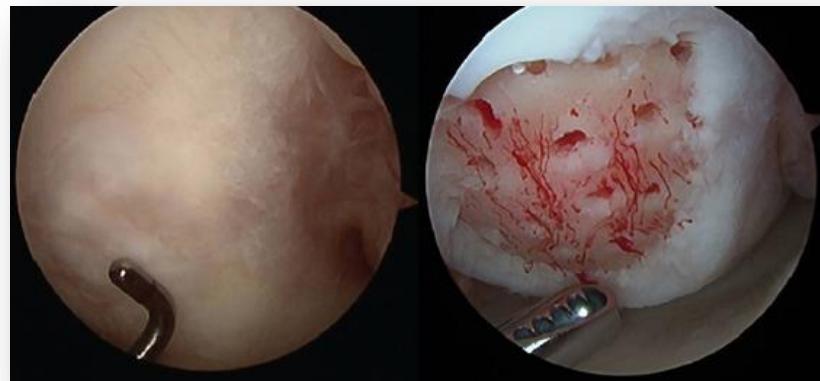
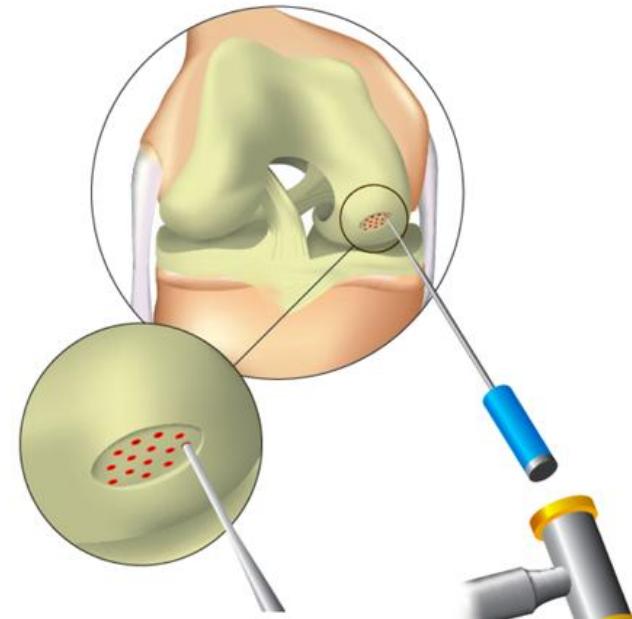
- Microfractures (+/- scaffolds)
- Cell therapy

## REPLACE:

- Osteochondral grafts
- Prosthetics

# MICROFRACTURES

- Drilling: *Priddie, 1959*
- Micro fracturing: *Steadman, 1994*



# MICROFRACTURES LIMITATIONS

## Clinical Efficacy of the Microfracture Technique for Articular Cartilage Repair in the Knee: An Evidence-Based Systematic Analysis

Kai Mithoefer, Timothy McAdams, Riley J. Williams, Peter C. Kreuz and Bert R. Mandelbaum  
*Am J Sports Med* 2009;37:2053 originally published online February 26, 2009  
DOI: 10.1177/0363546508328414

Mithoefer et al, *AJSM*, 2009

TABLE 4  
Overview of Reported Clinical Results  
After Microfracture

Clinical knee function	
Short-term clinical improvement rate ( $\leq 24$ mo)	75%-100%
Long-term clinical improvement rate ( $> 24$ mo)	67%-86%
Functional deterioration ( $> 24$ mo) <sup>a</sup>	47%-80%
Magnetic resonance imaging	
Complete cartilage fill	18%-95%
Poor cartilage fill	17%-57%
Complete peripheral integration	4%-8%
Subchondral bone hypertrophy	25%-49%
Macroscopic/microscopic repair cartilage assessment	
Macroscopic grading normal/near normal	45%-77%
Histology:	
Fibrocartilage	33%-57%
Fibrohyaline hybrid tissue	39%-64%
Complication rate	
Serious procedure-related complications	0%-13%
Failure/revision rate	
Less than 24 mo	2.5%
After 24 mo	2%-31%

<sup>a</sup>Despite deterioration, clinical function still better than before microfracture.

## Limitations:

High viability up to 2 years

- New cartilage type = fibrous
- Variable thickness
- Functional deterioration

# MICROFRACTURES + Scaffolds

- Increased interest : augmentation technique for microfractures
- « cell free »
- Numerous scaffolding materials...
  - MACI (Genzyme - Sanofi) – Collagen type I/III matrix
  - Maioresen (Fin-Ceramica) – Collagen type I/HA matrix
  - **Chondro-Gide (Geistlich)** - Collagen type I/III matrix
  - Hyalograft C (Fidia) – hyaluronic acid polymer matrix
  - CaRes (Arthro Kinetics) – Collagen type I matrix
  - BioCart II (Prochon Biotech) – Fibrin/Hyaluronan matrix
  - Cartipatch (Mions) – hydrogel (agarose/alginate)

## Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair

R. Dorotka M.D., U. Bindreiter M.D., K. Macfelda Ph.D., U. Windberger D.V.M.

and Professor S. Nehrer M.D.\*

*Medical University of Vienna, Department of Orthopedic Surgery, Waehringer Guertel 18-20,  
1090 Vienna, Austria*

### Summary

**Objective:** The purpose of the study was to determine whether the implantation of a scaffold would facilitate cartilage repair after microfracture in sheep over a period of 12 months. Furthermore, we investigated the effect of additional autologous cell augmentation of the implanted constructs.

**Methods:** Two chondral defects were produced in the medial femoral condyle of sheep without penetrating the subchondral bone. Twenty-seven sheep were divided into the following groups: seven served as untreated controls (Group 1), microfracture was created in 20 animals, seven of them without further treatment (Group 2), in six sheep the defects were additionally covered with a porcine collagen matrix (Group 3), and in seven animals the matrix was augmented with cultured autologous chondrocytes (Group 4). After 4 (11 sheep) and 12 months (16 sheep), the filling of the defects, tissue types, and semiquantitative scores were determined.

**Results:** The untreated defects revealed the least amount of defect fill. Defects treated with microfractures achieved better defect fill, while the additional use of the matrix did not increase the defect fill. The largest quantity of reparative tissue was found in the cell-augmented group. Semiquantitative scores were best in the cell-augmented group.

**Conclusion:** Microfracture treatment was observed to enhance the healing response. The implantation of a cell-seeded matrix further improved the outcome. The implantation of a collagen matrix alone did not enhance repair. Autologous cell implantation appears to be a very important aspect of the tissue engineering approach to cartilage defects.

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**Key words:** Cartilage tissue engineering, Scaffold, Chondrocyte, Microfracture.

**Animal model :** The implantation of a collagen matrix alone did not enhance repair

# CELL THERAPY

- INTRINSEQUER REPAIR:

ACI : Autologous chondrocytes implantation  
chondrocytes from an adjacent area

Clinical use

- EXTRINSEQUER REPAIR:

Stem cells: connectives tissues  
Bone marrow

Under  
Clinical trials

# CELL THERAPY: Autologous chondrocytes implantation



- Brittberg et al, *N Engl J Med*, 1994
- 30,000 patients throughout the world
- Processes :
  - Biopsy (1<sup>st</sup> surgery)
  - Cell expansion
  - Implantation (2<sup>nd</sup> surgery)
- 73.5 % still doing well at mean 12.8 yrs post surgery (n=224)

Peterson et al, *AJSM*, 2010

Moradi et al, *Arthroscopy*, 2012

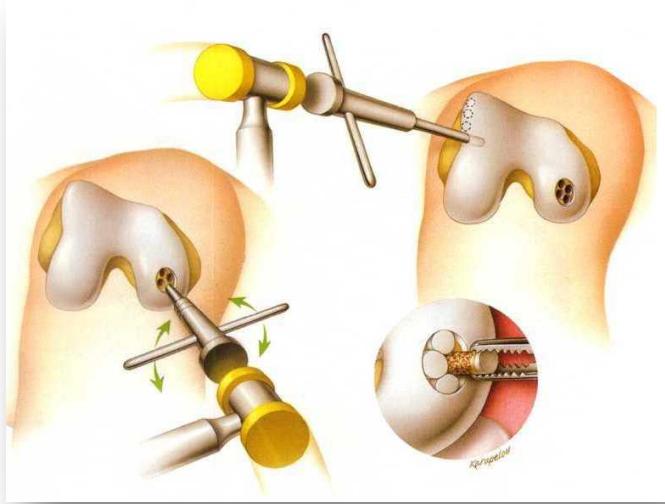
Bentley et al, *AJSM*, 2014

# CELL THERAPY: Autologous chondrocytes implantation

## An evolving process :

- 1<sup>st</sup> generation: cells injected under a periosteal cover
- 2<sup>nd</sup> generation: cells injected under a resorbable scaffold
- 3<sup>rd</sup> generation: cells cultivated directly into the scaffold  
(=> Arthroscopically placed)

# OSTEOCHONDRAL GRAFTS



- **AUTOGRAFT** : mosaic plasty
  - Donor morbidity
  - Cell viability
  - Small defects
- **ALLOGRAFT** :
  - Availability
  - Storage
  - Transmissible diseases
  - Bone resorption



# Comparative studies: ACI vs osteochondral graft

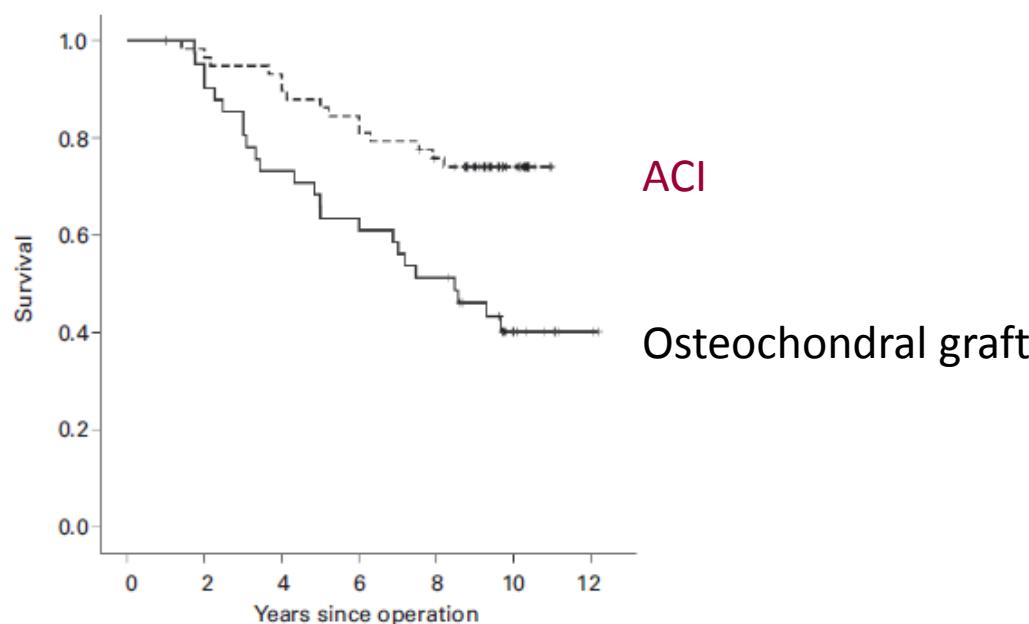
Bentley et al, *JBJS Br*, 2012



G. Bentley,  
L. C. Biant,

## ■ KNEE

**Minimum ten-year results of a prospective randomised study of autologous chondrocyte implantation *versus* mosaicplasty for symptomatic articular cartilage lesions of the knee**



# Comparative studies: ACI vs microfractures

## CURRENT CONCEPTS REVIEW Restoration of Articular Cartilage

Cathal J. Moran, MD, FRCS(Orth), Cecilia Pascual-Garrido, MD, Susan Chubinskaya, PhD, Hollis G. Potter, PhD, Russell F. Warren, MD, Brian J. Cole, MD, MBA, and Scott A. Rodeo, MD

**TABLE I Randomized Controlled Trials Comparing Different Cartilage Procedures in the Last Three Years**

Year	Study	Group 1*	Group 2*	No.	Follow-up (yr)	Clinical Outcome*	Other Findings*
2012	Bentley et al. <sup>50</sup>	OATS	ACI	100	10	Cincinnati score significantly better in ACI group ( $p = 0.02$ )	15% failed in ACI group vs. 55% in OATS group
2012	Crawford et al. <sup>51</sup>	Cartilage implant	Microfracture	30	2	IKDC, KOOS, and VAS significantly better in implant group ( $p = 0.0125$ )	76% in cartilage implant group vs. 44% in microfracture group responded to procedure
2011	Cole et al. <sup>52</sup>	Fragmented cartilage transplant	Microfracture	29	2	IKDC and KOOS significantly better in fragmented cartilage transplant group ( $p < 0.05$ )	MRI did not find difference between groups
2010	Basad et al. <sup>54</sup>	MACI	Microfracture	60	2	MACI group did significantly better than microfracture group ( $p = 0.005$ for Lysholm and $p = 0.04$ for Tegner)	
2010	Zeifang et al. <sup>55</sup>	MACI	ACI	21	2	No significant difference between groups	MOCART significantly better at 6 mo. for MACI group; no difference at 24 mo.
2010	Van Assche et al. <sup>57</sup>	CCI	Microfracture	67	2	No significant difference between groups	
2009	Gudas et al. <sup>53</sup>	OATS	Microfracture	50	4	OATS group did significantly better than microfracture group ( $p < 0.05$ )	Children with osteochondral lesions
2009	Saris et al. <sup>56</sup>	CCI	Microfracture	85	3	CCI group did significantly better than microfracture group ( $p = 0.048$ )	83% in CCI group vs. 62% in microfracture group responded to procedure

\*OATS = osteochondral autologous transplantation, ACI = autologous chondrocyte implantation, CCI = characterized chondrocyte implantation, MOCART = magnetic resonance observation of cartilage repair tissue scoring system, IKDC = International Knee Documentation Committee, KOOS = Knee Injury and Osteoarthritis Outcome Score, VAS = visual analog scale, MACI = matrix-assisted autologous chondrocyte implantation, and MRI = magnetic resonance imaging.

# WHAT IS AVAILABLE FOR CLINICAL USE AT THE CHUV?

## LIMITED CHOISE !

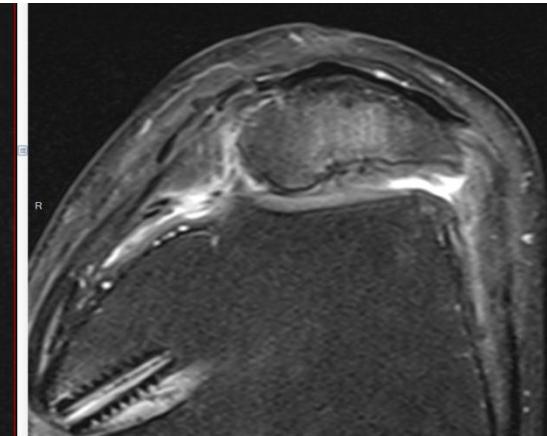
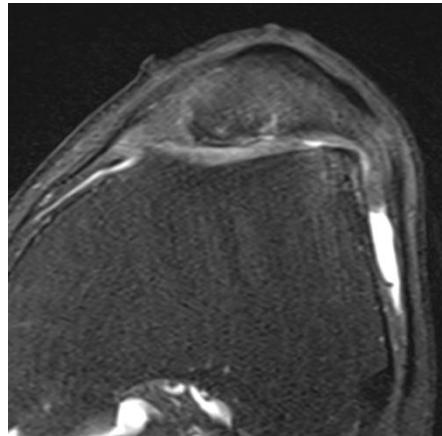
LARGE DEFECTS: Microfractures with scaffold



IRM Pre-operative



IRM post-operative (6 month)



# ACI is a real clinical need

## STRATEGY :

1. AQUIRE CHONDROCYTES CULTURE EXPERTISE
2. MID TERM : ACI CLINICAL TRIAL IN CHUV
3. LONG TERM: DEVELOP NEW CELL AND SCAFFOLD STRATEGIES

# 1- ADULT CHONDROCYTE CULTURE

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# AQUIRE CHONDROCYTE CULTURE EXPERTISE

## Brittberg et al. (1994):

Cell origin : Patient = **27 years** (range 14-48, 11 men/12 women)

Quantity : 200 to 300 mg from **healthy cartilage** =>  **$2.10^6$  cells/cm<sup>2</sup>**

Culture media : DMEM/Ham's F12 supplemented with ascorbic acid

glutamine

antibiotics

**autologous human serum**

Culture time : **21 days** (3 passages)



## Lausanne :

Cell origin : Patient = **53 years** (range 23-66, 5 men/6 women)

Quantity : 100 to 200 mg of cartilage from **total or partial arthroplasty** of knee

Culture media : DMEM/Ham's F12 supplemented with ascorbic acid

glutamine

antibiotics

**Lysat plaquettaire humain (hpL)**  
**ou Fetal Calf Serum (FCS)**

Culture time : **24.6 days** for hpL (3 passages)

**50.2 days** for FCS (3 passages)

# CULTURE PROCESS

Donnor



P0 ( $T25\text{cm}^2$ )

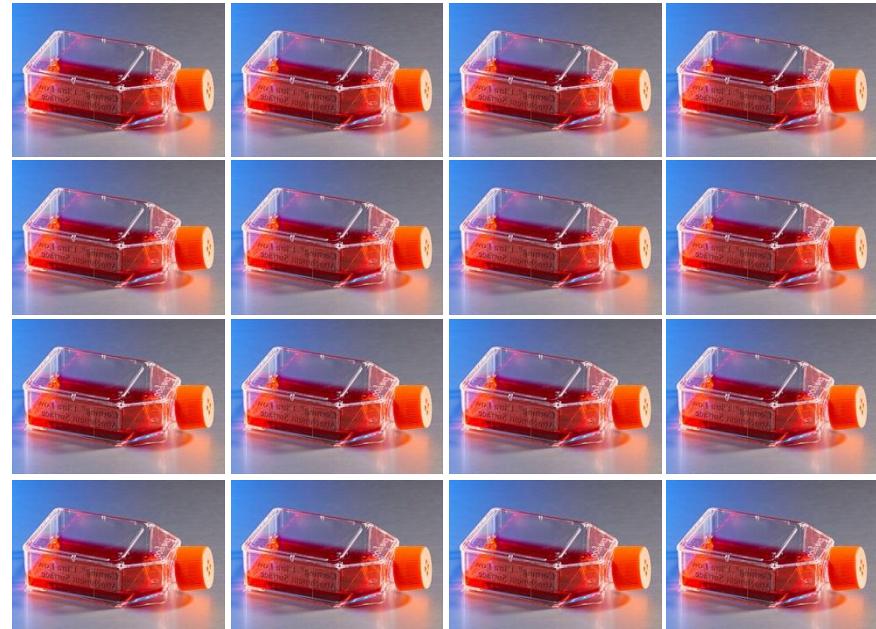


## Sample preparation

Tissue fragmentation

Tissue digestion

Subculture ( $T75\text{cm}^2$ , max. P4)



Cryopreservation



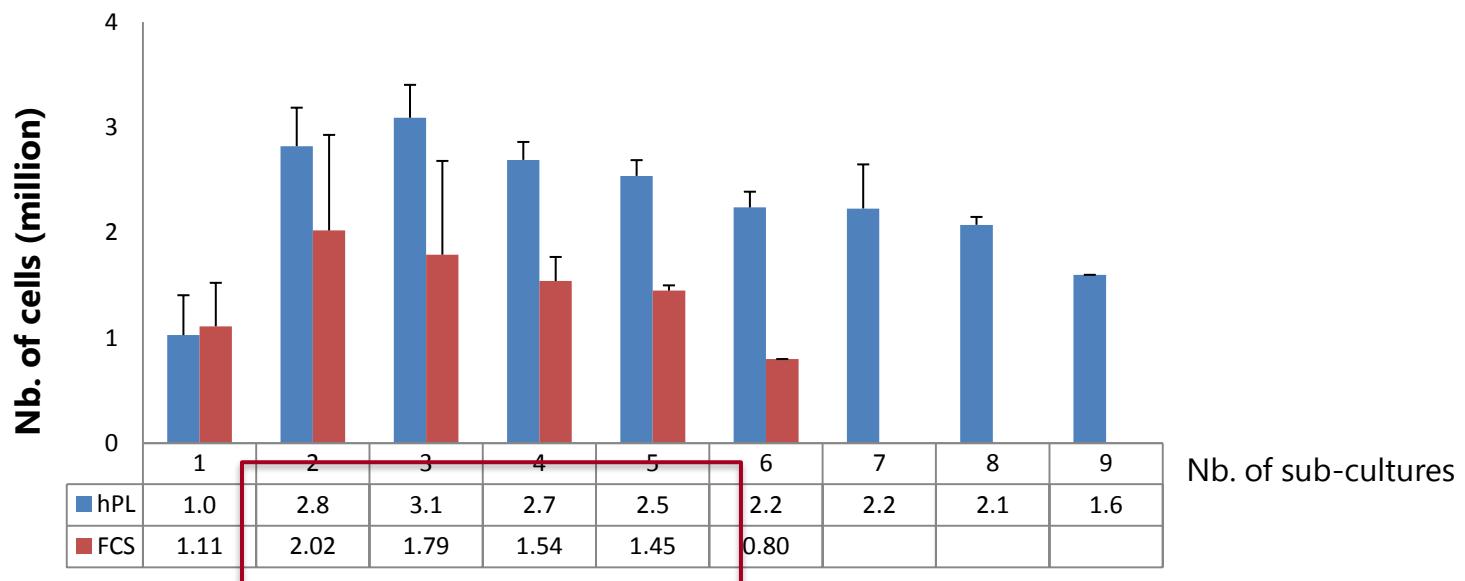
Injection



# CELL GROWTH

## Nb. of cells (85% de confluence)

n= 16



## Time between each sub-culture (days)

n= 16

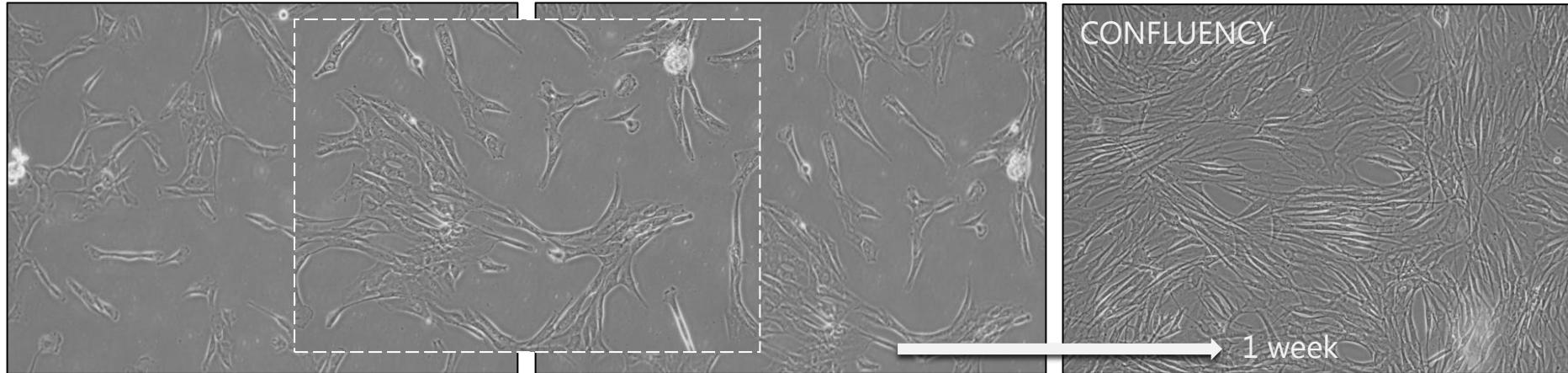
Nb. of sub-cultures

Nb. of sub-cultures	hPL	FCS
1	11.4	15.6
2	6.6	18.3
3	6.6	15.7
4	8.4	12.3
5	9	16.0
6	14.2	13.3
7	15.5	13.3
8	16	13.3
9	20	13.3

# MONOLAYER CULTURE: Cell morphology

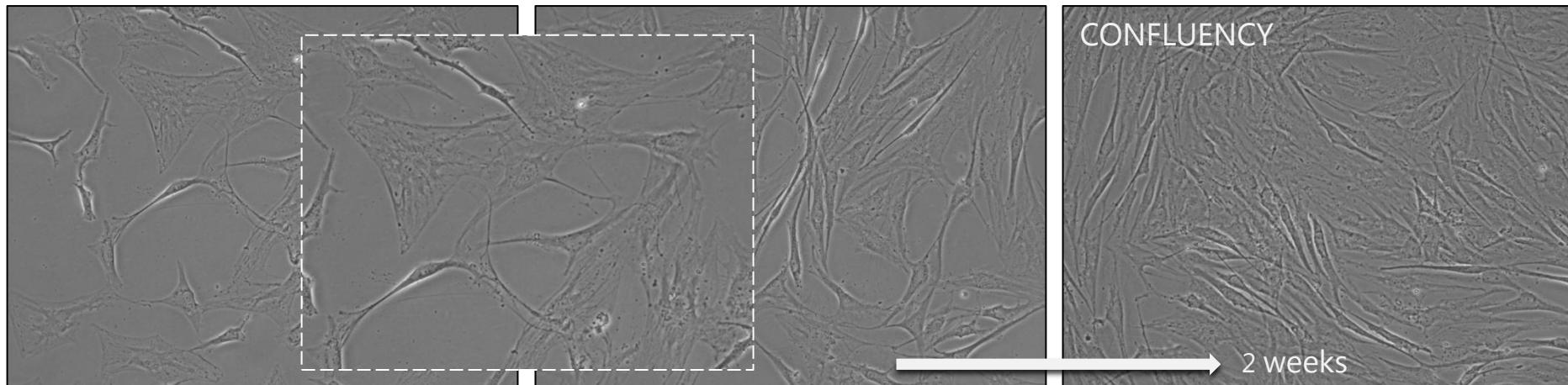
Passage 2

Brittberg + 10% hPL



Passage 2

Brittberg + 10% FCS

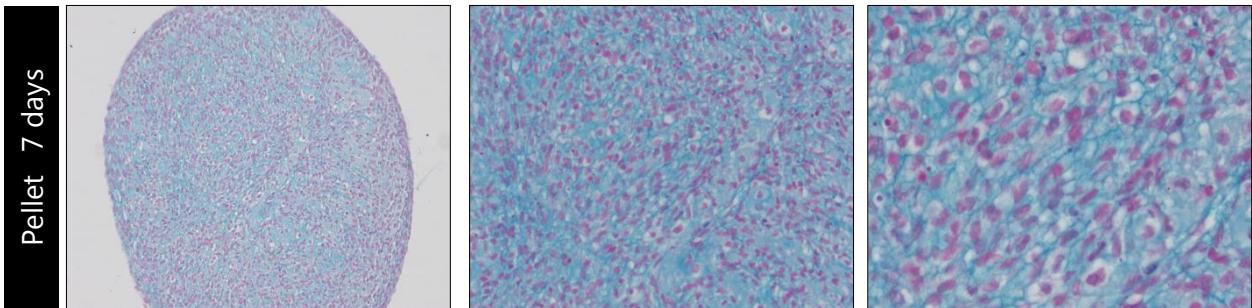




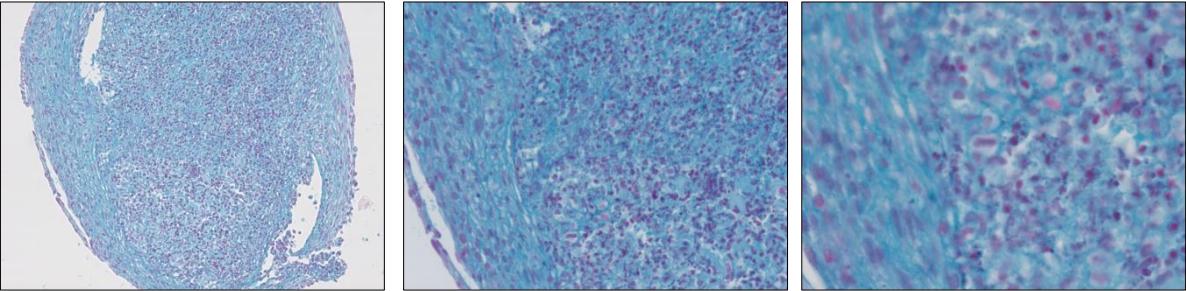
Blue Alcian staining  
(n=4)

# 3D-CULTURE : Pellets (0.5 millions of cells)

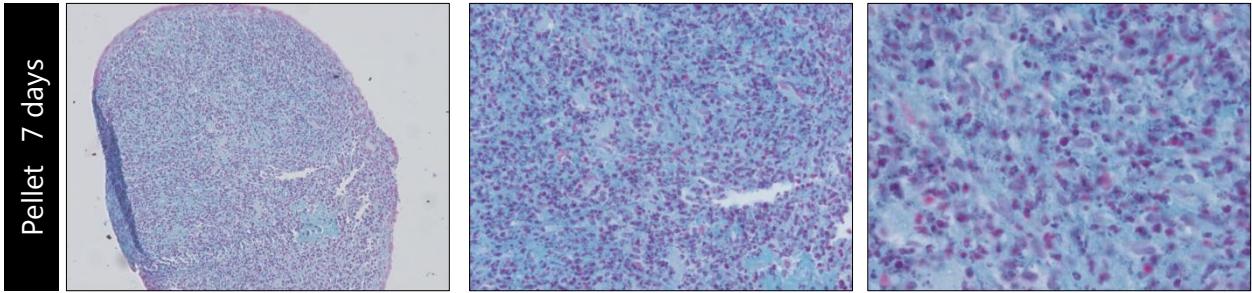
FCS



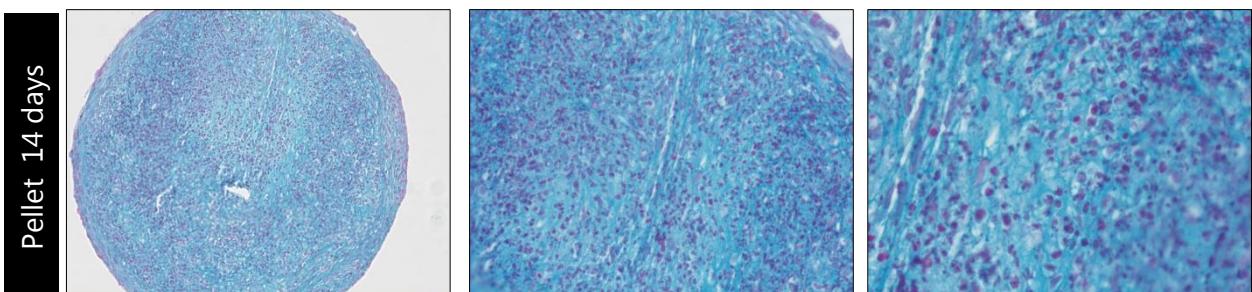
Pellet 14 days



hpL



Pellet 14 days

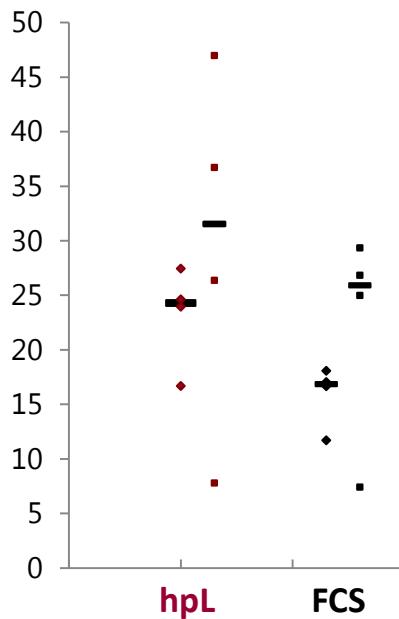




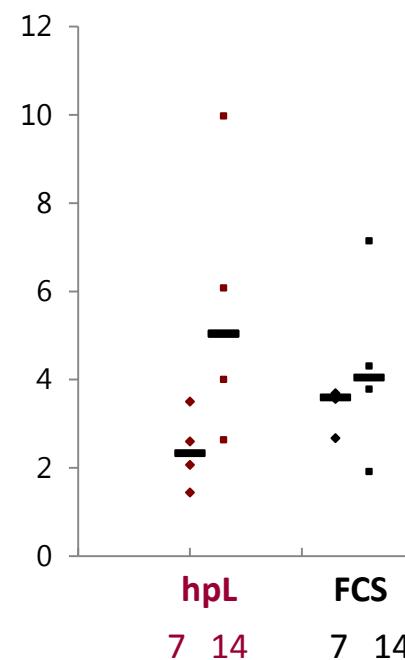
# 3D-CULTURE : Pellets (0.5 millions of cells)

Relative quantification / GAPDH normalized (n=4)

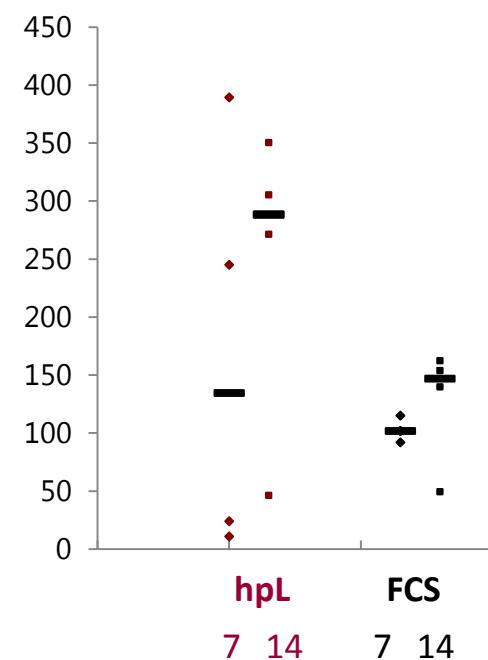
SOX9



ACAN



COL2-a1



Time (days):

7 14 7 14

# CULTURE EFFICIENCY

$2.10^6$  cells/cm<sup>2</sup>



P0 (T25cm<sup>2</sup>)  
1,5-3.10<sup>5</sup> cells



$10^6$  cells

P1 (T75cm<sup>2</sup>)  
3-5.10<sup>5</sup> cells



$2-2.5.10^6$  cells

$2-2.5.10^6$  cells

$2-2.5.10^6$  cells

Cryopreservation  
 $10^6$  cells/ml



P2 (T75cm<sup>2</sup>)  $5.10^5$  cells



$24-30.10^6$  cells

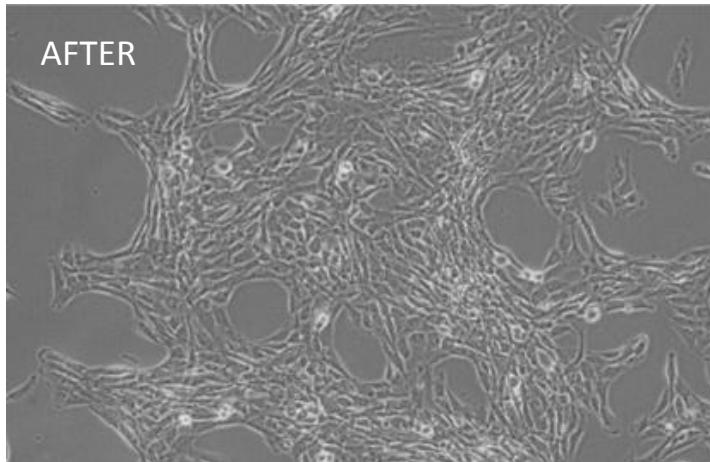
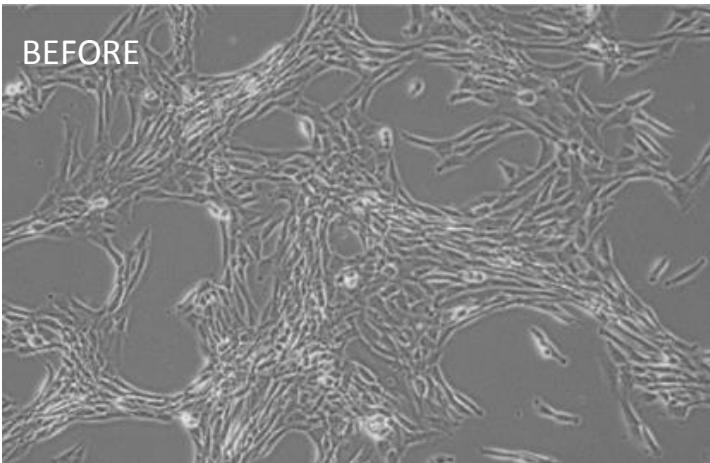


7-10 days

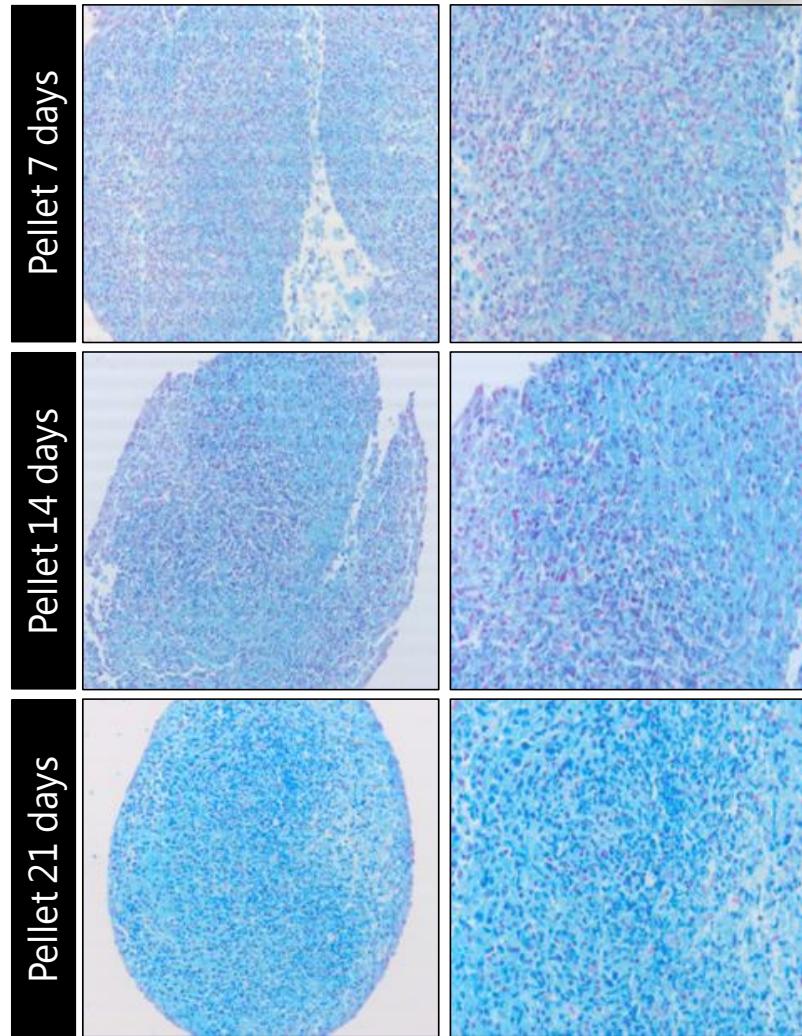
7 days

# CRYOPRESERVATION

~20% cell loss after thawing



hpL





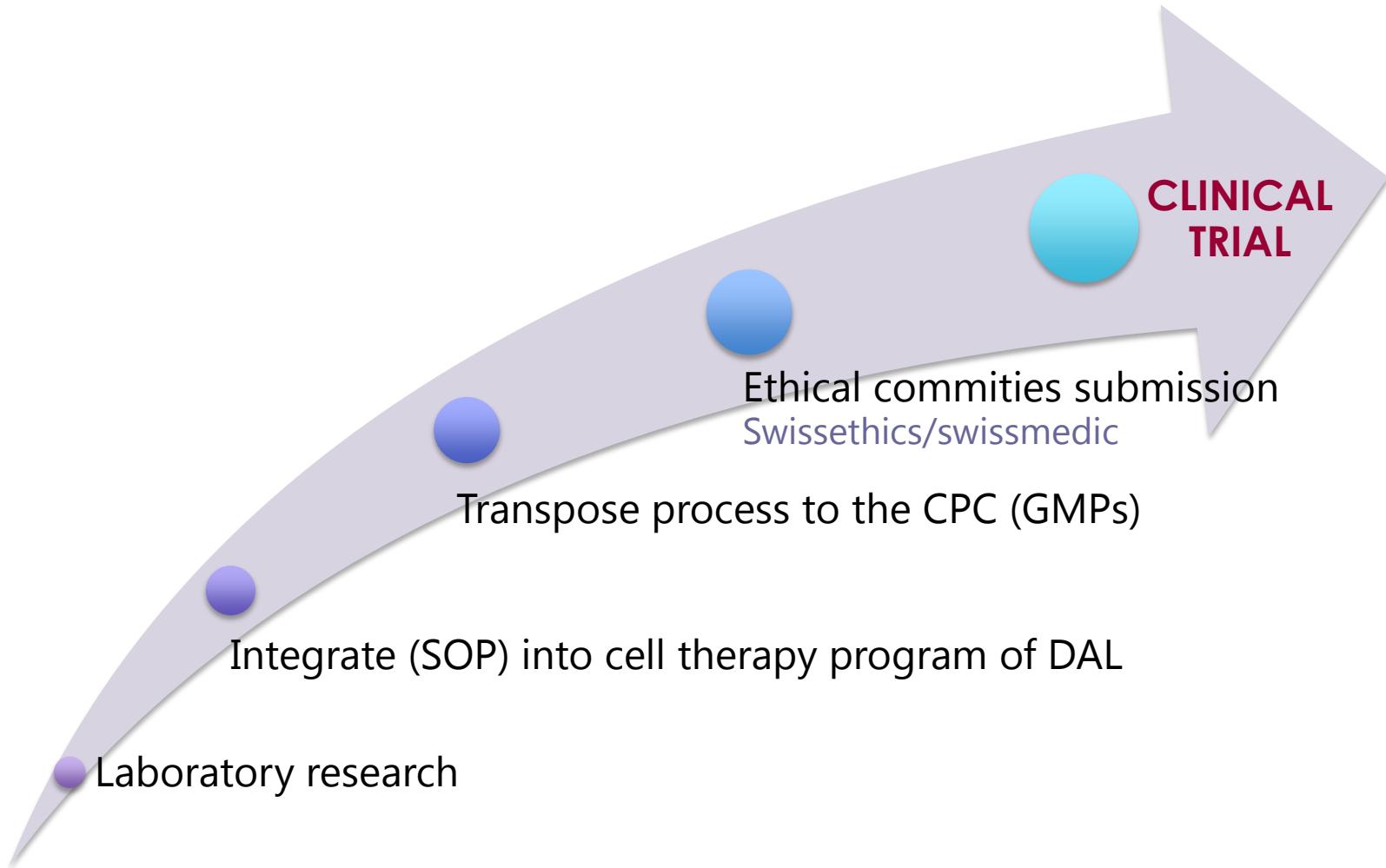
From laboratory  
research to clinic

## GMP guidelines

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# FROM LABORATORY RESEARCH TO CLINIC



# STANDARD OPERATING PROCEDURES (SOP)

## Préparation du Milieu de culture

### Table des matières

1 Matériel ...	1
2 Solutions ...	1
3 Marche à suivre ...	1

### 1 Matériel

Flux laminaire de classe A  
PipetBoy  
Pipettes stériles 25ml (Falcon #357525)  
Pipettes stériles 10ml (Falcon #357551)

### 2 Solutions

- Dulbecco's Modified Eagle Medium (D-MEM) (1x); liquid (High Glucose 4.5g/l) with 0.110g/l Na Pyr, Invitrogen 41966-029), stocké à 4°C
- L-Glutamine 200 mM (100x), Invitrogen 25030-024, aliquots de 5ml stockés à -20°C
- Serum Fetal Bovine (FBS) 500ml, aliquots de 50ml stockés à -20°C

### 3 Marche à suivre

Préparation du milieu de culture: .....

Nombre de bouteilles préparées: .....

DMEM: 500ml      Lot: .....      Exp: .....  
FCS:      50ml      Lot: .....      Exp: .....  
L-Glutamine      5ml      Lot: .....      Exp: .....

Milieu du .....      Lot: .....      Exp: .....

### Validation

TITRE DU DOCUMENT	SOP Préparation du milieu de culture		
AUTEUR(S)	C. Scaletta		
VALIDATION	L. Laurent-Applegate		
DATE DE LA VERSION	27/09/2012		

*Standardise culture processes :*

= documentation reporting instructions for the entire process:

- Cell isolation
- Cell culture
- Cell storage
- Cell thawing
- Cell delivery
- All the controls needed

Working base for the CPC

= transpose all processes with Good Manufacturing Practice (GMP) Guidelines

# WHAT IS GMP ?

## **GOOD MANUFACTURING PRACTICE**

It is a question of **quality assurance**

GMP guidelines provide guidance for manufacturing, testing, and quality assurance in order to ensure that a product is of high quality and do not pose any risk to the public/patient.

Other good-practice systems:

- Good laboratory practice (GLP)
- Good clinical practice (GCP)
- Good regulatory practice (GRP)
- Good distribution practice (GDP)
- Good transportation practice (GTP)

# WHAT'S INVOLVE GMP GUIDELINES ?

**GMP**

Defined and validated manufacturing processes

Specific storage and transportation

Trained and qualified staff

Qualified facilities

Written and approved procedures

Full traceability

Internal audit system

Records and claims

**Cell Production Center (CPC)**

Dr J-F. BRUNET-Manager

Dr L. WASELLE- Production manager



# To adjust culture procedures according to GMP guidelines

Sample preparation: Tissue fragmentation and digestion



Culture



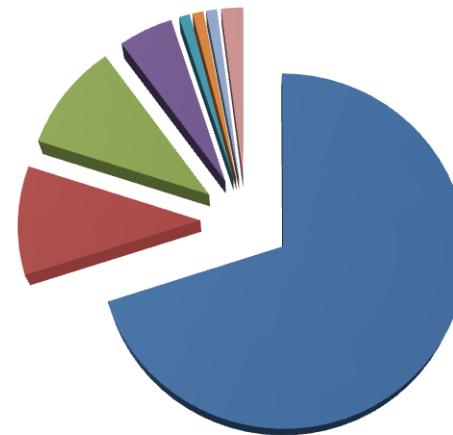
Cryopreservation



Injection

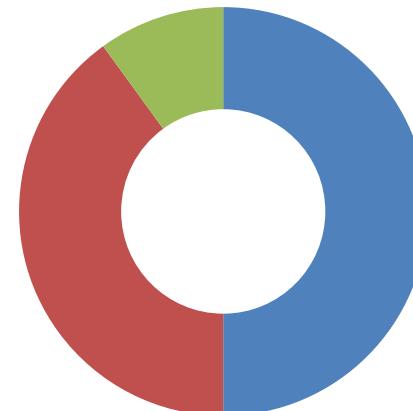


Culture media



- Water
- Salts
- hpL
- Sugar
- Glutamine
- Growth Factors
- Aminoacids

Freezing media



- Basic culture medium
- hpL
- DMSO

# Adapting exemple according to GMP guidelines

## Fetal Bovine Serum

FBS is **gold standard**, used since 1940/50 (growth factors, attachment factors, nutrients):

- Animal origin
- Great variability between batches
- Risk of contamination (virus ou prions)

= **ETHICAL ISSUES !!!**

## ALTERNATIVE SOLUTIONS

- Commercial defined medium (with recombinant proteins)
- Human platelet rich plasma (hPRP)
- **Human platelet lysate (hPL)**

## Freezing media

DMSO (Dimethyl Sulfoxide) is a toxic cryoprotectant that needs several washes to be eliminated

»»» improper use in clinic !!!

## ALTERNATIVE SOLUTIONS

- Use sugars
- Commercial freezing media (clinical grade)

# Adapting exemple according to GMP guidelines

## **WORKING ENVIRONNEMENT:**

Class A module



# Conclusions



- Specific questions
- Establish the objectives
- How to solve the problem ?
- Which are the tools?
  - Pre-clinic studies
  - Cells
  - Animals
- SOPs
- GMPs Guidelines
- Data analysis
- Conclusions
- Ethical documentation
- Ethical submission
  - Swissethics
  - Swissmedic
- Ethical authorisation

**SEVERAL YEARS !!!!**

Cell production center(CPC),  
Dr Jean-Francois BRUNET  
Manager



Dr Laurent WASELLE  
Production manager



Production team



Quality control team

THANK YOU FOR YOUR  
ATTENTION

Unité de Thérapie Regenerative (UTR),  
Pr Lee LAURENT-APPLEGATE

