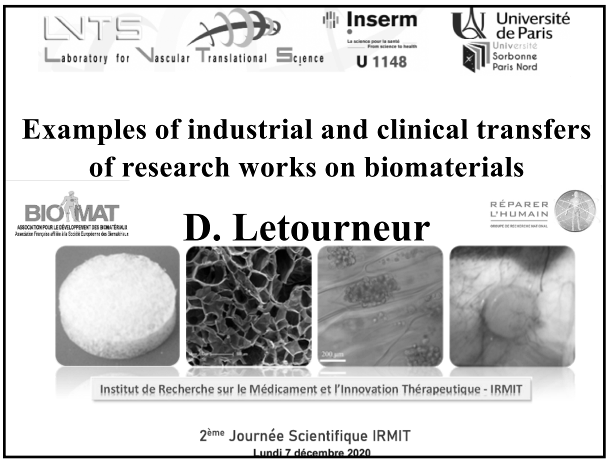
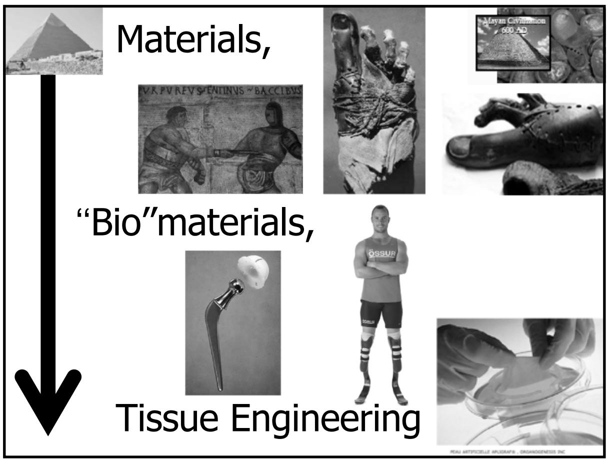
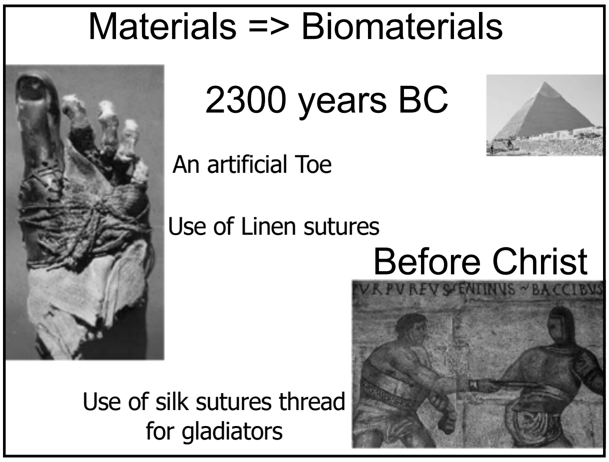

LVTS Laboratory for Vascular Translational Science
Inserm U 1148
Université de Paris
Université Sorbonne Paris Nord
Examples of industrial and clinical transfers of research works on biomaterials
BIOMAT
RÉPARER L'HUMAIN
D. Letourneur
Institut de Recherche sur le Médicament et l'Innovation Thérapeutique - IRMIT
2ème Journée Scientifique IRMIT
Lundi 7 décembre 2020

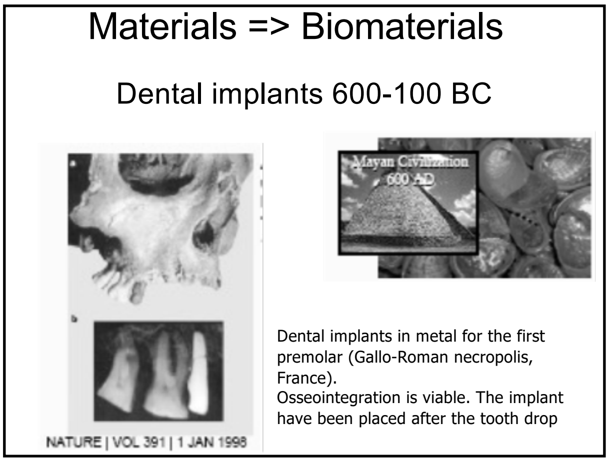
1


Materials,
"Bio"materials,
Tissue Engineering
Mayan Civilization 600 AD

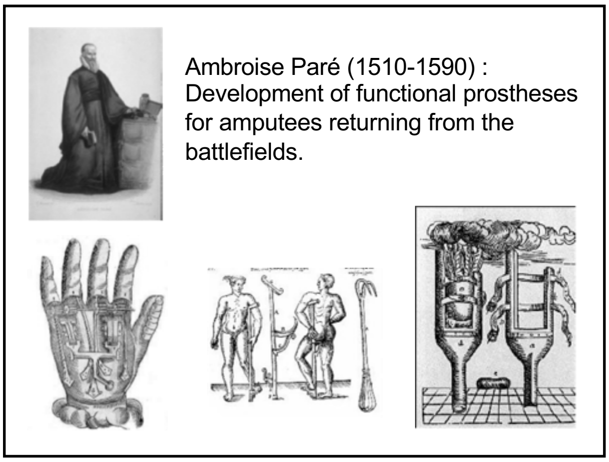
2


Materials => Biomaterials
2300 years BC
An artificial Toe
Use of Linen sutures
Before Christ
Use of silk sutures thread for gladiators

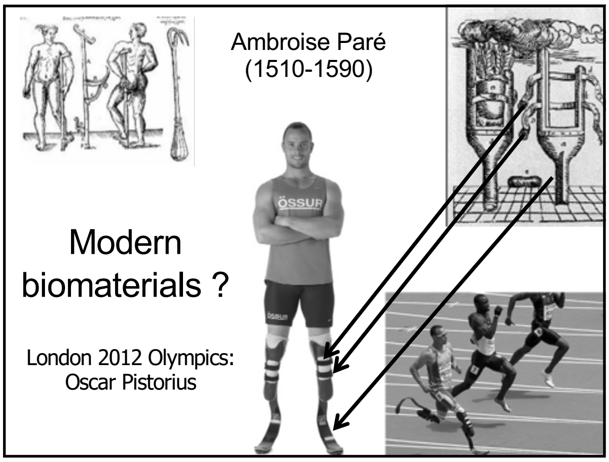
3


Materials => Biomaterials
Dental implants 600-100 BC
Mayan Civilization 600 AD
NATURE | VOL 391 | 1 JAN 1998
Dental implants in metal for the first premolar (Gallo-Roman necropolis, France).
Osseointegration is viable. The implant have been placed after the tooth drop

4


Ambroise Paré (1510-1590) : Development of functional prostheses for amputees returning from the battlefields.

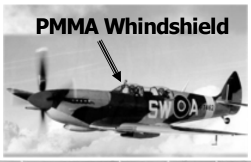
5


Ambroise Paré (1510-1590)
Modern biomaterials ?
London 2012 Olympics: Oscar Pistorius
ÖSSUR

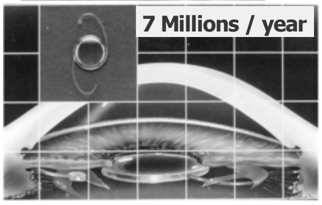
6

Modern «bio»materials

Sir Harold Ridley (1906-2001)
Ophtalmologist



1950



7

Prosthesis:
Latin : pro (instead of)
and thesis (to put)

Greek: from prostithenai,
prosthe-, *to add* : tithenai, *to place*;

=> Total or partial replacement of an organ, limb or restore function

8

BIOMATERIALS

According to Chester definition
(European Society for Biomaterials 1986) :

A non-living material for use in a medical device and for replacing or treating a tissue, organ or function with a contact time greater than three weeks.

⇒ **Medical Device : ANSM**

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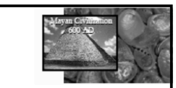
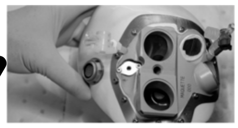
Materials,



"Bio"materials,

CARMAT



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Example 1

From biomaterial design to clinical use :

An expansible aortic ring

EVOLVING TECHNOLOGY/BASIC SCIENCE

An expansible aortic ring for a physiological approach to conservative aortic valve surgery

Journal of Thoracic and Cardiovascular Surgery 2009

Emmanuel Lansac, MD, PhD,[a,b] Isabelle Di Centa, MD,[c] François Raoux, MD,[d] Neil Bulman-Fleming,[e] Adrian Ranga,[e] Aïcha Abed, MSc,[a,f] Maguette Ba, MD,[a] Anthony Paolitto,[e] Didier Letourneur, PhD,[a,f] and Anne Meddahi-Pellé, MD, PhD[a,g]

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Example 1

From biomaterial design to clinical use :

An expansible aortic ring

PATENT : Aortic ring and ancillary device for implanting it

Aortic ring made of a flexible, suturable and biocompatible material, having a length, in the implanted state, making it possible to maintain a normal aortic ring diameter, said ring comprising, or being able to be combined with, means for holding the ring in a closed position in its site of implantation, in particular in a subvalvular plane.

Fr patent 2003
US patent 2005

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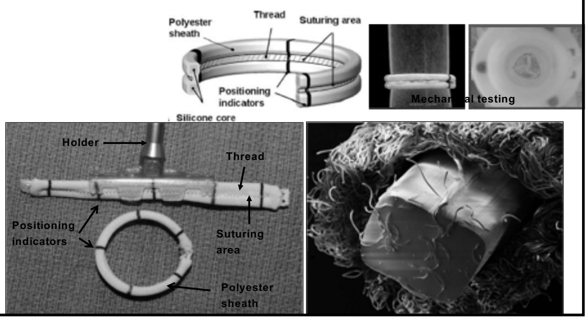
EVOLVING TECHNOLOGY/BASIC SCIENCE

An expansible aortic ring for a physiological approach to conservative aortic valve surgery *J Thoracic & Cardiovascular Surgery 2009*

Emmanuel Lansac, MD, PhD,[a,b] Isabelle Di Centa, MD,[c] François Raoux, MD,[d] Neil Bulman-Fleming,[e] Adrian Ranga,[e] Aicha Abed, MSc,[a,f] Maguette Ba, MD,[a] Anthony Paolitto,[e] Didier Letourneur, PhD,[a,f] and Anne Meddahi-Pellé, MD, PhD[a,d]



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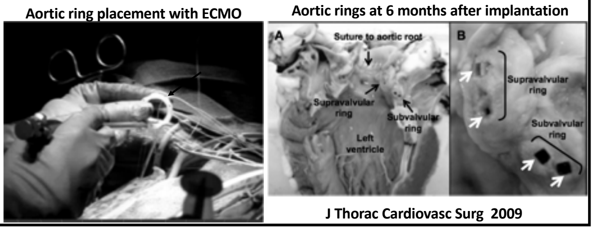
Aortic rings in sheep (6 months)

Aortic ring placement with ECMO

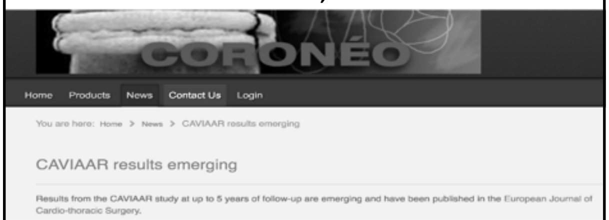
Aortic rings at 6 months after implantation



J Thorac Cardiovasc Surg 2009

14

Aortic rings in man
from 2010
600 patients, multi-center clinical trials
Extra-Aortic®, Coroneo Inc



Eur J Cardiothorac Surg. 2015
An expansible aortic ring to preserve aortic root dynamics after aortic valve repair.
J Thorac Cardiovasc Surg. 2015
Standardized approach to valve repair using an expansible aortic ring versus mechanical Bentall: early outcomes of the CAVIAAR multicentric prospective cohort study.

On going 10-year follow-up

15

Lessons from Example 1

From biomaterial design to clinical use :
An expansible aortic ring

PATENT : Aortic ring and ancillary device for implanting it
Fr patent 2003 - Owner name: APHP
US patent 2005
2007 Owner name: Coroneo Inc, Canada

Extensions US 2013 0073033 **:**
EXPANDABLE ANNULOPLASTY RING AND ASSOCIATED RING HOLDER
Owner name: Coroneo Inc, Canada

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Lessons from Example 1

From biomaterial design to clinical use :
An expansible aortic ring

PATENT : Aortic ring and ancillary device for implanting it
Fr patent 2003 - Owner name: APHP
US patent 2005
2007 Owner name: Coroneo Inc, Canada

Extensions US 2013 0073033 **:**
EXPANDABLE ANNULOPLASTY RING AND ASSOCIATED RING HOLDER
Owner name: Coroneo Inc, Canada

5-year clinical trials 2010-2015
10-year clinical trials 2010-2020

+ Several MDs, Several Engineers

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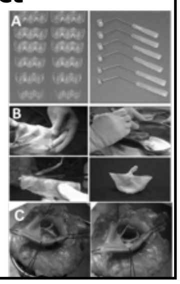
Lessons from Example 1

From biomaterial design to clinical use ...
...and back to bench for a
New Research Project

Autologous pericardium valve
A: Moulds
B. Valve process
C. ex vivo valve implantation
left side: close, right side: open

Publication submitted. Clinical trial anticipated



+ Another New Research Project

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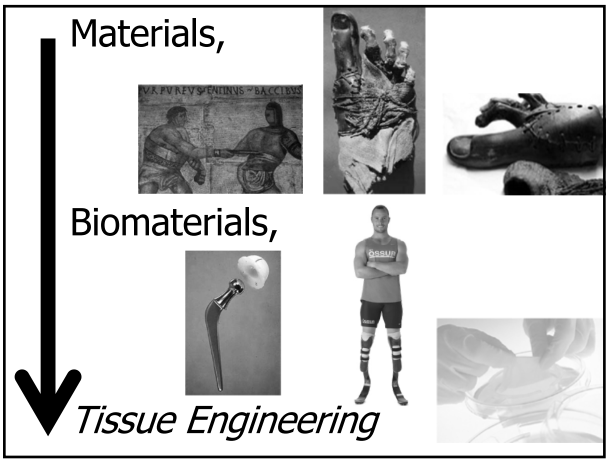

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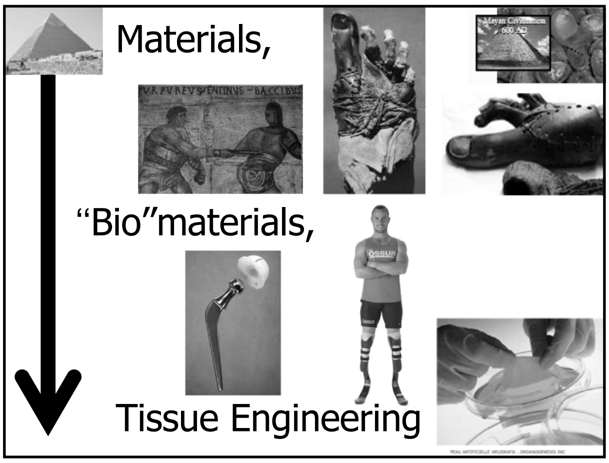

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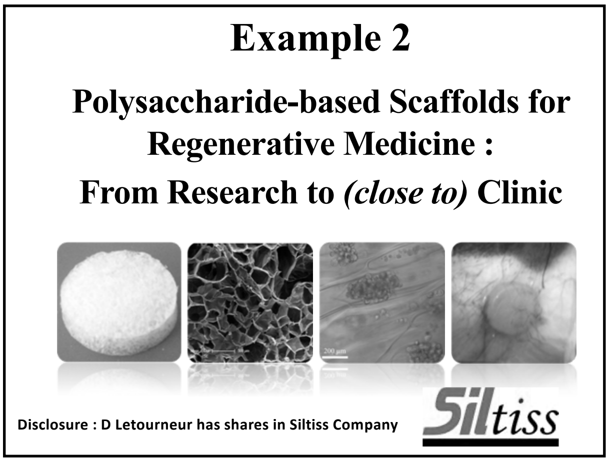

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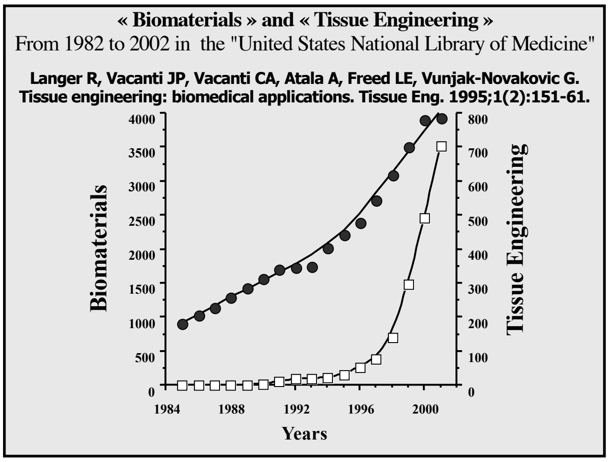

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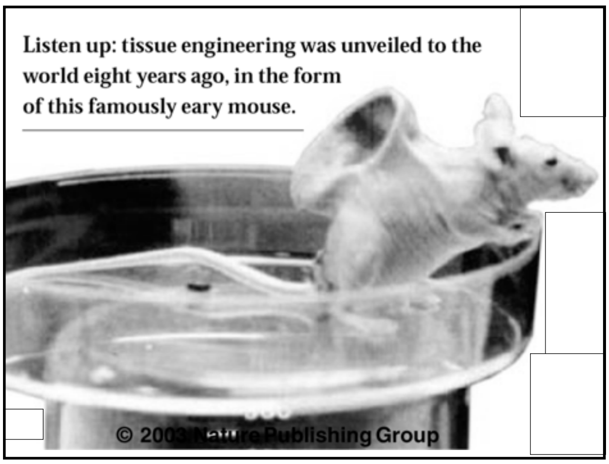

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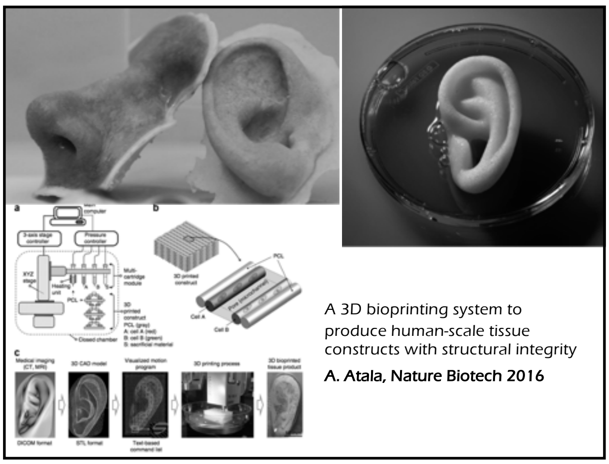

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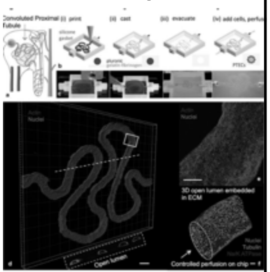
Human kidneys on a chip

Bioprinting of 3D Convoluted Renal Proximal Tubules on Perfusable Chips
KA. Homan et al., Scientific Reports (October 2016)



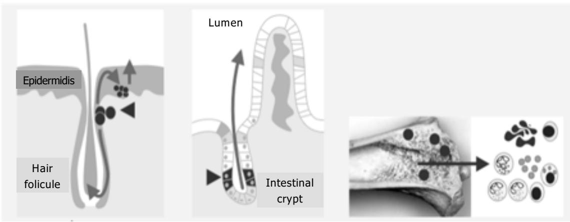
« A bioprinting method for creating 3D human renal proximal tubules in vitro that are fully embedded within an extracellular matrix and housed in perfusable tissue chips, allowing them to be maintained for greater than two months »

25

Several organs in culture

26

Human Stem Cells



Epidermidis	**Intestine**	**Blood cells**
30 days	**10^8 cells/day**	**10^{12} cells/day**

27

human hair implants

Nature Comm 2012

Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches

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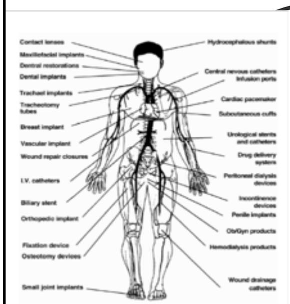
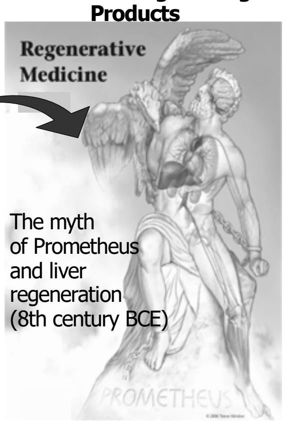
Future ?

Better BIOMATERIALS

And Tissue-engineering Products

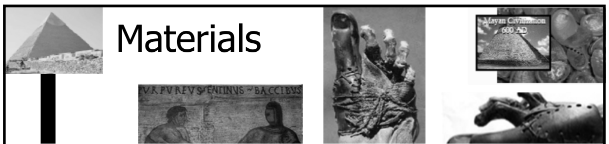




The myth of Prometheus and liver regeneration (8th century BCE)

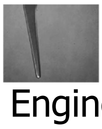
29

Materials



"*A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine.*"

D.F. Williams / Biomaterials 30 (2009) 5897–5909



Tissue Engineering products

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Shift of paradigm

- From replacement/destruction (graft, cancer tumour…)
- To construction/repair
  - Cell repair (gene therapy)
  - Cell engineering (stem cells)
  - Increasing self-healing & defense properties
  - Tissue engineering

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The clinical transfers to patients in Regenerative Medecine

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Reconstructing the epidermis

The three-cell model

Including the skin's defence cells, the Langerhans cells, in a reconstructed epidermis remained a real challenge because these cells divide little *in vivo* and do not multiply at all *in vitro*. In 1992, the discovery of precursors of these cells in umbilical cord blood and in the peripheral blood of adults and the possibility of making them multiply *in vitro* removed this obstacle.

EPISKIN®

Reconstructing an immunosensitive epidermis

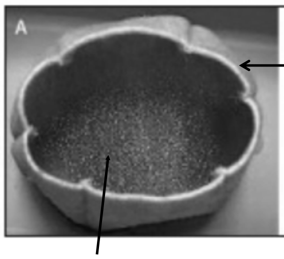
In 1996, L'Oréal researchers involved in a European programme in cooperation with Inserm (French National Medical Research Institute), the Karolinska Institute in Stockholm and the University of Bonn used precursors of Langerhans cells derived from blood. By placing these precursors in a culture medium with melanocytes and keratinocytes, then seeding these cells onto the DED support, the scientists succeeded in constructing a model of the epidermis with a functional horny layer which included the three cell types found in a normal epidermis.

How is a three-cell model reconstructed ?

33

1st clinical trial of a tissue-engineering bladder

Atala et al, *The Lancet*, 2006, 367: 1241



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Tissue Engineered Arteriovenous shunt in humans

Nicolas L'Heureux, Cytograft, CA, USA

Human tissue-engineered blood vessels for adult arterial revascularization

Nicolas L'Heureux[1], Nathalie Dusserre[1], Gerhardt Konig[1], Braden Victor[2], Paul Keire[3], Thomas N Wight[3], Nicolas A F Chronos[4], Andrew E Kyles[5], Clare R Gregory[5], Grant Hoyt[6], Robert C Robbins[6] & Todd N McAllister[1]

Nature Medicine, 2006

NEJM, 2008

Lancet, 2009

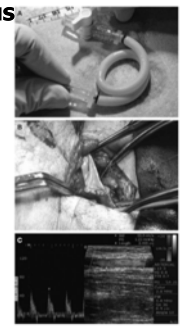

Figure 1 The first clinical use of a tissue-engineered blood vessel for high pressure arterial revascularization. (A) A completely autologous graft was implanted as an arteriovenous shunt between the humeral artery and the axillary vein. (B) The tissue-engineered blood vessel resembled native tissue and had normal surgical handling and suturing properties. The vessel synthesized met safety criteria and showed excellent flow under routine Doppler surveillance. (C) At 6 months, the shunt maintains high flow without signs of restenosis or aneurysm.

Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study

Summary
Background Application of a tissue-engineered vascular graft for small-diameter vascular reconstruction has been a long awaited and much anticipated advance for vascular surgery. We report results after a minimum of 6 months of follow-up for the first ten patients implanted with a completely biological and autologous tissue-engineered vascular graft.

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Materials and/or Cells

Tissue Engineering Products

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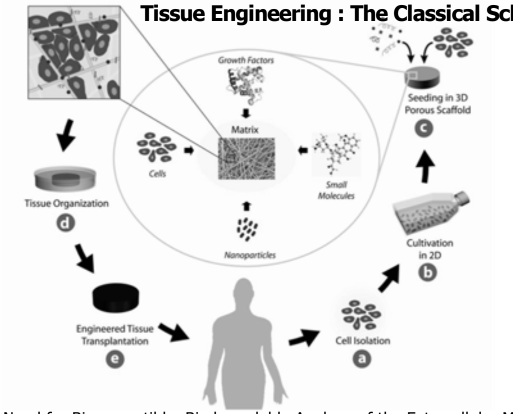
Tissue Engineering : The Classical Scheme



Need for Biocompatible, Biodegradable Analogs of the Extracellular Matrix
=> Scaffolds

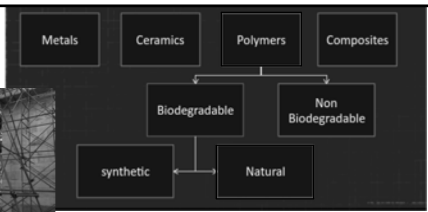
37

Chemistry

BIODEGRADABLE SCAFFOLDS

Biology

38



Scaffolds

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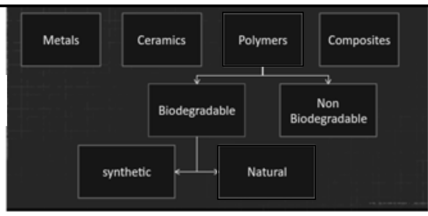
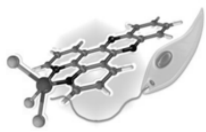
Scaffolds



Polysaccharides for Biocompatible, Biodegradable Analogs of the Extracellular Matrix :
«cell niches»

Biology



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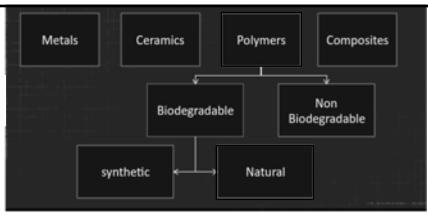
Scaffolds



Polysaccharides for Biocompatible, Biodegradable Analogs of the Extracellular Matrix :
«cell niches»

Polysaccharides	Origin	Function
Cellulose	Plants	Mechanical Support
Amylose	Plants	Energy reservoir
Dextran	Synthesized – bacteria	Matrix for growth
Chitin	Insects, crustaceans	Structural integrity
Glycosaminoglycans	Connective tissues	Mechanical support/motility

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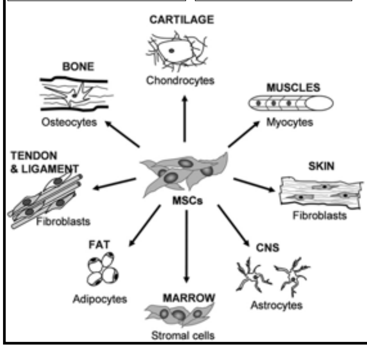
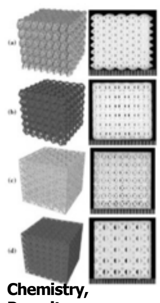
Biology **Chemistry**

STEM CELLS **ADULT CELLS** **SCAFFOLDS**





Chemistry, Porosity, Degradability ...

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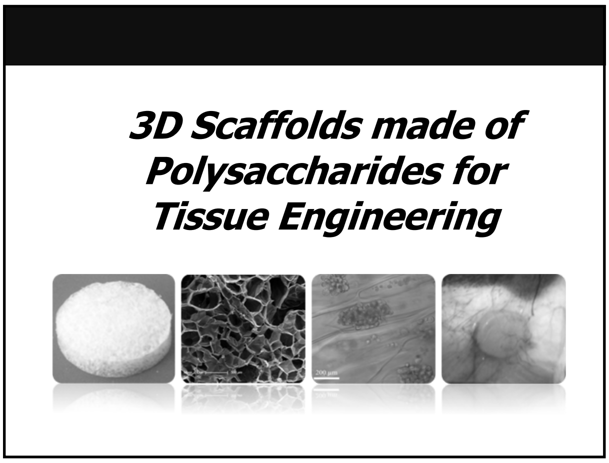

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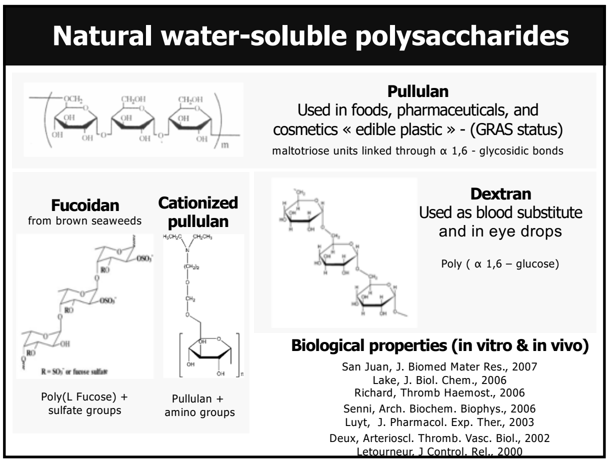

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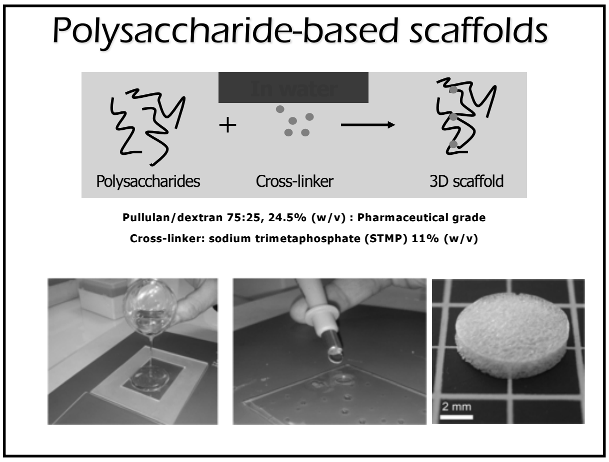

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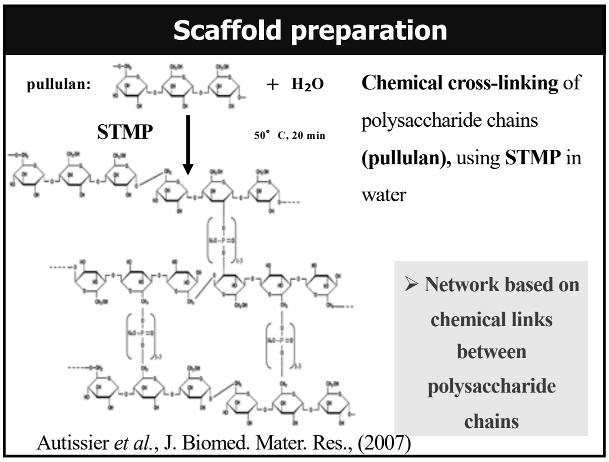

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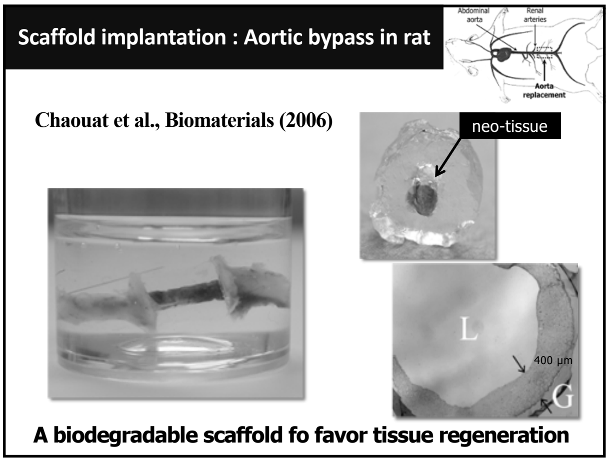

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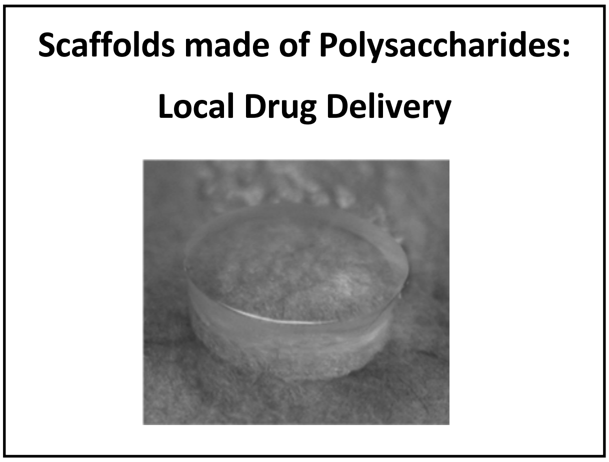

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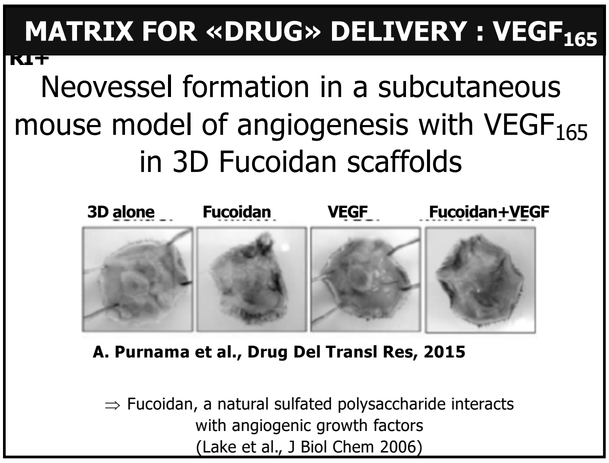

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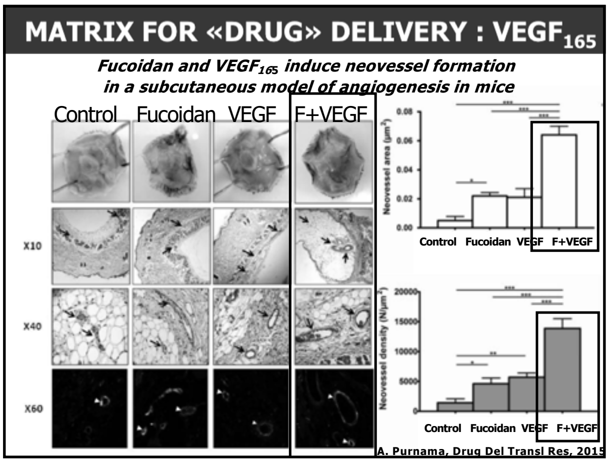

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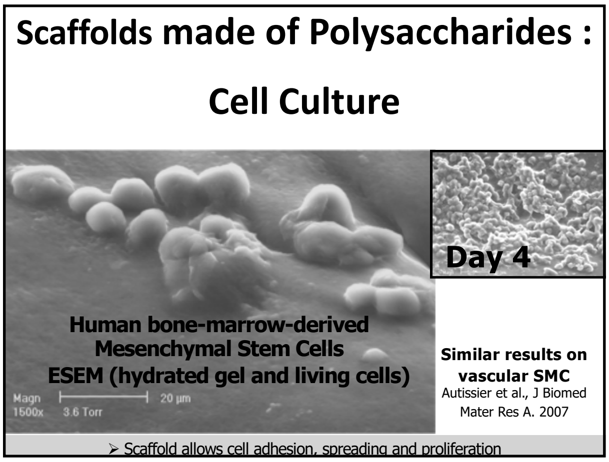

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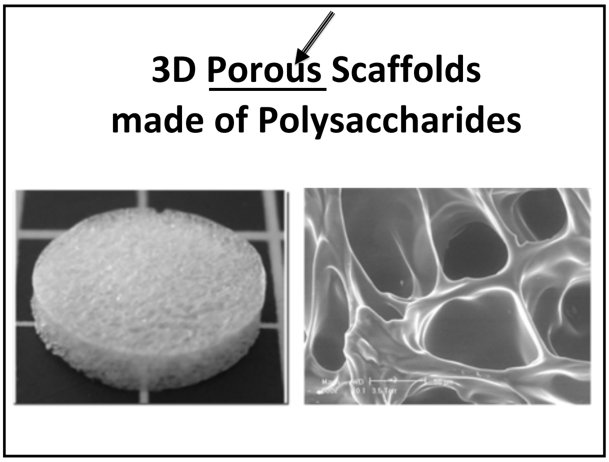

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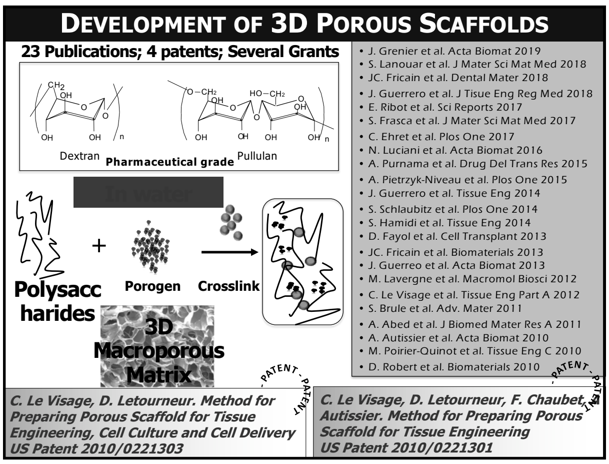

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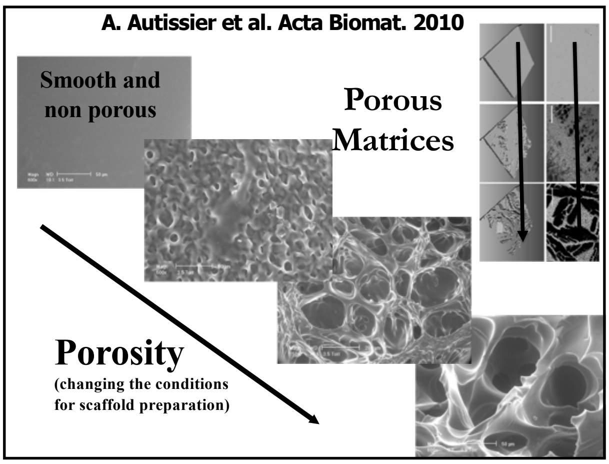

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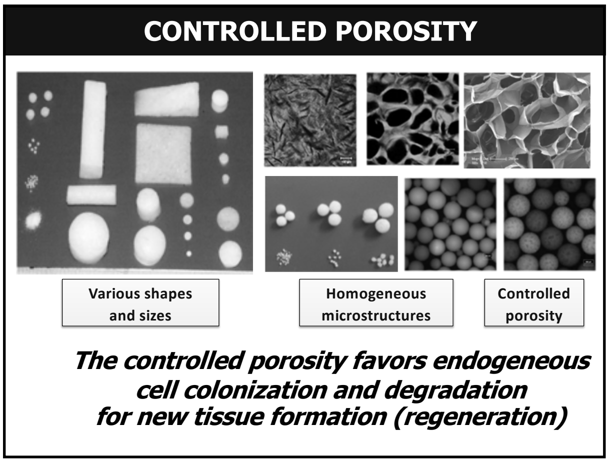

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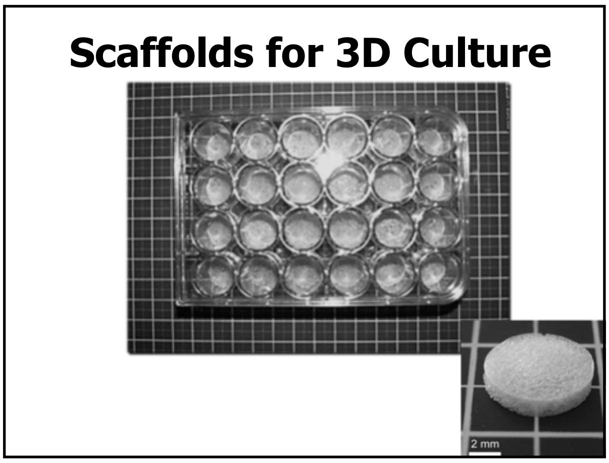

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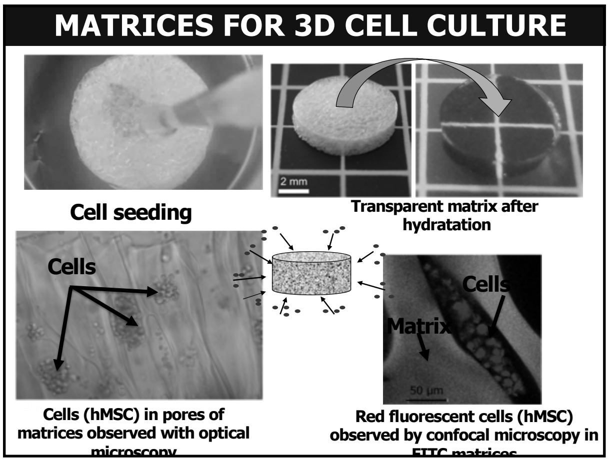

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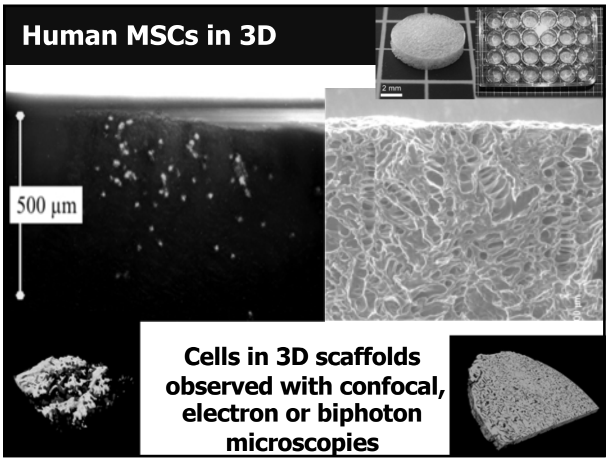

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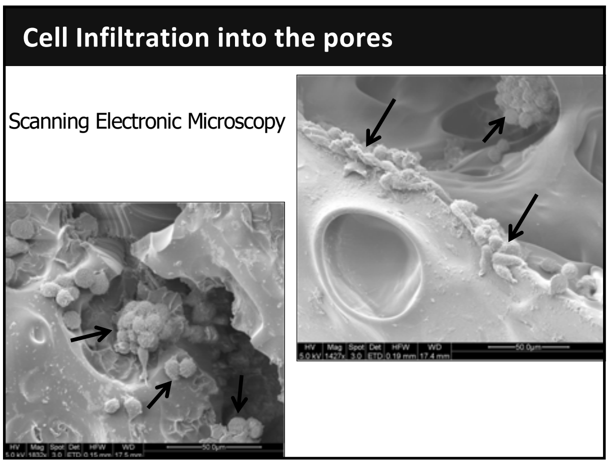
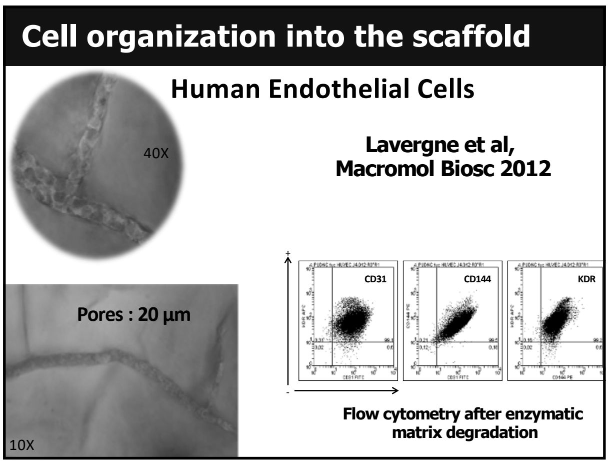


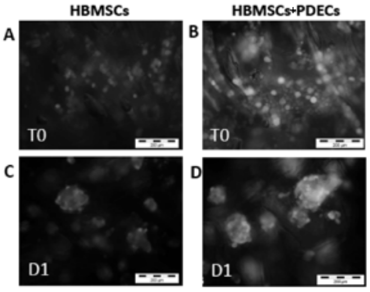
59



60

2 types of cells within the matrix (in vitro)

Human bone marrow mesenchymal stem cells (HBMSCs)
+Human progenitor-derived endothelial cells (PDECs)



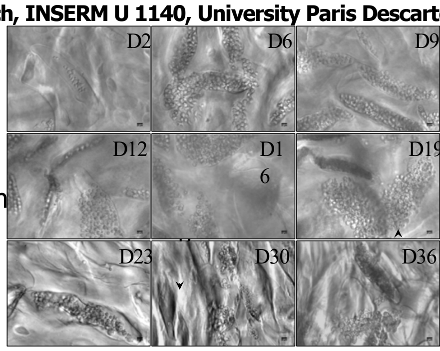
J. Guerrero et al., Acta Biomat. 2013

61

Culture of human Megakaryocytes in 3D

D Baruch, INSERM U 1140, University Paris Descartes

In 3D
>1 month



In Liquid <16 days

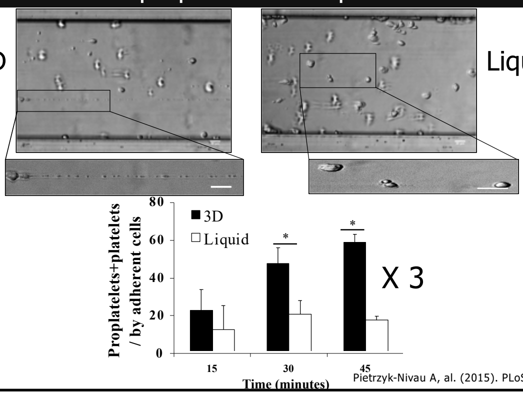
Pietrzyk-Nivau A, al. (2015) Three-Dimensional Environment Sustains Hematopoietic Stem Cell Differentiation into Platelet-Producing Megakaryocytes. PLoS ONE 10(8): e0136652.

62

Megakaryocyte deformations and reorganization into proplatelets and platelets

3D

Liquid



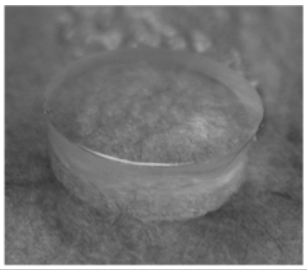
X 3

Pietrzyk-Nivau A, al. (2015). PLoS ONE

63

3D (porous) Scaffolds made of Polysaccharides :

Biodegradation

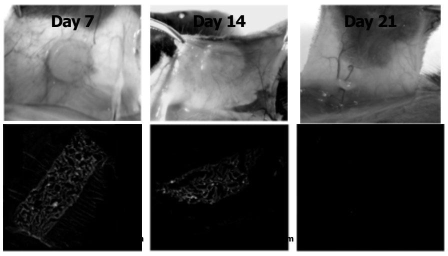


64

IN VIVO DEGRADATION (SUBCUTANEOUS IN MICE)

Macroscopic observation

Fluorescence observation on cross-sections



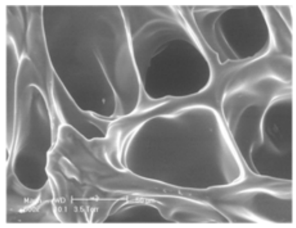
Degradation rate

- **Increases with porosity**
- **Decreases with crosslinker content**

Degradation varies with the implantation site and injury

65

3D Porous Biodegradable Scaffolds made of Polysaccharides



Biomedical uses ?

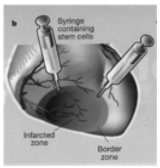
66

3D Scaffolds made of Polysaccharides for Cardiac Cell Therapy

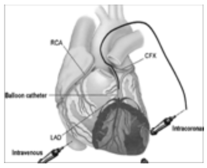
67

How to Deliver Cells to the Infarcted Myocardium ?

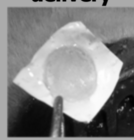
Intramyocardial injection
↓
Local delivery
But limited engraftment



Intracoronary injection
↓
Less invasive
But limited engraftment



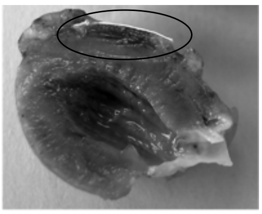
3D scaffold as a cardiac patch
↓
Local and efficient cell delivery



68

In vivo implantation (rat model)

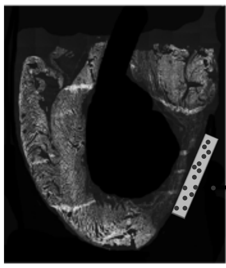
6mm diameter porous scaffold stained with alcian blue



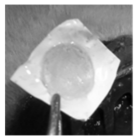
Scaffold implanted onto the heart

69

Transfer of autologous MSCs into the infarcted area



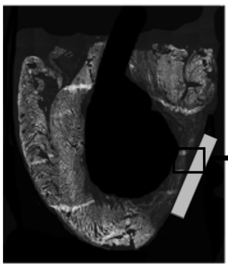
Autofluorescence - Objective x4



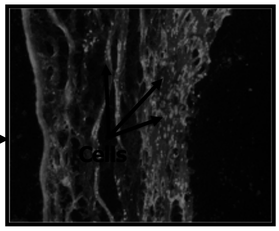
Cellularized Patch for autologous cell therapy (Bone Marrow derived Mesemchymal Stem Cells)

70

Transfer of MSCs into the infarcted area (2 months) + total scaffold biodegradation



Autofluorescence - Objective x4



Merged image of green autofluorescence and red labeled cells in an infarcted myocardial wall - Objective x10

C. Le Visage et al., Tissue Eng, 2012

71

Matrix for Cell Delivery in Heart

RI+

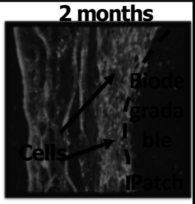
Cardiac Patch for Local Cell Delivery

In rat

	2 months	
	EC	Patch
Residual cell number	61 300 (12400-87400)	163 200 (17500-2213000)
Engraftment (%)	6.1%	16.3%

BETTER CELL TRANSFERT than direct injection

2 months

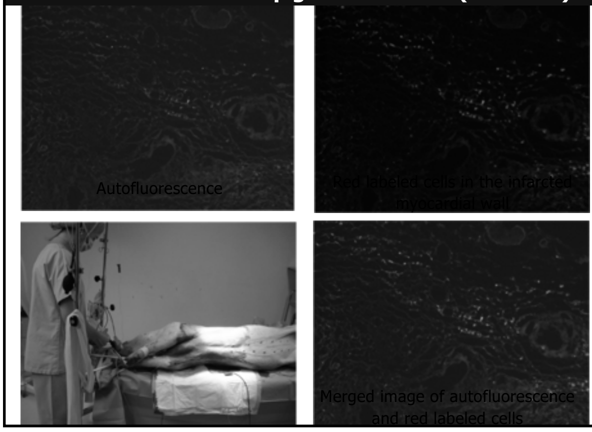


C. Le Visage et al., Tissue Eng, 2012

In Pig (autologous cell therapy)

72

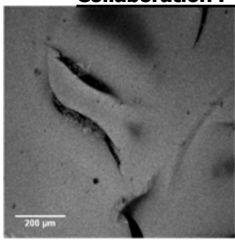
Transfer of cells into the pig infarcted area (2 months)

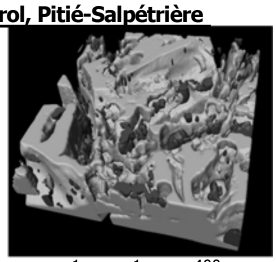


73

In vitro culture of hES cells in porous scaffolds

Collaboration F Norol, Pitié-Salpétrière





1 mm x 1 mm x 400 µm

Undifferentiated cells cultured w/o feeder layer for 2 weeks

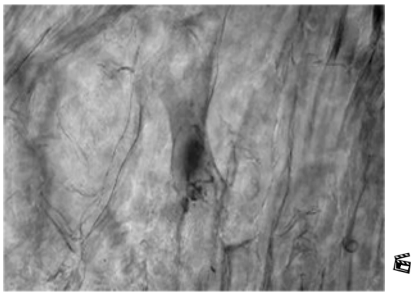
=> 3D Cell culture for ES cells

S Hamidi et al., *Tissue Eng*, 2014

74

Beating Areas in 3D Scaffolds *in vitro*

S Hamidi et al., *Tissue Eng*, 2014

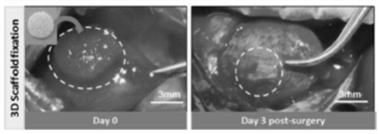


hES-derived cardiomyocytes retain their contractility *in vitro* in 3D conditions for weeks (Pharmaco-toxicological assays)

75

Organ Repair, Hemostasis, and In Vivo Bonding of Medical Devices by Aqueous Solutions of Nanoparticles

3D scaffold fixed on beating heart with nanoparticles



A 3D polysaccharide scaffold fixed to a beating heart

A. Meddahi-Pellé, A. Legrand, A. Marcellan, L. Louedec, D. Letourneur, and L. Leibler

PATENT

A. Pellé et al, Angewandte Chemie Inter Ed, 2014

After 3 days

76

3D Scaffolds made of Polysaccharides for Vascular Tissue Engineering

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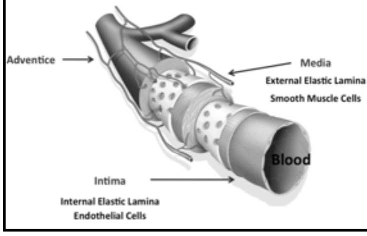
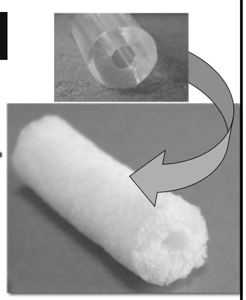
Porous Tubular Scaffolds with Polysaccharides for Vascular Tissue Engineering

BLOOD VESSEL





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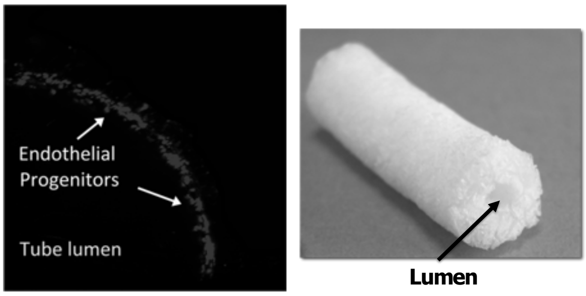
3D VASCULAR TISSUE ENGINEERING IN VITRO

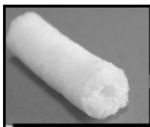


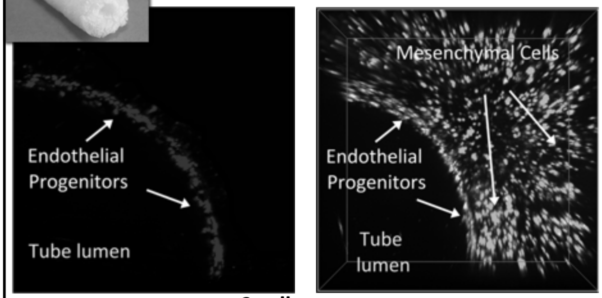
3D reconstruction on the full tube

Collaboration
F Gazeau, C Wilhelm, Univ. P7 - CNRS

79

3D VASCULAR TISSUE ENGINEERING (in vitro)





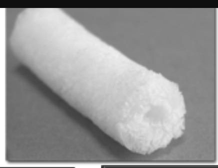
2 cell types :
human endothelial progenitors + mesenchymal stem cells

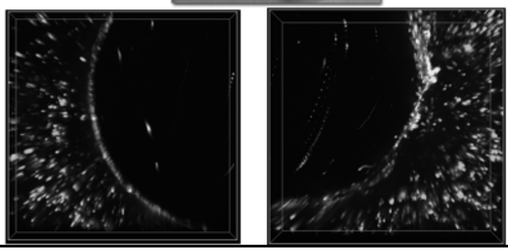
D. Fayol et al. Cell Transpl 2013

80

IN VITRO BLOOD VESSEL ENGINEERING

Human endothelial progenitors (red) in the lumen, and MSCs (green) in the internal structure





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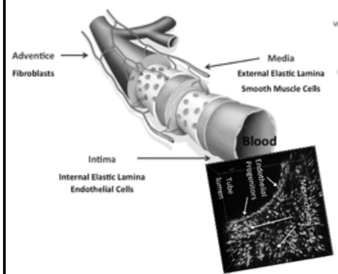
IN VITRO BLOOD VESSEL ENGINEERING



D Fayol et al, Nov 2013

Cover

Cell Transplantation

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3D Scaffolds made of Polysaccharides for Skin Tissue Engineering

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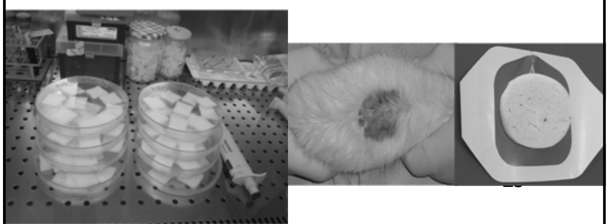
MATRICES FOR SKIN REGENERATION

Skin Regeneration

Burns or irradiations (rat)



Ongoing study with a private company
ANR 2017 Healskin + PhD Cifre

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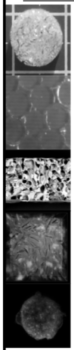
3D Scaffolds made of Polysaccharides for Liver Repair

ANR RHU iLite

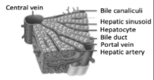
85

RHU Ilite (2017-2021)

Inserm



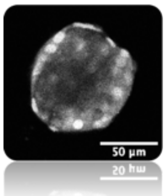
iLite
Innovation in Liver Tissue Engineering



Porous 3D polysaccharide matrices as a scaffolds for hepatic organoids

T. Simon-Yarza et al. Mater Sci Eng C . 2021

ML Labour et al. Int J Mol Sci. 2020

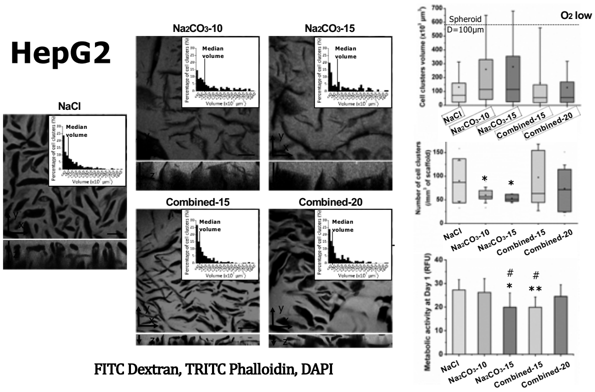


Camille Le Guilcher
Marie-Nöelle Labour
Soraya Lanouar
Rachida Aïd
Teresa Simon-Yarza

86

3D scaffolds for liver spheroids

HepG2



FITC Dextran, TRITC Phalloidin, DAPI

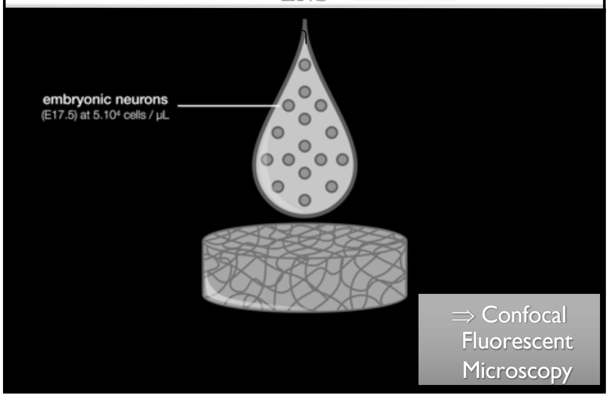
ML Labour et al., Int J Mol Sci. 2020

87

3D Scaffolds made of Polysaccharides for Brain Repair

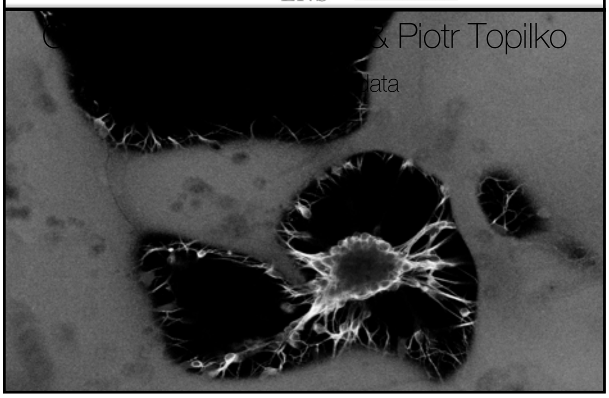
88

Inserm La science pour la santé From science to health | Fondation Bettencourt Schueller | ENS | IBENS | Sorbonne Université Créateurs de futurs depuis 1257



89

Inserm La science pour la santé From science to health | Fondation Bettencourt Schueller | ENS | IBENS | Sorbonne Université Créateurs de futurs depuis 1257



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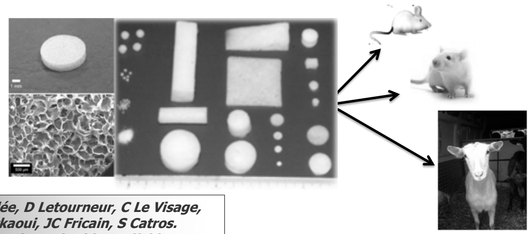
3D Scaffolds made of Polysaccharides for Bone Tissue Engineering

91

Bone repair with *NON CELLULARIZED* MATRICES

A macroporous osteoinductive polysaccharide – based matrix produced at different sizes and shapes



J Amédée, D Letourneur, C Le Visage, SM Derkaoui, JC Fricain, S Catros. Porous polysaccharide scaffold comprising hydroxyapatite and use for bone formation EP2011/064924 (2011) (WO 2012)

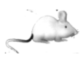
PATENT - PATENT

with Inserm U1026; Bordeaux

92

Matrix+HA without cells induces bone formation in non osseous site (*small and large animals)*

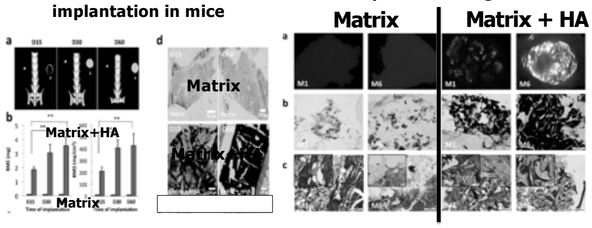
with Inserm U1026; Bordeaux



JC Fricain et al, *Biomaterials* 2013

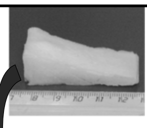
Formation of a mineralized bone tissue after subcutaneous implantation in mice

Formation of a mineralized bone tissue after intramuscular implantation in goat

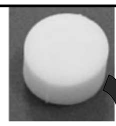


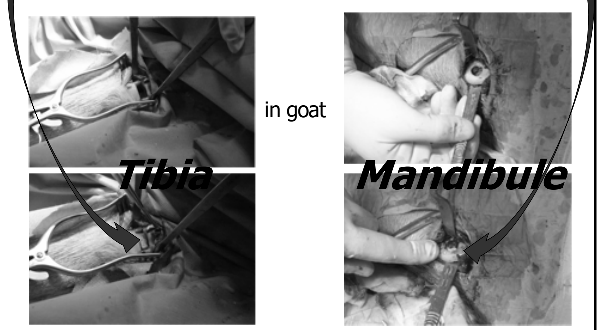
=> Osteoinductive Matrix+HA

93



Orthopedic use
Bone tissue engineering





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3D Scaffolds for Tissue Engineering

- *3D Matrices*

- MicroMatrices : Microsystems for Injectable drug/cell delivery

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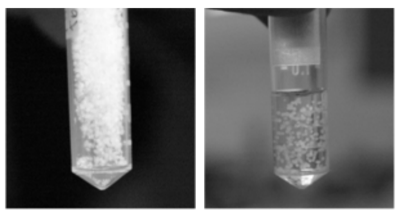
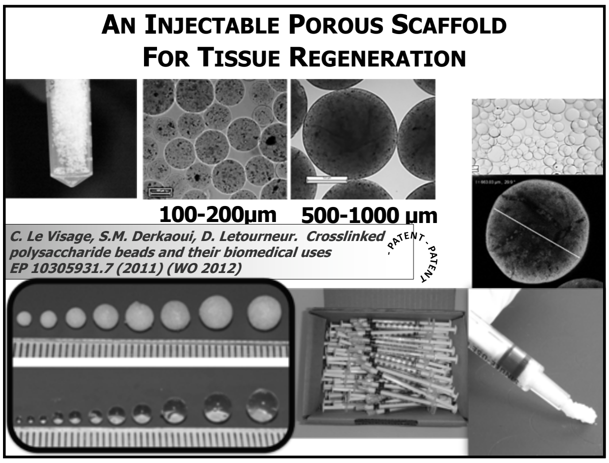
3D POROUS POLYSACCHARIDE BEADS

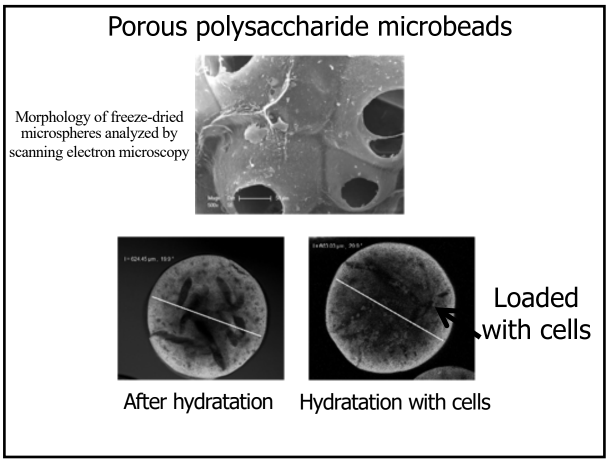


Brulé et al, Advanced Materials 2011

96



97



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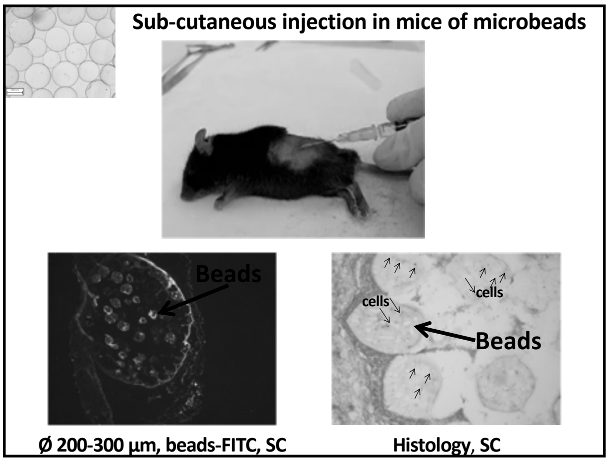
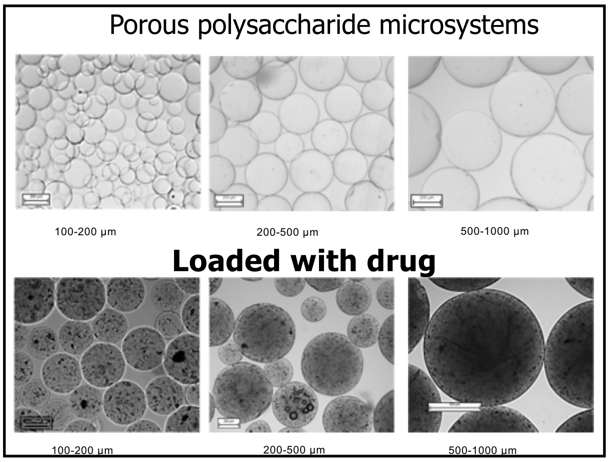
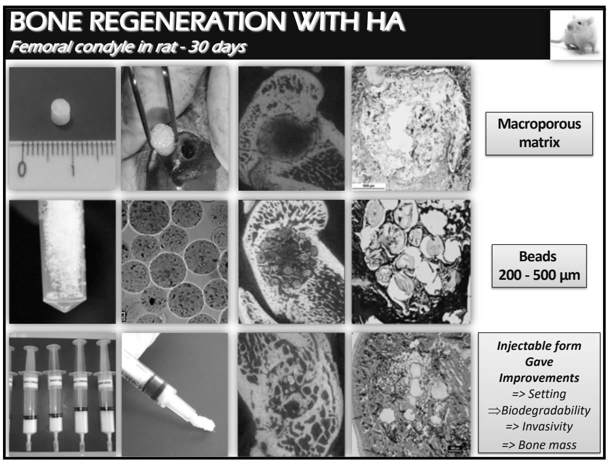


99

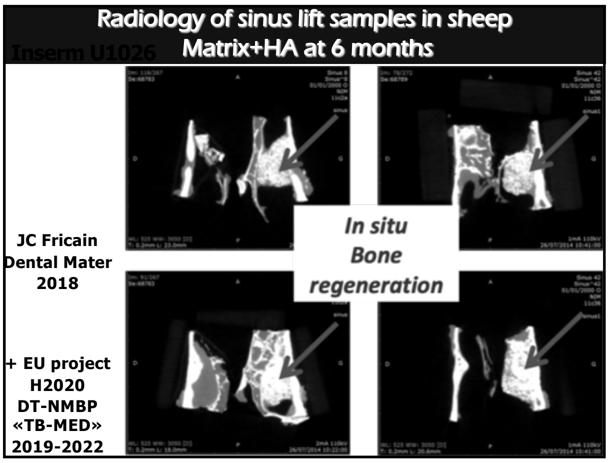


100

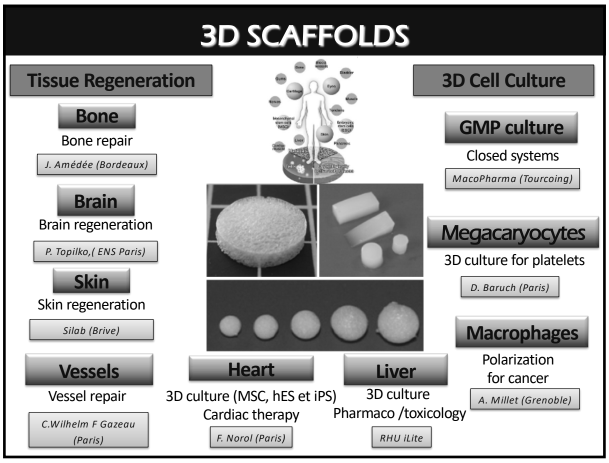


101

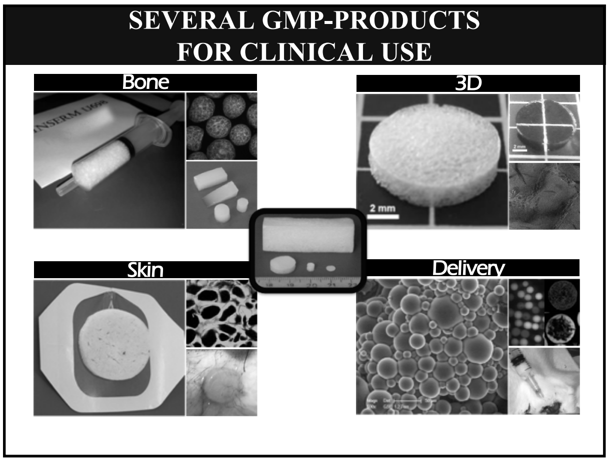
102



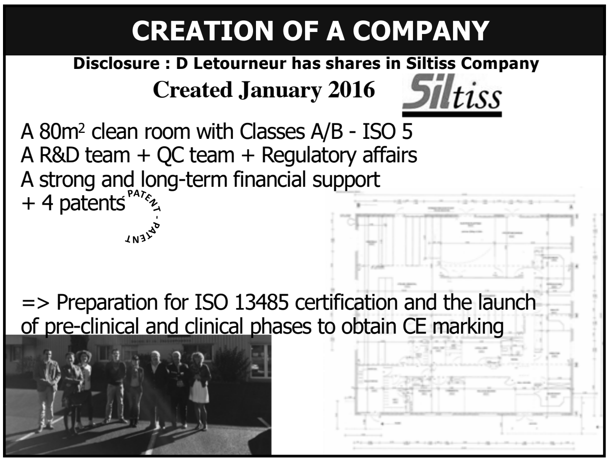
103



104



105



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COMPANY

A 80m² clean room with Classes A/B - ISO 5
A R&D team
A strong and long-term financial support

A Production site (500m²)

109

Example 3

Nanosystems made of Polysaccharides for Drug Delivery And Molecular Imaging

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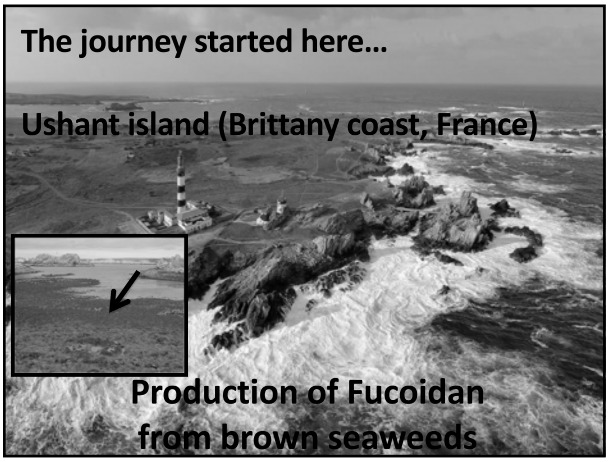
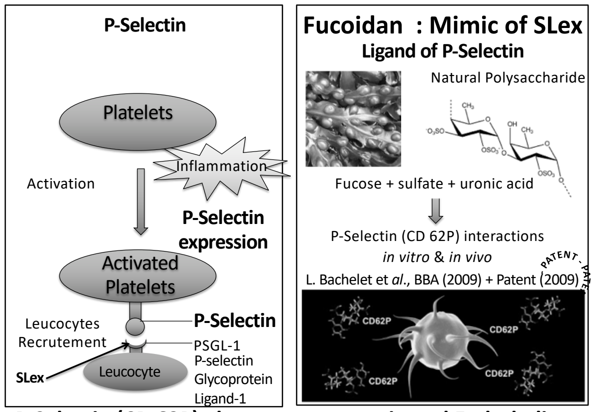


111



P-Selectin (CD 62P) also present on activated Endothelium

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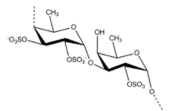
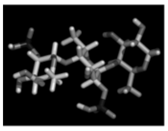
FACILITIES



Fucoidan :
sulfated polyfucose



2015 : Agreement for LMW fucoidans
=> Pharmaceutical use ingredient (ANSM)

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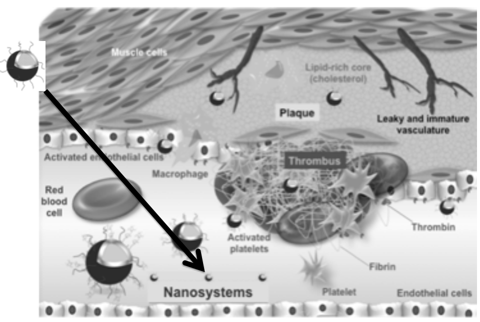
Targeted Nanosystems for Imaging of Thrombus



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FP7
2013-2018
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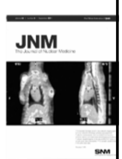
Coordinator

NanoAthero



AKA Silva et al, Theranostics + Cover June 2014
F. Rouzet et al, J Nucl Med + Cover Sept 2011

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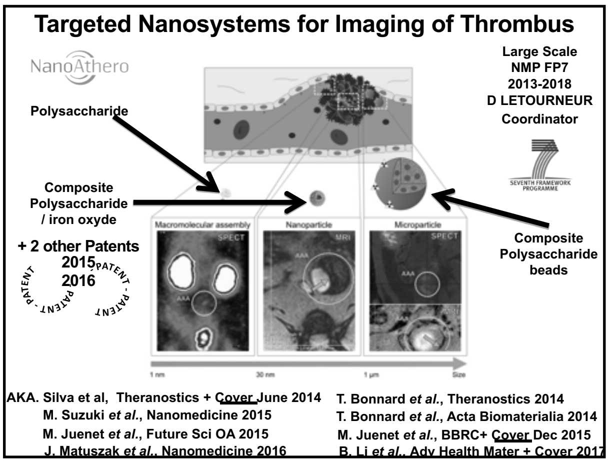

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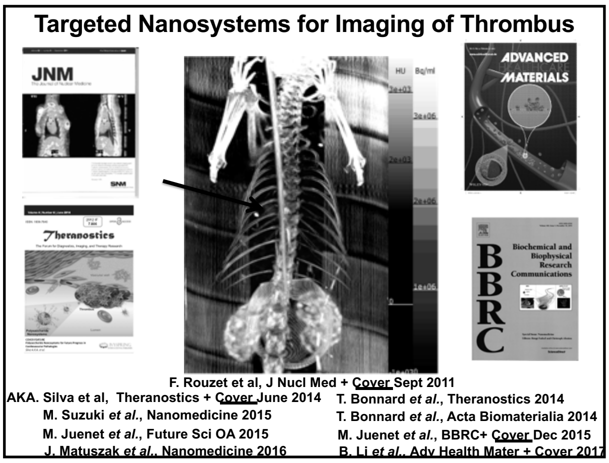

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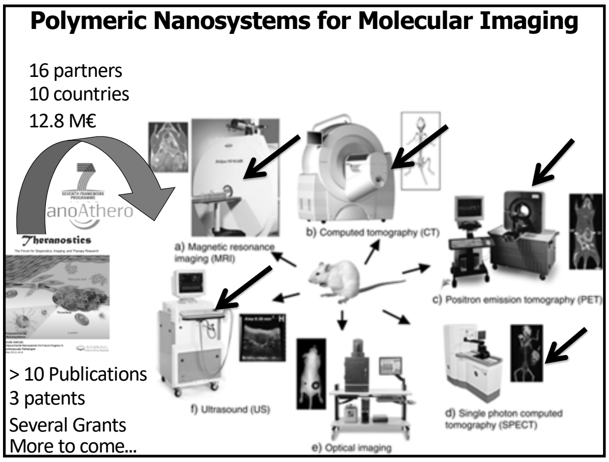

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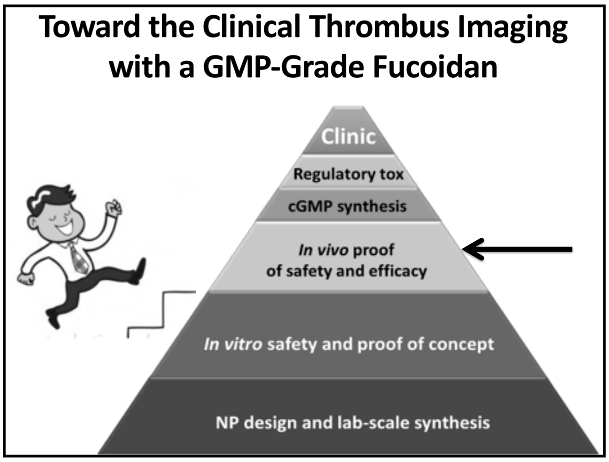

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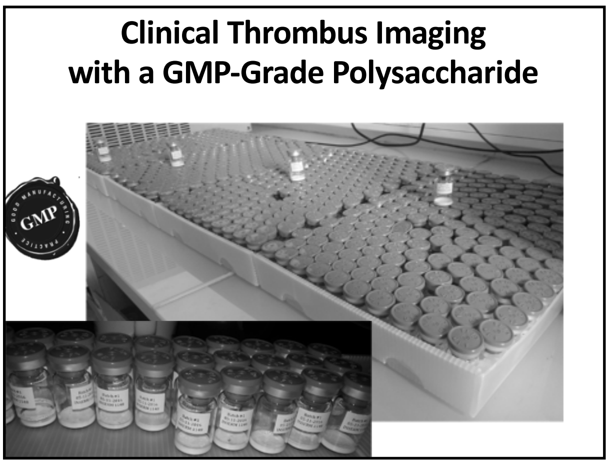

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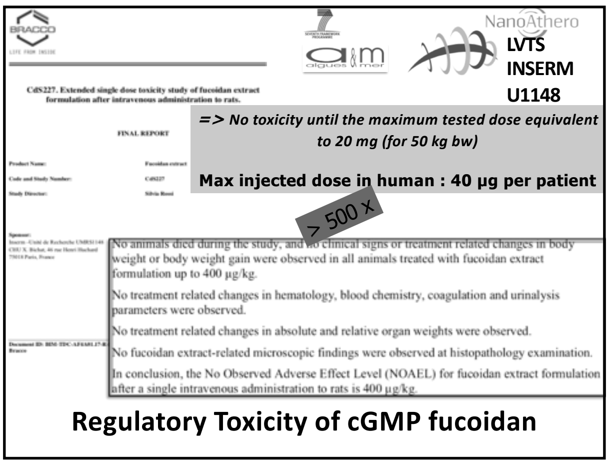

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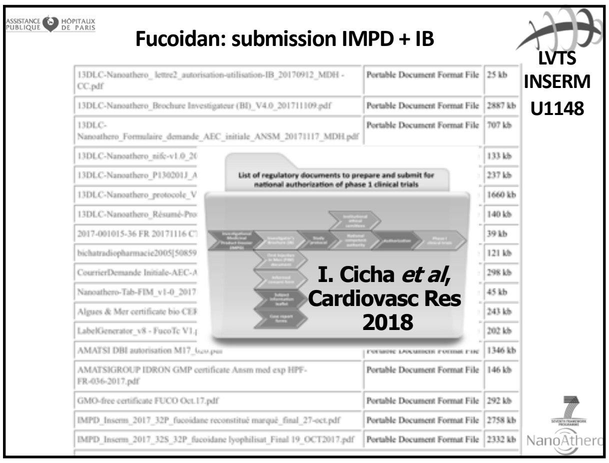

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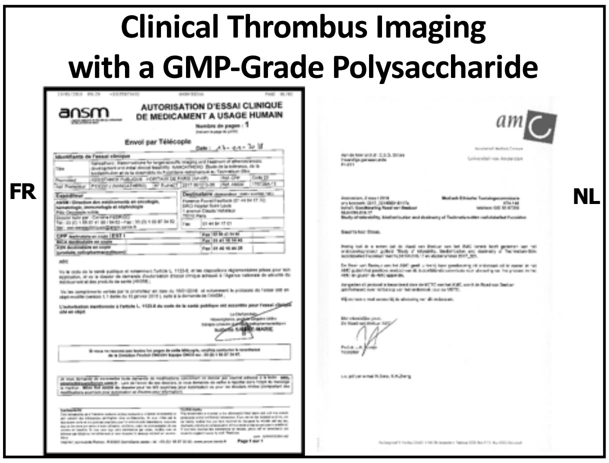

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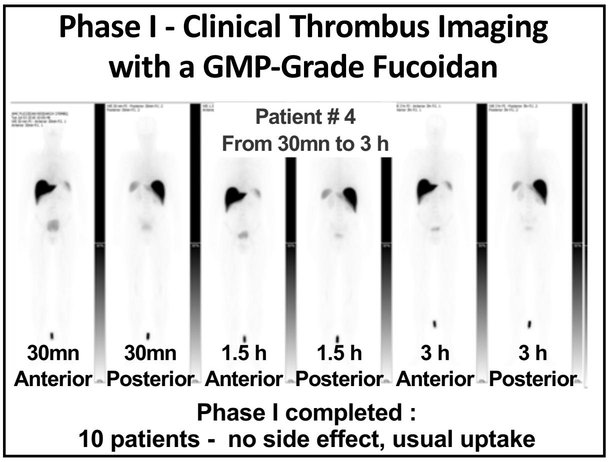

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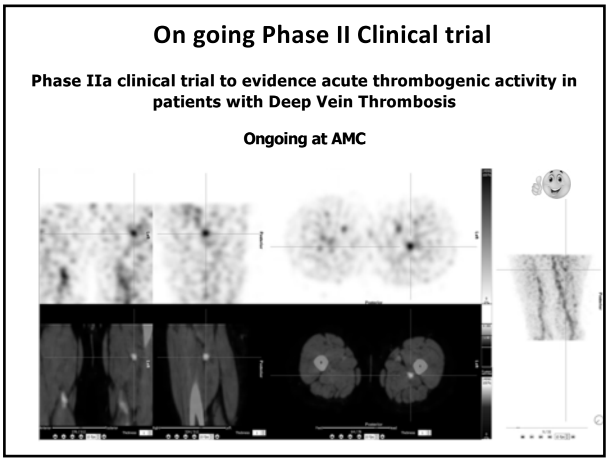

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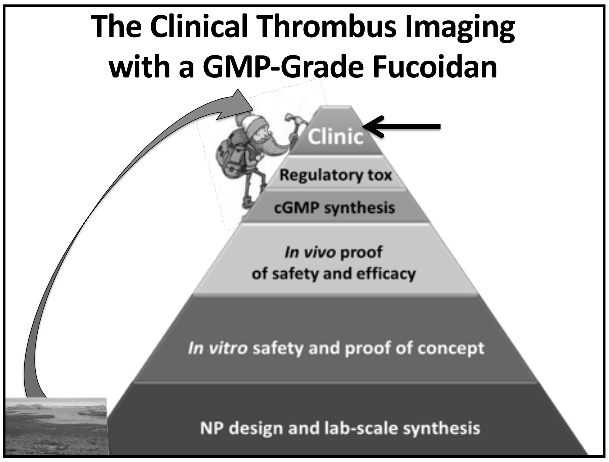

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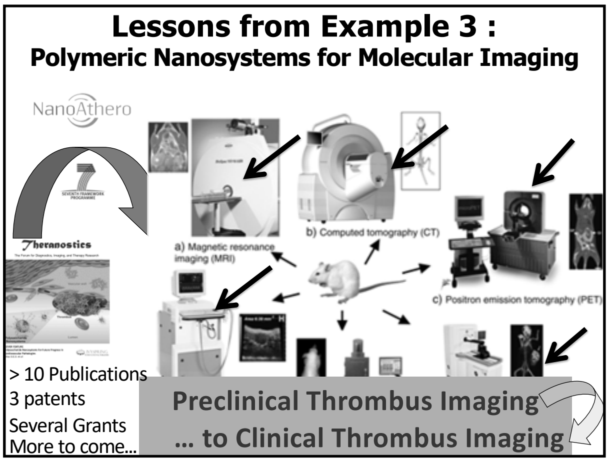

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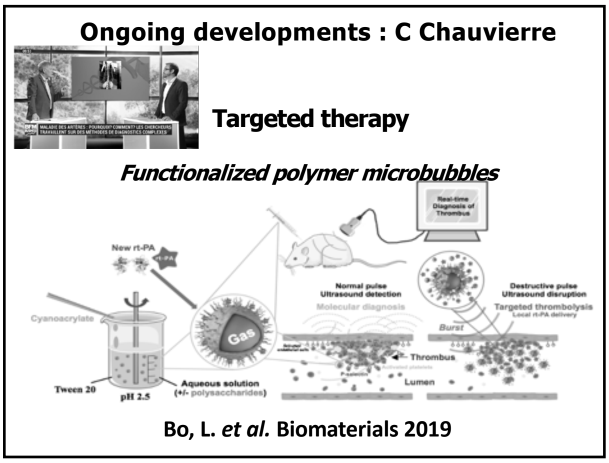

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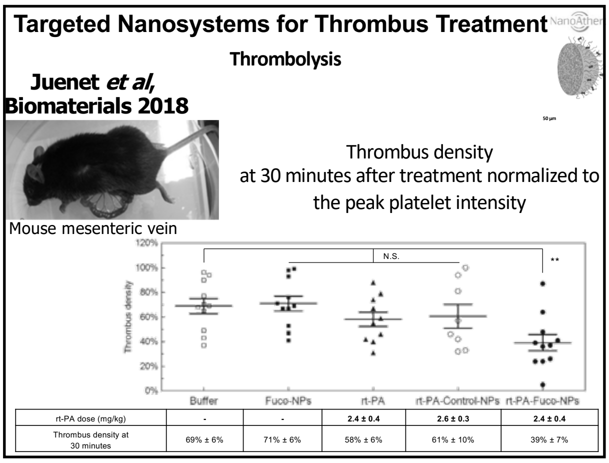

rt-PA dose (mg/kg)	-	-	**2.4 ± 0.4**	**2.6 ± 0.3**	**2.4 ± 0.4**
Thrombus density at 30 minutes	69% ± 6%	71% ± 6%	58% ± 6%	61% ± 10%	39% ± 7%

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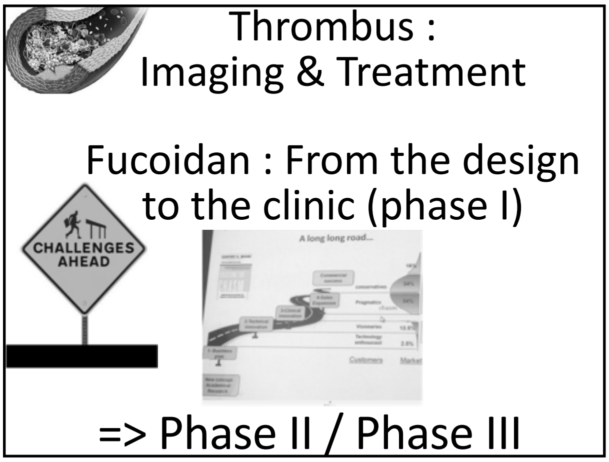

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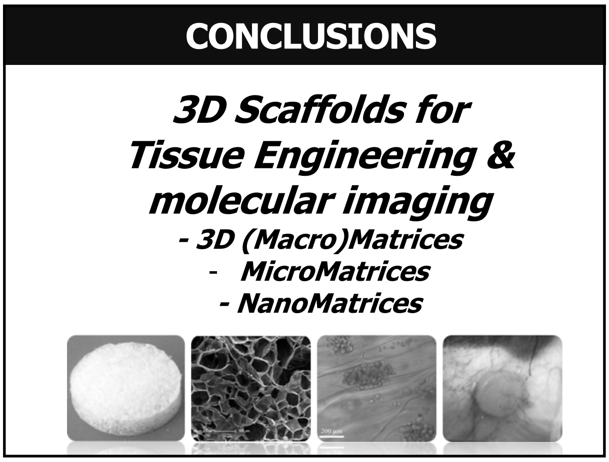

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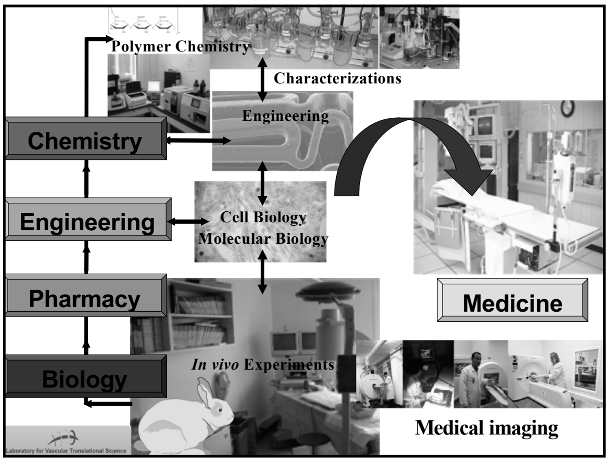

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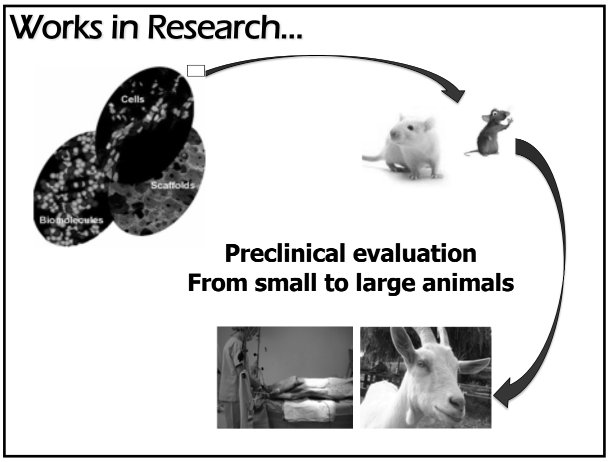

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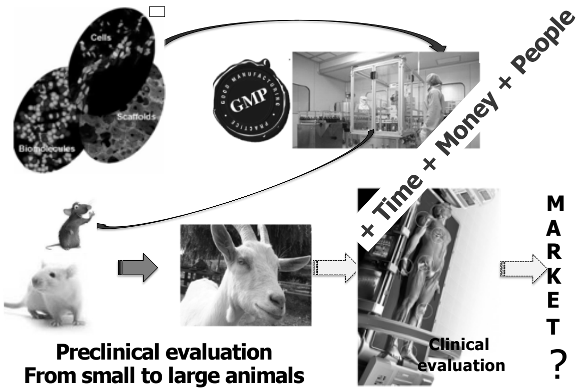
Challenges From Research to the Market



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How to produce ?

The largest organ, which 4-5 square meters, is the skin

For a single patient ?

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Improvements in manufacturing efficiencies are helping to enable commercially viable business models. Here a technician from Cytograft Tissue Engineering uses an iPad-based quality and tracking system to oversee automated feeding in a closed system bioreactor to produce an off-the-shelf, allogeneic blood vessel.

GMP Production of Tissue Engineering Products

Cytograft Tissue Engineering's team works to produce tissue engineered blood vessels for clinical use in a GMP facility in Novato, California.

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Plenty of ideas…

…for a profitable product !!!

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A strong IP

Or well-protected (?) know-how

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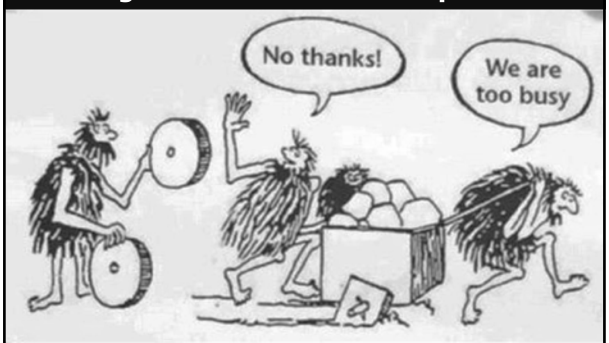
A strong IP Portfolio with new protections



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A strong IP Portfolio with new protections

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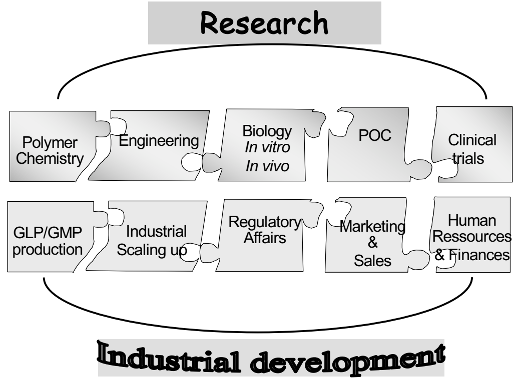
A need of large expertise



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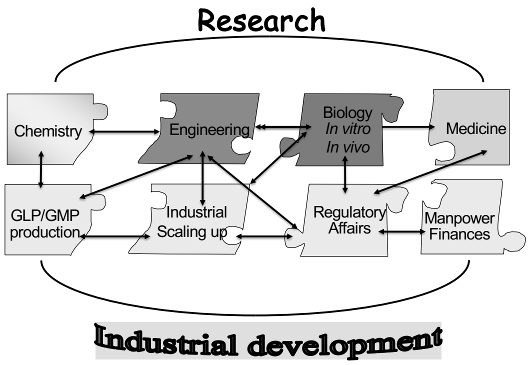
A need of large expertise



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Examples of industrial and clinical transfers of research works on biomaterials

D. Letourneur

Inserm U1148, Université Sorbonne Paris Nord, Université de Paris

CHU X Bichat, 75018 Paris, France

BIOMAT — RÉPARER L'HUMAIN

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- ✓ ANR BIOTECS (2009); ANR Emergence Bio (2009); ANR Blanc (2010); ANR TECSAN (2010) (X2) ; ANR Emergence (2010) (X2); ANR France-Singapour (2010); ANR TECSAN (2012); ANR Emergence (2012); ANR Labcom (2013); ANR RPIB (2013); ANR Défi Santé (2014) (X2); ANR Renouveau Industriel (2015); ANR Défi Santé (2017); ANR Astrid (2019); ANR Santé (2020)
- ✓ FUI11/Medicen(2011); SATT IdFInov (2014); RHU Ilite (2016)

Large Scale NMP 2013-2018 NanoAthero

- ✓ European Community : FP7 (2008) Health Large Scale (Coordinator); FP7 (2009) NMP; FP7 (2010) Health; FP7 (2012) NMP Large Scale (Coordinator); H2020 TB-MED (2019)

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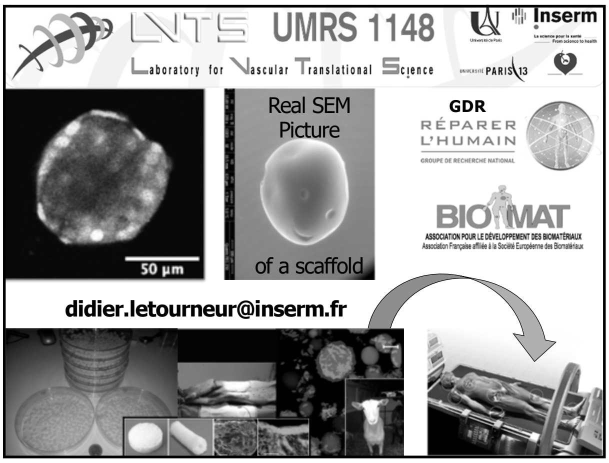
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Real SEM Picture
of a scaffold
50 µm
GDR
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La science pour la santé
From science to health
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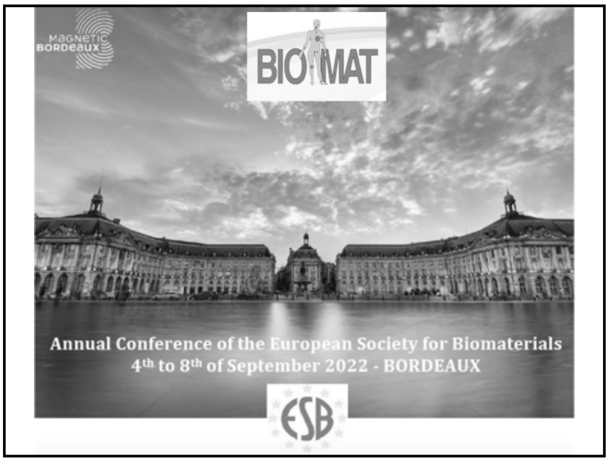
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