

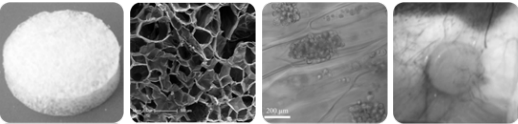


Examples of industrial and clinical transfers of research works on biomaterials


D. Letourneur




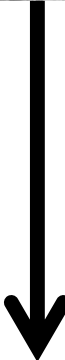
Institut de Recherche sur le Médicament et l'Innovation Thérapeutique - IRMIT

2^{ème} Journée Scientifique IRMIT
Lundi 7 décembre 2020

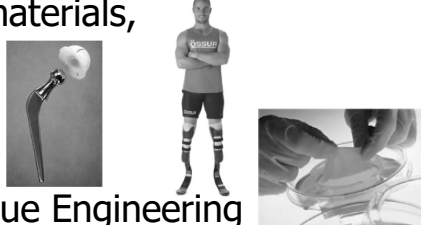
1


Materials,





"Bio"materials,




Tissue Engineering

2

Materials => Biomaterials


2300 years BC



An artificial Toe

Use of Linen sutures

Before Christ

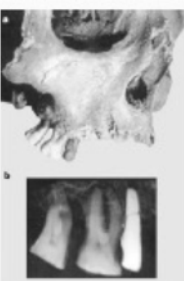



Use of silk sutures thread for gladiators

3

Materials => Biomaterials

Dental implants 600-100 BC






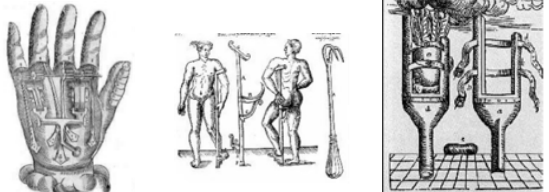
Dental implants in metal for the first premolar (Gallo-Roman necropolis, France).
Osseointegration is viable. The implant have been placed after the tooth drop

NATURE | VOL 391 | 1 JAN 1998

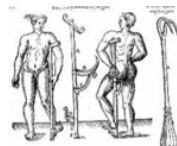
4




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



6

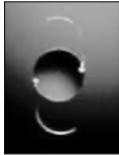
Modern «bio»materials



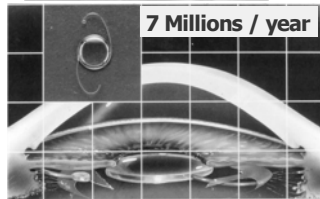
Sir Harold Ridley (1906-2001)
Ophthalmologist



PMMA Whindshield



1950



7 Millions / year

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

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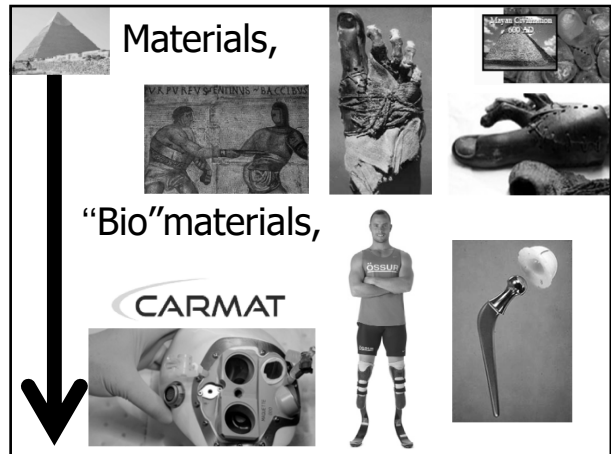
BIOMATERIALS

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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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 Rangan,^f Aicha Abed, MSc,^{g,h} Muguette Ba, MD,^g Anthony Paolitto,^g Didier Letourneur, PhD,^{g,h} and
Anne Meddahi-Pellé, MD, PhD^{g,h}

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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,^{1,2} Isabelle Di Centa, MD,² François Raoux, MD,⁴ Neil Bulman-Fleming,⁵ Adrian Ranga,⁶ Aicha Abed, MSc,^{2,3} Maguette Ba, MD,² Anthony Paolitto,² Didier Letourneur, PhD,^{2,3} and Anne Meddahi-Pellé, MD, PhD^{2,3}

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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.

Ongoing 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
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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**

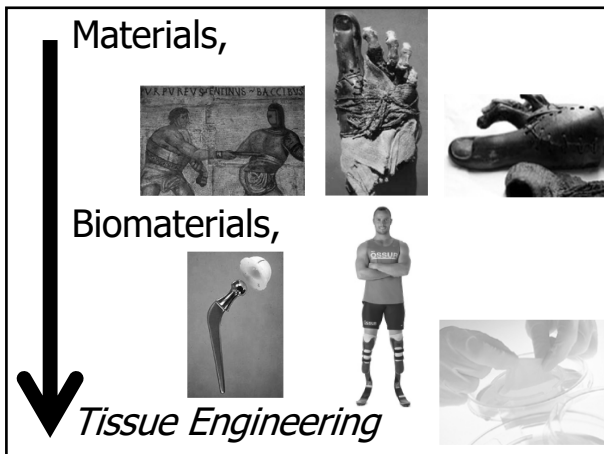
Autologous pericardium valve

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

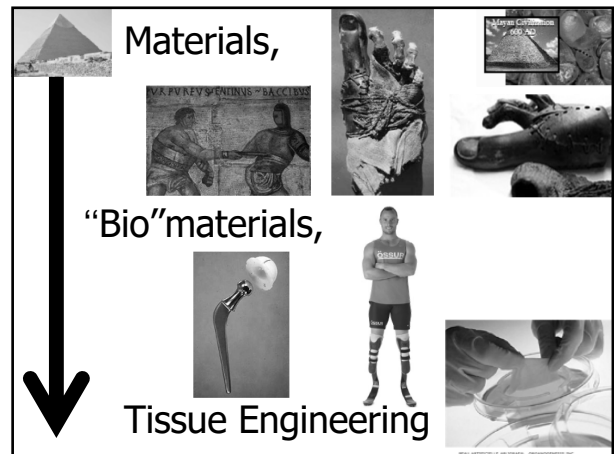
Publication submitted. Clinical trial anticipated

+ Another New Research Project

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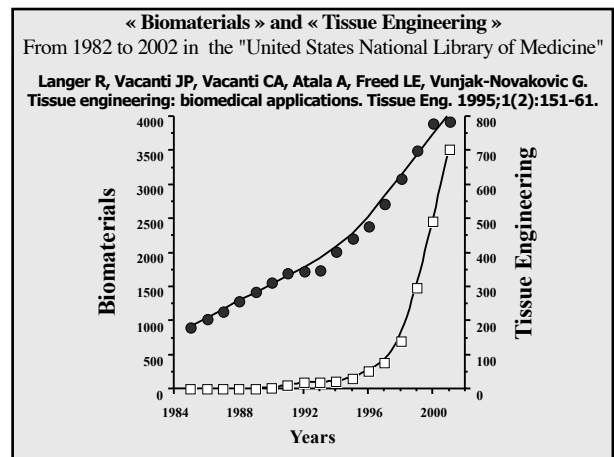
20

Example 2

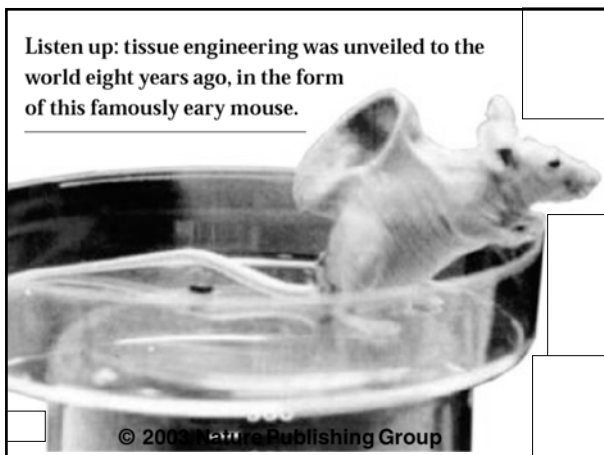
Polysaccharide-based Scaffolds for Regenerative Medicine : From Research to (close to) Clinic

Disclosure : D Letourneur has shares in Siltiss Company **Siltiss**

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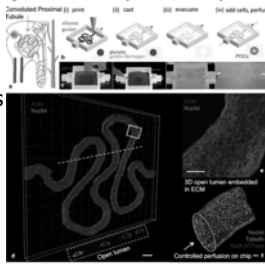
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A 3D bioprinting system to produce human-scale tissue constructs with structural integrity
A. Atala, *Nature Biotech* 2016

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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 »

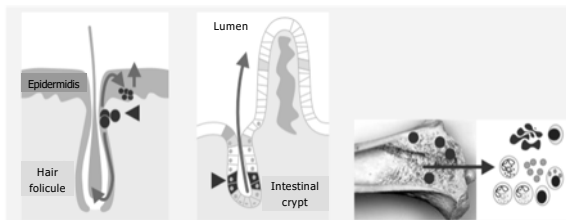
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Several organs in culture

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Human Stem Cells



Epidermis
30 days

Intestine
 10^8 cells/day

Blood cells
 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



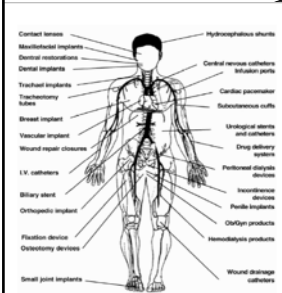
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Future ?

Better **BIOMATERIALS**

And Tissue-engineering Products

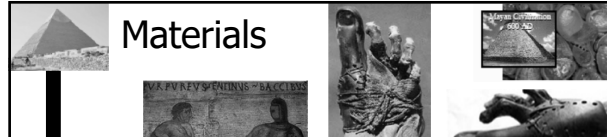
Regenerative Medicine



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

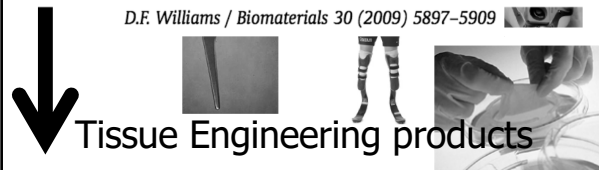
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Materials



"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

30

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 Medicine

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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 sending these cells onto the DEJ 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?

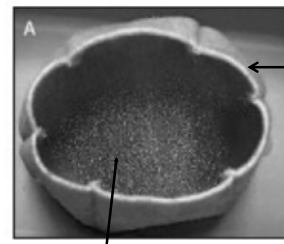


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1st clinical trial of a tissue-engineering bladder

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

collagen/ PLGA



Urothelial cells

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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'Heureux¹, Nathalie Dussort², Gerardo Kovic³, Braden Victor⁴, Paul Katic⁵, Thomas N Wright⁶, Nicola A F Chronos⁷, Andrew E Kiko⁸, Clare R Gregory⁹, Grant Hoyt⁹, Robert C Robbins⁹ & Todd N McAllister¹

Nature Medicine, 2006

NEJM, 2008

Lancet, 2009

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

Todd N McAllister, Neesh Monsoorwalli, Sagar A Gauria, Hisham Wajsbaybawi, Nathalie Dussort, Akshat Maruti, Ruyard Dugali, Alexander Havel, Arnon Avila, Alexander Goren, Jorg Anwerli, Atila Enche, Maria Zentgraf, Leif Cople, Lisa M Anselmetti, Michel Chouhan

Summary
The potential application of a tissue-engineered vascular graft for modification of vascular reconstruction has been demonstrated and such anticipated advance for vascular surgery. We report results after a minimum of 4 months of follow-up for the first ten patients implanted with a completely biological and autologous tissue-engineered vascular graft.

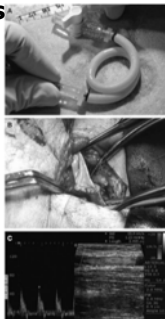


Figure 1 The first clinical use of a tissue-engineered blood vessel for high pressure arterial revascularization. (A) A completely autologous graft was implanted as an arteriovenous shunt between the humeral artery and the axillary vein. (B) The tissue-engineered blood vessel resembled native tissue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

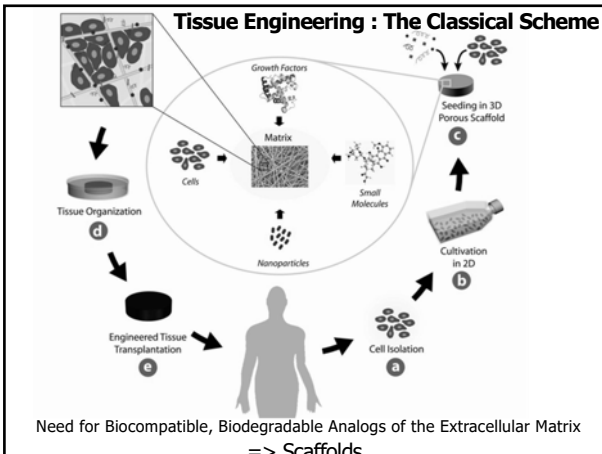
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Materials and/or Cells

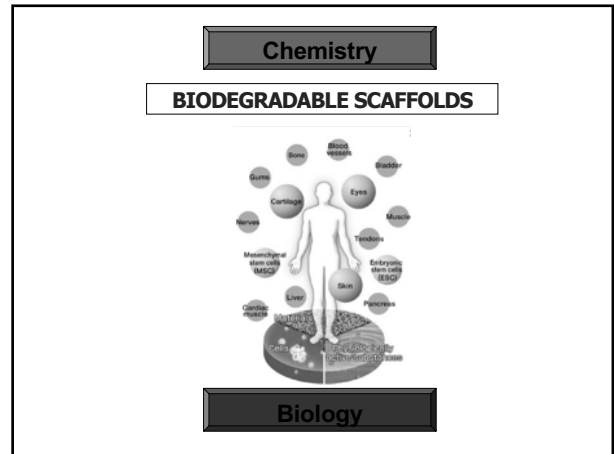


Tissue Engineering Products

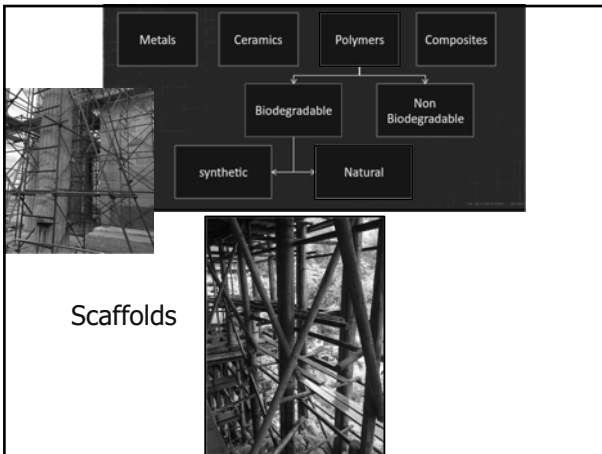
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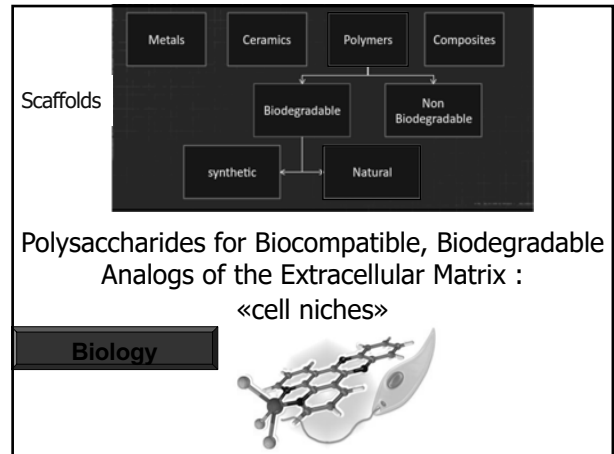
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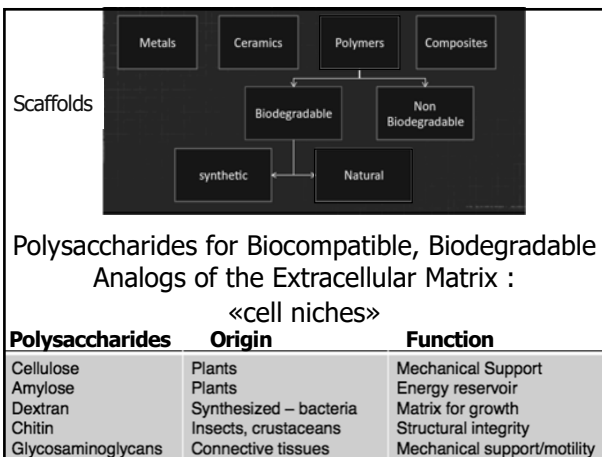
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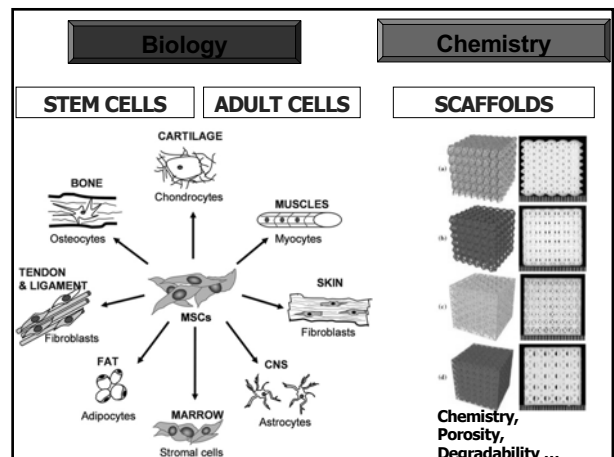
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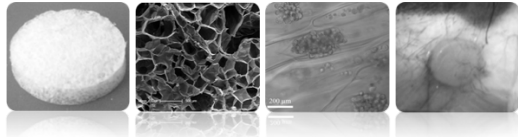


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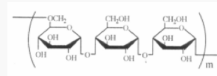
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3D Scaffolds made of Polysaccharides for Tissue Engineering



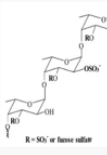
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Natural water-soluble polysaccharides



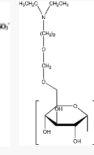
Pullulan
Used in foods, pharmaceuticals, and cosmetics « edible plastic » - (GRAS status)
maltotriose units linked through α 1,6 - glycosidic bonds

Fucoidan
from brown seaweeds

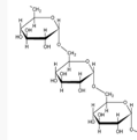


Poly(L Fucose) + sulfate groups

Cationized pullulan



Pullulan + amino groups



Dextran
Used as blood substitute and in eye drops

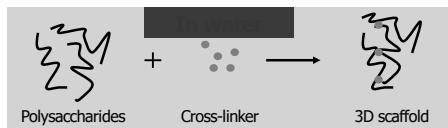
Poly (α 1,6 - glucose)

Biological properties (in vitro & in vivo)

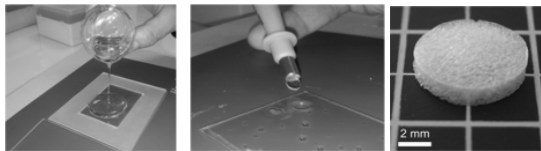
San Juan, J. Biomed Mater Res., 2007
Lake, J. Biol. Chem., 2006
Richard, Thromb Haemost., 2006
Senni, Arch. Biochem. Biophys., 2006
Luyt, J. Pharmacol. Exp. Ther., 2003
Deux, Arterioscl. Thromb. Vasc. Biol., 2002
Letourneur, J Control. Rel., 2000

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Polysaccharide-based scaffolds

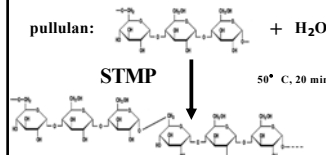


Pullulan/dextran 75:25, 24.5% (w/v) : Pharmaceutical grade
Cross-linker: sodium trimetaphosphate (STMP) 11% (w/v)



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Scaffold preparation



Chemical cross-linking of polysaccharide chains (pullulan), using STMP in water



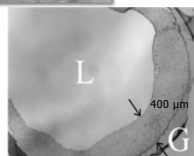
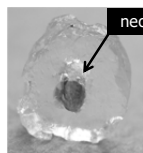
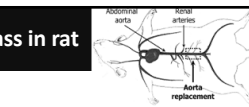
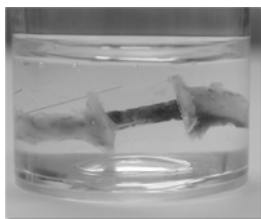
➤ Network based on chemical links between polysaccharide chains

Autissier *et al.*, J. Biomed. Mater. Res., (2007)

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Scaffold implantation : Aortic bypass in rat

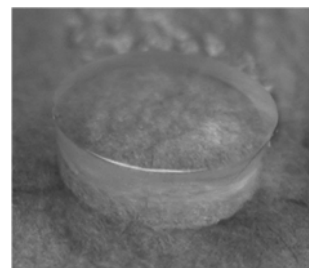
Chaouat *et al.*, Biomaterials (2006)



A biodegradable scaffold fo favor tissue regeneration

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Scaffolds made of Polysaccharides: Local Drug Delivery



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MATRIX FOR «DRUG» DELIVERY : VEGF₁₆₅

Neovessel formation in a subcutaneous mouse model of angiogenesis with VEGF₁₆₅ in 3D Fucoidan scaffolds

3D alone Fucoidan VEGF Fucoidan+VEGF

A. Purnama et al., Drug Del Transl Res, 2015

⇒ Fucoidan, a natural sulfated polysaccharide interacts with angiogenic growth factors (Lake et al., J Biol Chem 2006)

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MATRIX FOR «DRUG» DELIVERY : VEGF₁₆₅

Fucoidan and VEGF₁₆₅ induce neovessel formation in a subcutaneous model of angiogenesis in mice

Control Fucoidan VEGF F+VEGF

X10

X40

X60

Neovessel area (µm²)

Neovessel density (N/mm²)

Control Fucoidan VEGF F+VEGF

A. Purnama, Drug Del Transl Res, 2015

50

Scaffolds made of Polysaccharides : Cell Culture

Day 4

Human bone-marrow-derived Mesenchymal Stem Cells ESEM (hydrated gel and living cells)

Magn 1500x 3.6 Torr 20 µm

Similar results on vascular SMC Autissier et al., J Biomed Mater Res A. 2007

> Scaffold allows cell adhesion, spreading and proliferation

51

3D Porous Scaffolds made of Polysaccharides

52

DEVELOPMENT OF 3D POROUS SCAFFOLDS

23 Publications; 4 patents; Several Grants

Dextran Pullulan

Pharmaceutical grade

Polysaccharides + Porogen + Crosslink → 3D Macroporous Matrix

- J. Grenier et al. Acta Biomater 2019
- S. Lanouar et al. J Mater Sci Mat Med 2018
- J.C. Fricain et al. Dental Mater 2018
- J. Guerrero et al. J Tissue Eng Reg Med 2018
- E. Ribot et al. Sci Reports 2017
- S. Frasca et al. J Mater Sci Mat Med 2017
- C. Ehret et al. Plos One 2017
- N. Luciani et al. Acta Biomater 2016
- A. Purnama et al. Drug Del Trans Res 2015
- A. Pietrzyk-Niveau et al. Plos One 2015
- J. Guerrero et al. Tissue Eng 2014
- S. Schlaubitz et al. Plos One 2014
- S. Hamidi et al. Tissue Eng 2014
- D. Fayol et al. Cell Transplant 2013
- J.C. Fricain et al. Biomaterials 2013
- J. Guerrero et al. Acta Biomater 2013
- M. Lavergne et al. Macromol Biosci 2012
- C. Le Visage et al. Tissue Eng Part A 2012
- S. Brule et al. Adv. Mater 2011
- A. Abed et al. J Biomed Mater Res A 2011
- A. Autissier et al. Acta Biomater 2010
- M. Poirier-Quinot et al. Tissue Eng C 2010
- D. Robert et al. Biomaterials 2010

C. Le Visage, D. Letourneur. Method for Preparing Porous Scaffold for Tissue Engineering, Cell Culture and Cell Delivery US Patent 2010/0221303

C. Le Visage, D. Letourneur, F. Chaubet, Autissier. Method for Preparing Porous Scaffold for Tissue Engineering US Patent 2010/0221301

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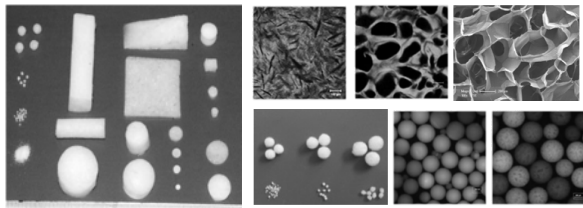
A. Autissier et al. Acta Biomater. 2010

Smooth and non porous Porous Matrices

Porosity
(changing the conditions for scaffold preparation)

54

CONTROLLED POROSITY



Various shapes and sizes

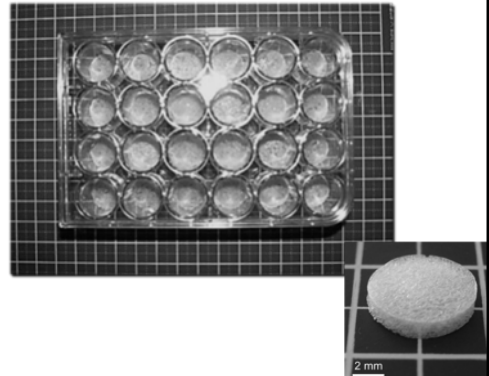
Homogeneous microstructures

Controlled porosity

The controlled porosity favors endogeneous cell colonization and degradation for new tissue formation (regeneration)

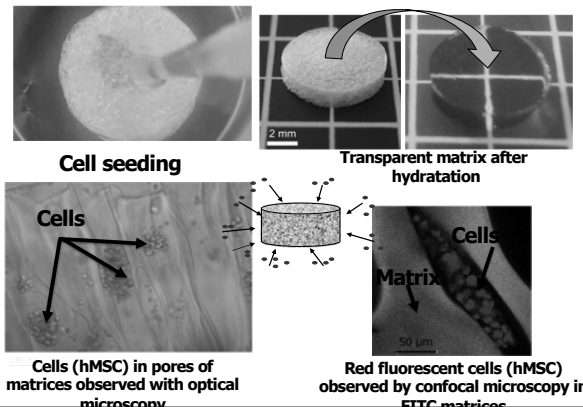
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Scaffolds for 3D Culture



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MATRICES FOR 3D CELL CULTURE



Cell seeding

Transparent matrix after hydration

Cells

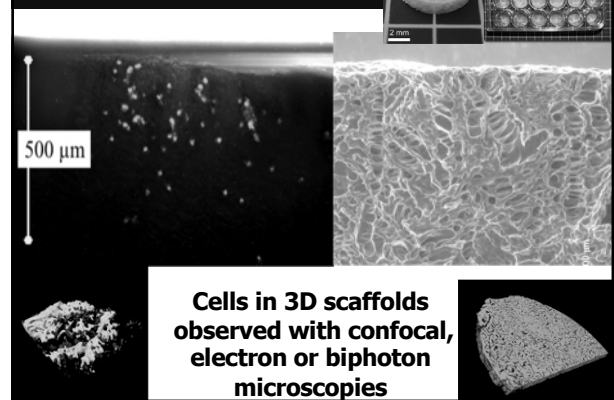
Cells

Cells (hMSC) in pores of matrices observed with optical microscopy

Red fluorescent cells (hMSC) observed by confocal microscopy in FITC matrices

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Human MSCs in 3D

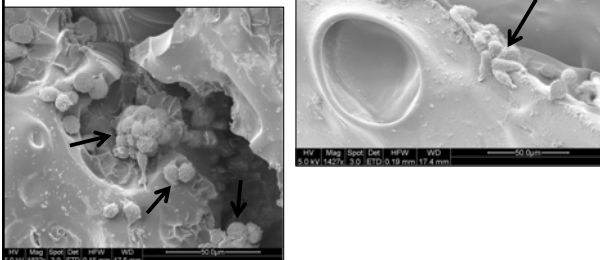


Cells in 3D scaffolds observed with confocal, electron or biphoton microscopies

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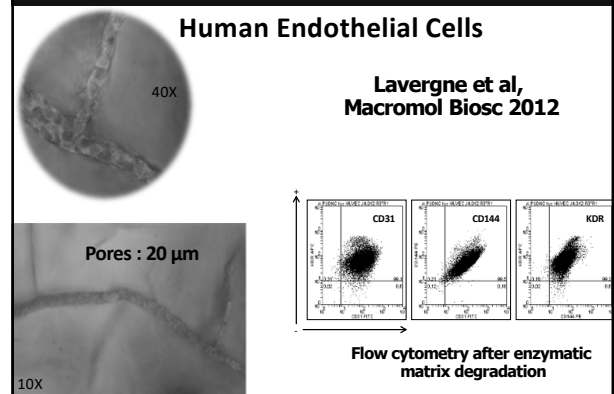
Cell Infiltration into the pores

Scanning Electronic Microscopy



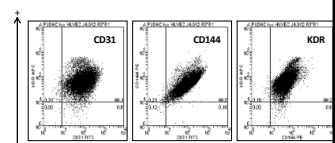
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Cell organization into the scaffold



Human Endothelial Cells

Lavergne et al, Macromol Biosc 2012



Flow cytometry after enzymatic matrix degradation

60

2 types of cells within the matrix (in vitro)

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

Seeding

Day 1

J. Guerrero et al., Acta Biomater. 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, et 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**

Proplatelets+platelets /by adherent cells

Time (minutes)

15 30 45

X 3

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

63

**3D (porous) Scaffolds made of Polysaccharides :
Biodegradation**

64

IN VIVO DEGRADATION (SUBCUTANEOUS IN MICE)

Macroscopic observation

Day 7 Day 14 Day 21

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

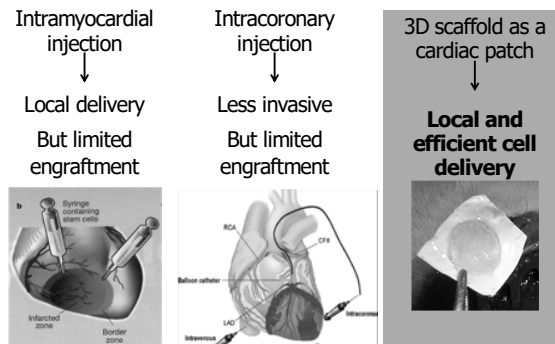
Biomedical uses ?

66

3D Scaffolds made of Polysaccharides for Cardiac Cell Therapy

67

How to Deliver Cells to the Infarcted Myocardium ?

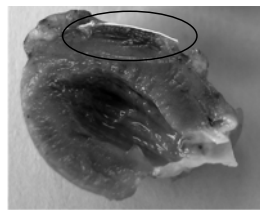


68

In vivo implantation (rat model)



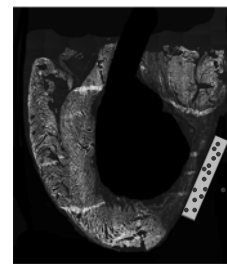
6mm diameter porous scaffold stained with alcian blue



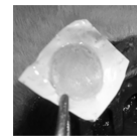
Scaffold implanted onto the heart

69

Transfer of autologous MSCs into the infarcted area



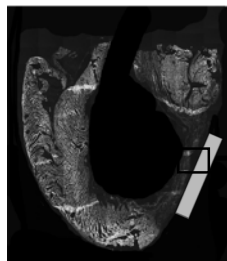
Autofluorescence - Objective x4



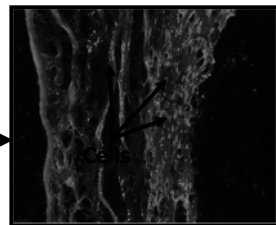
Cellularized Patch for autologous cell therapy (Bone Marrow derived Mesenchymal Stem Cells)

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

Cardiac Patch for Local Cell Delivery

In rat

	2 months	
	EC	Patch
Residual cell number	61 300 (12400-87400)	163 200 (17500-271300)
Engraftment (%)	6.1%	16.3%

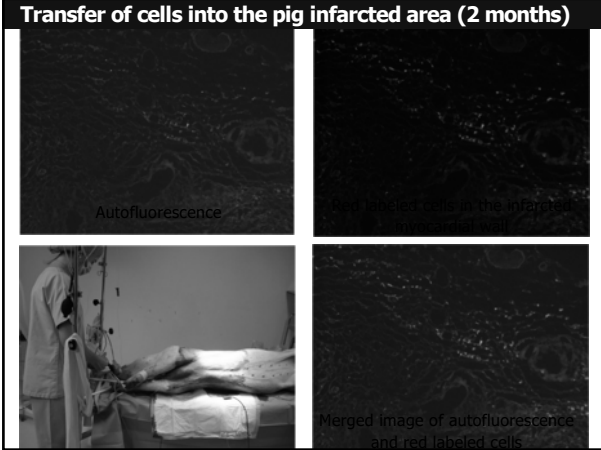
BETTER CELL TRANSFERT than direct injection

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

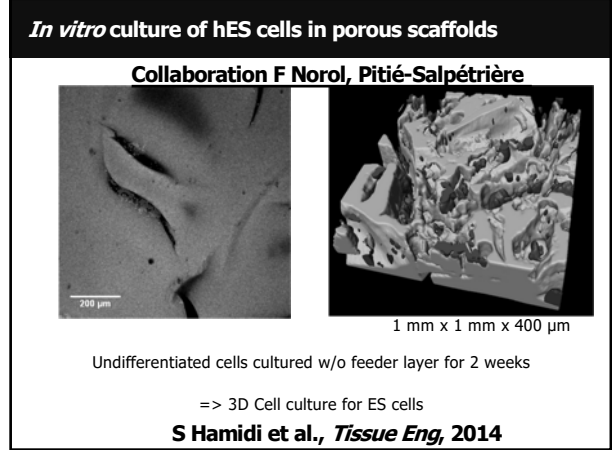
In Pig (autologous cell therapy)



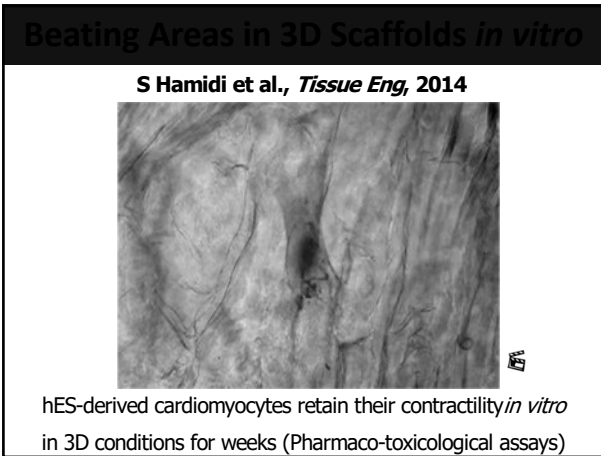
72



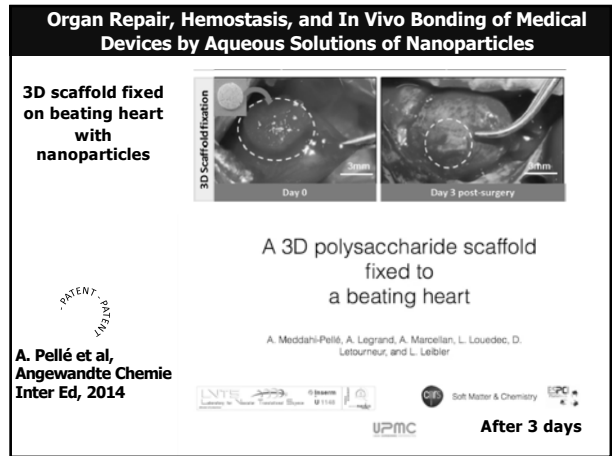
73



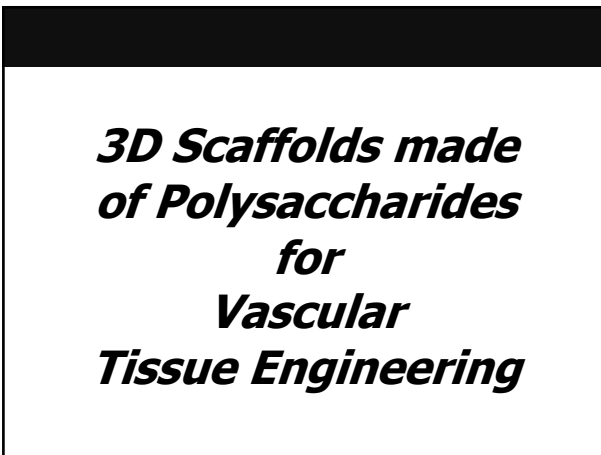
74



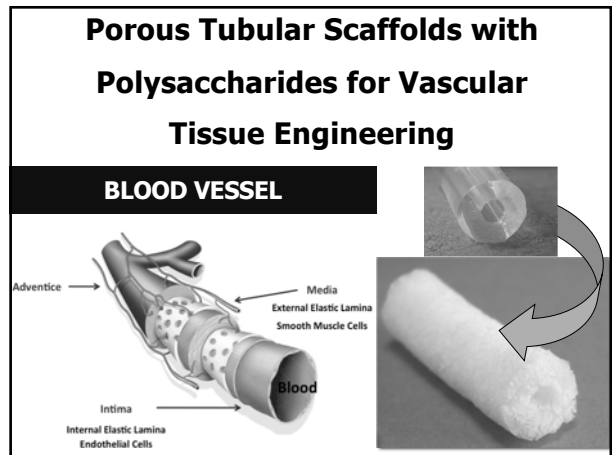
75



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78

3D VASCULAR TISSUE ENGINEERING IN VITRO

Endothelial Progenitors
Tube lumen

Lumen

3D reconstruction on the full tube

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

79

3D VASCULAR TISSUE ENGINEERING (in vitro)

Endothelial Progenitors
Tube lumen

Mesenchymal Cells
Endothelial Progenitors
Tube lumen

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

81

IN VITRO BLOOD VESSEL ENGINEERING

Adventitia Fibroblasts
Intima Internal Elastic Lamina Endothelial Cells
Media External Elastic Lamina Smooth Muscle Cells
Blood

Volume 20, Number 11
ISSN 0883-9887
E-ISSN 1550-3892

CELL TRANSPLANTATION
The Regenerative Medicine Journal

D Fayol et al, Nov 2013
Cover
Cell Transplantation

82

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

84

3D Scaffolds made of Polysaccharides for Liver Repair



85

RHU iLite (2017-2021)



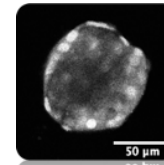
iLite

Innovation in Liver Tissue Engineering

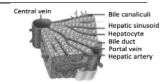
Porous 3D polysaccharide matrices as a scaffolds for hepatic organoids

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

ML Labour et al.
Int J Mol Sci. 2020



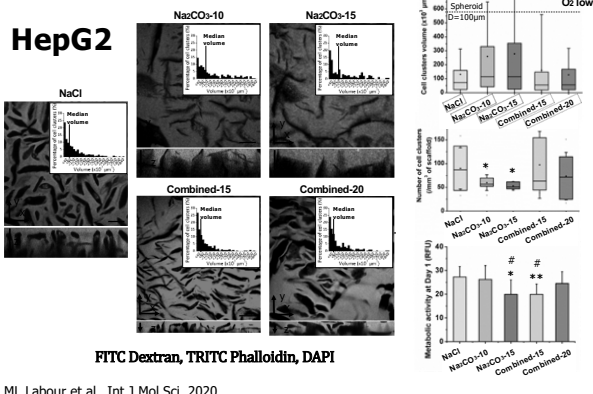
Camille Le Guilcher
Marie-Noëlle Labour
Soraya Lanouar
Rachida Aïd
Teresa Simon-Yarza



86

3D scaffolds for liver spheroids

HepG2



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

87

3D Scaffolds made of Polysaccharides for Brain Repair

88

embryonic neurons (E17.5) at $5 \cdot 10^4$ cells / μL

⇒ Confocal Fluorescent Microscopy

89

by Piotr Topilko

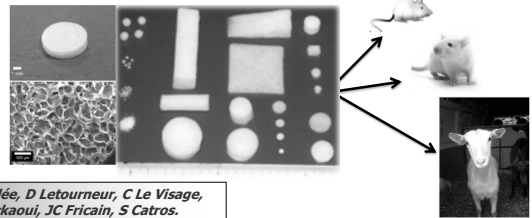
90

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)

with Inserm U1026; Bordeaux

92

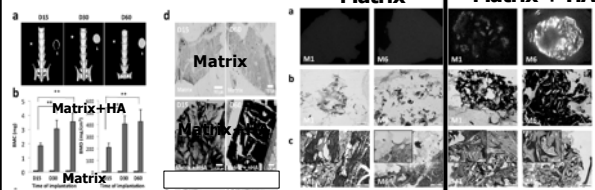
Matrix+HA without cells induces bone formation in non osseous site (*small and large animals*)

with Inserm U1026; Bordeaux

JC Fricain et al, *Biomaterials* 2013

Formation of a mineralized bone tissue after subcutaneous implantation in mice

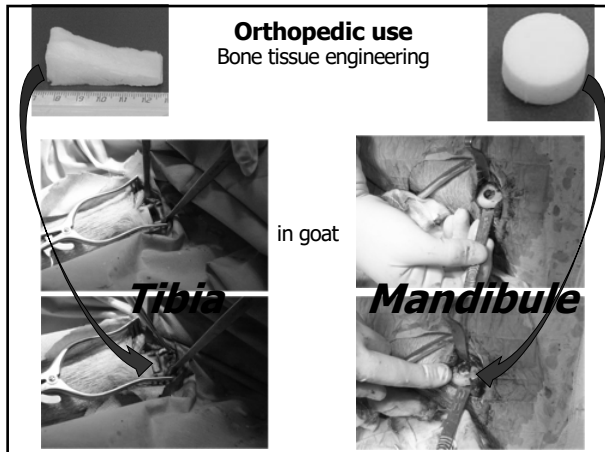
Formation of a mineralized bone tissue after intramuscular implantation in goat



=> Osteoinductive Matrix+HA

93

Orthopedic use
Bone tissue engineering



94

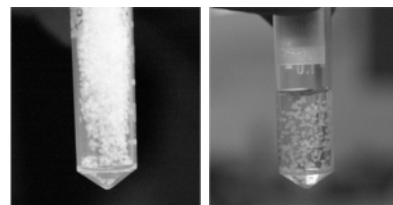
3D Scaffolds for Tissue Engineering

- 3D Matrices

- **MicroMatrices :**
Microsystems for
Injectable drug/cell delivery

95

3D POROUS POLYSACCHARIDE BEADS



Brulé et al, *Advanced Materials* 2011

96

AN INJECTABLE POROUS SCAFFOLD FOR TISSUE REGENERATION

100-200µm 500-1000 µm

C. Le Visage, S.M. Derkaoui, D. Letourneur. Crosslinked polysaccharide beads and their biomedical uses EP 10305931.7 (2011) (WO 2012)

97

Porous polysaccharide microbeads

Morphology of freeze-dried microspheres analyzed by scanning electron microscopy

Loaded with cells

After hydration Hydration with cells

98

Sub-cutaneous injection in mice of microbeads

Ø 200-300 µm, beads-FITC, SC **Histology, SC**

99

Porous polysaccharide microsystems

100-200 µm 200-500 µm 500-1000 µm

Loaded with drug

100-200 µm 200-500 µm 500-1000 µm

100

BONE REGENERATION WITH HA

Femoral condyle in rat - 30 days

Macroporous matrix

Beads 200 - 500 µm

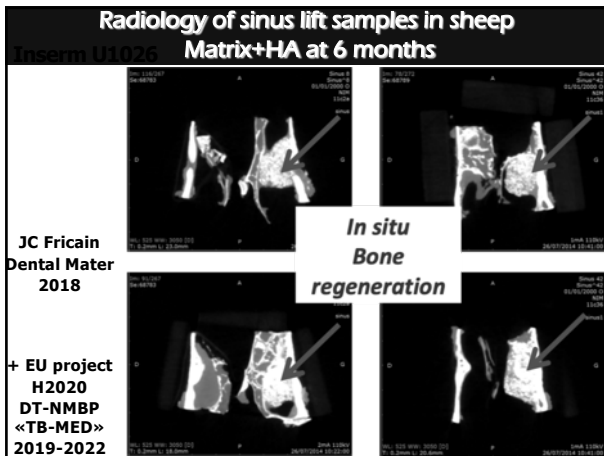
Injectable form Gave Improvements
=> Setting
=> Biodegradability
=> Invasivity
=> Bone mass

101

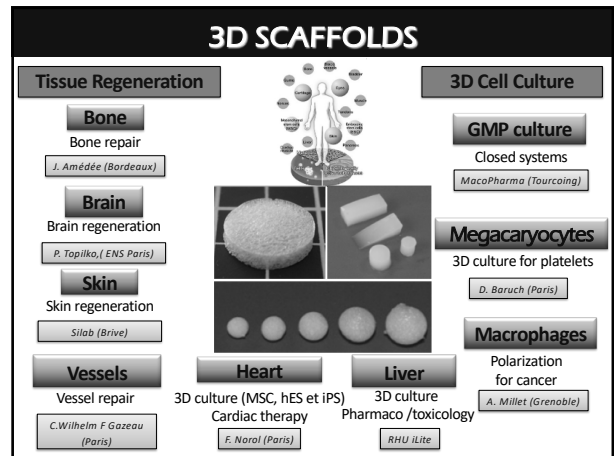
INJECTABLE 3D POROUS SCAFFOLDS FOR BONE IN SHEEP (60-70 KG)

With Inserm U1026 Bordeaux

102



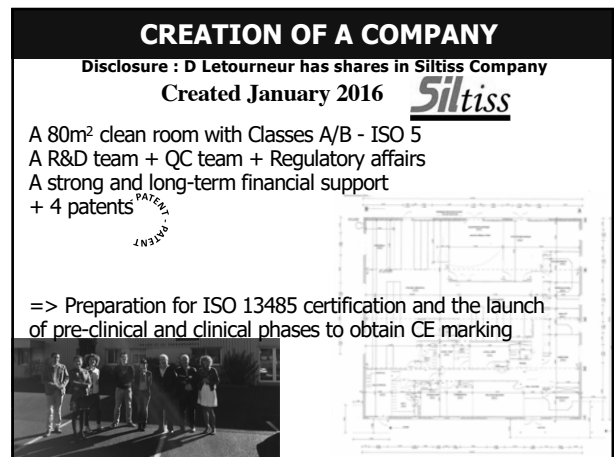
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COMPANY 

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)



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Example 3

Nanosystems made of Polysaccharides for Drug Delivery And Molecular Imaging

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The journey started here...

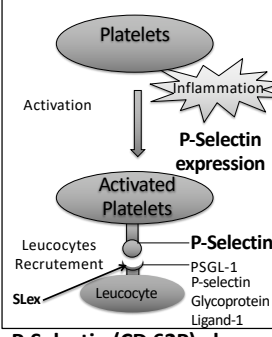
Ushant island (Brittany coast, France)



Production of Fucoidan from brown seaweeds

111

P-Selectin

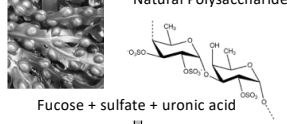


Platelets → Activation → Inflammation → P-Selectin expression → Activated Platelets → Leucocyte Recruitment → Leucocyte

Leucocyte: P-Selectin, PSGL-1, P-selectin Glycoprotein Ligand-1

Fucoidan : Mimic of SLeX Ligand of P-Selectin

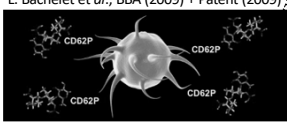
Natural Polysaccharide



Fucose + sulfate + uronic acid

P-Selectin (CD 62P) interactions *in vitro* & *in vivo*


L. Bachelet *et al.*, BBA (2009) + Patent (2009)




CD62P

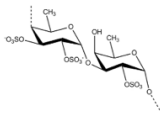
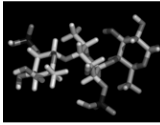
P-Selectin (CD 62P) also present on activated Endothelium

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 **FACILITIES**



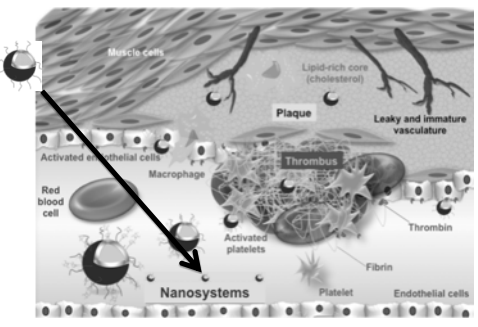
Fucoidan : sulfated polyfucose

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

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Targeted Nanosystems for Imaging of Thrombus



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

SEVERE FRAMEWORK PROGRESS

Large Scale NMP FP7 2013-2018
 D LETOURNEUR Coordinator

NanoAthero

JNM

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Targeted Nanosystems for Imaging of Thrombus

NanoAthero

Polysaccharide

Composite Polysaccharide / iron oxide

+ 2 other Patents 2015, 2016

Large Scale NMP FP7 2013-2018 D LETOURNEUR Coordinator

SEVENTH FRAMEWORK PROGRAMME

AKA. Silva et al., Theranostics + Cover June 2014
M. Suzuki et al., Nanomedicine 2015
M. Juenet et al., Future Sci OA 2015
J. Matuszak et al., Nanomedicine 2016

T. Bonnard et al., Theranostics 2014
T. Bonnard et al., Acta Biomaterialia 2014
M. Juenet et al., BBRC+ Cover Dec 2015
B. Li et al., Adv Health Mater + Cover 2017

115

Targeted Nanosystems for Imaging of Thrombus

F. Rouzet et al., J Nucl Med + Cover Sept 2011

AKA. Silva et al., Theranostics + Cover June 2014
M. Suzuki et al., Nanomedicine 2015
M. Juenet et al., Future Sci OA 2015
J. Matuszak et al., Nanomedicine 2016

T. Bonnard et al., Theranostics 2014
T. Bonnard et al., Acta Biomaterialia 2014
M. Juenet et al., BBRC+ Cover Dec 2015
B. Li et al., Adv Health Mater + Cover 2017

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Polymeric Nanosystems for Molecular Imaging

16 partners
10 countries
12.8 M€

a) Magnetic resonance imaging (MRI)
b) Computed tomography (CT)
c) Positron emission tomography (PET)
f) Ultrasound (US)
e) Optical imaging
d) Single photon computed tomography (SPECT)

> 10 Publications
3 patents
Several Grants
More to come...

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Toward the Clinical Thrombus Imaging with a GMP-Grade Fucoidan

Clinic
Regulatory tox
cGMP synthesis
In vivo proof of safety and efficacy
In vitro safety and proof of concept
NP design and lab-scale synthesis

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Clinical Thrombus Imaging with a GMP-Grade Polysaccharide

GMP

119

Regulatory Toxicity of cGMP fucoidan

BRADICO
am
NanoAthero
LVTS
INSERM
U1148

C6S27. Extended single dose toxicity study of fucoidan extract formulation after intravenous administration to rats.

FINAL REPORT

Product Name: Fucoidan extract
Code and Study Number: C-4027
Study Director: Silvia Boud

=> No toxicity until the maximum tested dose equivalent to 20 mg (for 50 kg bw)

Max injected dose in human : 40 μg per patient

> 500 x

No animals died during the study, and no clinical signs or treatment related changes in body weight or body weight gain were observed in all animals treated with fucoidan extract formulation up to 400 μg/kg.
No treatment related changes in hematology, blood chemistry, coagulation and urinalysis parameters were observed.
No treatment related changes in absolute and relative organ weights were observed.
No fucoidan extract-related microscopic findings were observed at histopathology examination.
In conclusion, the No Observed Adverse Effect Level (NOEL) for fucoidan extract formulation after a single intravenous administration to rats is 400 μg/kg.

120

Fucoidan: submission IMPD + IB

13DL-C-Nanoathero_ame2_ autorisation-utilisation-IB_20170912_MDI - CC.pdf	Portable Document Format File	25 kb
13DL-C-Nanoathero_Bochure Investigateur (BI)_V4.0_201711109.pdf	Portable Document Format File	2887 kb
13DL-C-Nanoathero_Formulaire_demande_AEC_initiale_ANSM_20171117_MDI1.pdf	Portable Document Format File	707 kb
13DL-C-Nanoathero_ame-v1.0_20		133 kb
13DL-C-Nanoathero_P1302011_A		217 kb
13DL-C-Nanoathero_protocole_V		1660 kb
13DL-C-Nanoathero_Résumé-Pro		140 kb
2017-001015-36-FR-20171116-CT		39 kb
Ischaradipharmsiac200550859		121 kb
CourrierDemande Initiale-AEC-A		298 kb
Nanoathero-Tab-FDM_v1.0_2017		45 kb
Algers & Mer certificate bio CER		243 kb
LabelGenerator_v8 - Fucote V1.1		202 kb
AMAFSI DBI autorisation M17_2017		1346 kb
AMAFSINGROUP IDRON GMP certificate Ansm med exp HPP-FR-016-2017.pdf	Portable Document Format File	146 kb
GMO-free certificate FUCO Oct.17.pdf	Portable Document Format File	292 kb
DMPD_Insem_2017_32P_fucoidane recombinat marqui_final_27-oct.pdf	Portable Document Format File	2758 kb
DMPD_Insem_2017_32S_32P_fucoidane lyophilisat_Final 19_OCT2017.pdf	Portable Document Format File	2332 kb

List of regulatory documents to prepare and submit for national authorization of phase 1 clinical trials

I. Cicha et al, Cardiovasc Res 2018

NanoAthero

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Clinical Thrombus Imaging with a GMP-Grade Polysaccharide

FR NL

ANSM
AUTORISATION D'ESSAI CLINIQUE DE MEDICAMENT A USAGE HUMAIN
N° de dossier: 17-001015-36-FR-20171116-CT

Envoi par Téléscope

amc

ANSM
N° de dossier: 17-001015-36-FR-20171116-CT

ANSM
N° de dossier: 17-001015-36-FR-20171116-CT

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Phase I - Clinical Thrombus Imaging with a GMP-Grade Fucoidan

Patient # 4
From 30mn to 3 h

30mn 30mn 1.5 h 1.5 h 3 h 3 h

Anterior - Posterior - Anterior - Posterior - Anterior - Posterior

Phase I completed :
10 patients - no side effect, usual uptake

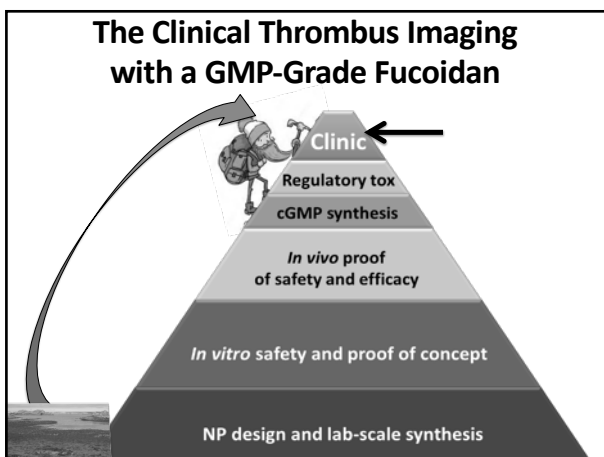
123

On going Phase II Clinical trial

Phase IIa clinical trial to evidence acute thrombogenic activity in patients with Deep Vein Thrombosis

Ongoing at AMC

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Lessons from Example 3 : Polymeric Nanosystems for Molecular Imaging

NanoAthero

7hermosities

a) Magnetic resonance imaging (MRI)

b) Computed tomography (CT)

c) Positron emission tomography (PET)

> 10 Publications
3 patents
Several Grants
More to come...

Preclinical Thrombus Imaging ... to Clinical Thrombus Imaging

126

Ongoing developments : C Chauvierre

Targeted therapy

Functionalized polymer microbubbles

Bo, L. *et al.* Biomaterials 2019

127

Targeted Nanosystems for Thrombus Treatment

Juenet *et al.*, Biomaterials 2018

Thrombolysis

Thrombus density at 30 minutes after treatment normalized to the peak platelet intensity

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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Thrombus : Imaging & Treatment

Fucoidan : From the design to the clinic (phase I)

CHALLENGES AHEAD

A long long road...

=> Phase II / Phase III

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CONCLUSIONS

3D Scaffolds for Tissue Engineering & molecular imaging

- 3D (Macro)Matrices
- MicroMatrices
- NanoMatrices

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Works in Research...

Cells, Scaffolds, Bio-molecules

Preclinical evaluation From small to large animals

132



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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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GMP Production of Tissue Engineering Products

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.

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

No thanks!

We are too busy

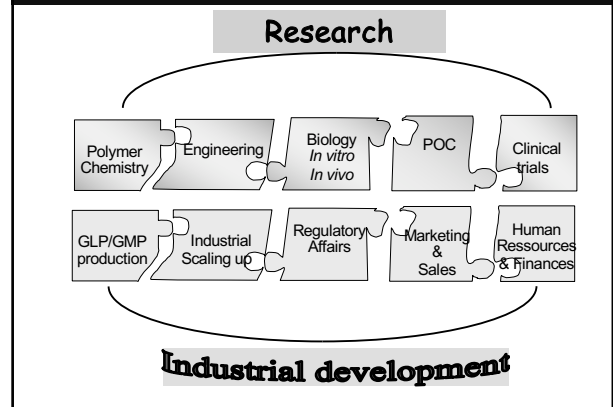
138

A strong IP Portfolio with new protections



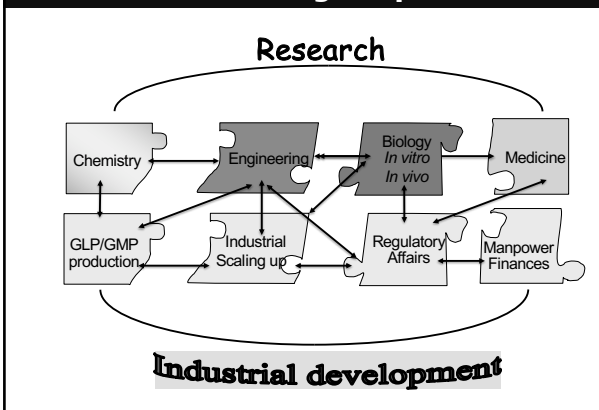
139

A need of large expertise



140

A need of large expertise



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Acknowledgements



143

Acknowledgements

LNTS - U 1148 Inserm - Team 3

- ✓ Fondation Avenir (2007;2008;2010;2011); Fondation Rech Méd. (2010, 2017); Agence Biomédecine (2007;2008;2010;2012); Lefoulon-Delalande (2014)
- ANR
 - ✓ 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)
- COMPETITIVITE
 - ✓ 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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LNTS UMRS 1148
 Laboratory for Vascular Translational Science


 Real SEM Picture
 of a scaffold

GDR RÉPARER L'HUMAIN
 GROUPE DE RECHERCHE NATIONAL

BIO MAT
 ASSOCIATION POUR LE DÉVELOPPEMENT DES BIOMATÉRIAUX
 Association Française affiliée à la Société Européenne des Biomaterials

didier.letourneur@inserm.fr



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 GDR

aviesan
 alliance nationale pour les sciences de la vie et de la santé



Inserm
 La science pour la santé
 Pour accéder à la santé


RÉPARER L'HUMAIN

GROUPE DE RECHERCHE NATIONAL






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 MAGNETIC BORDEAUX


BIO MAT

Annual Conference of the European Society for Biomaterials
 4th to 8th of September 2022 - BORDEAUX


ESB



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