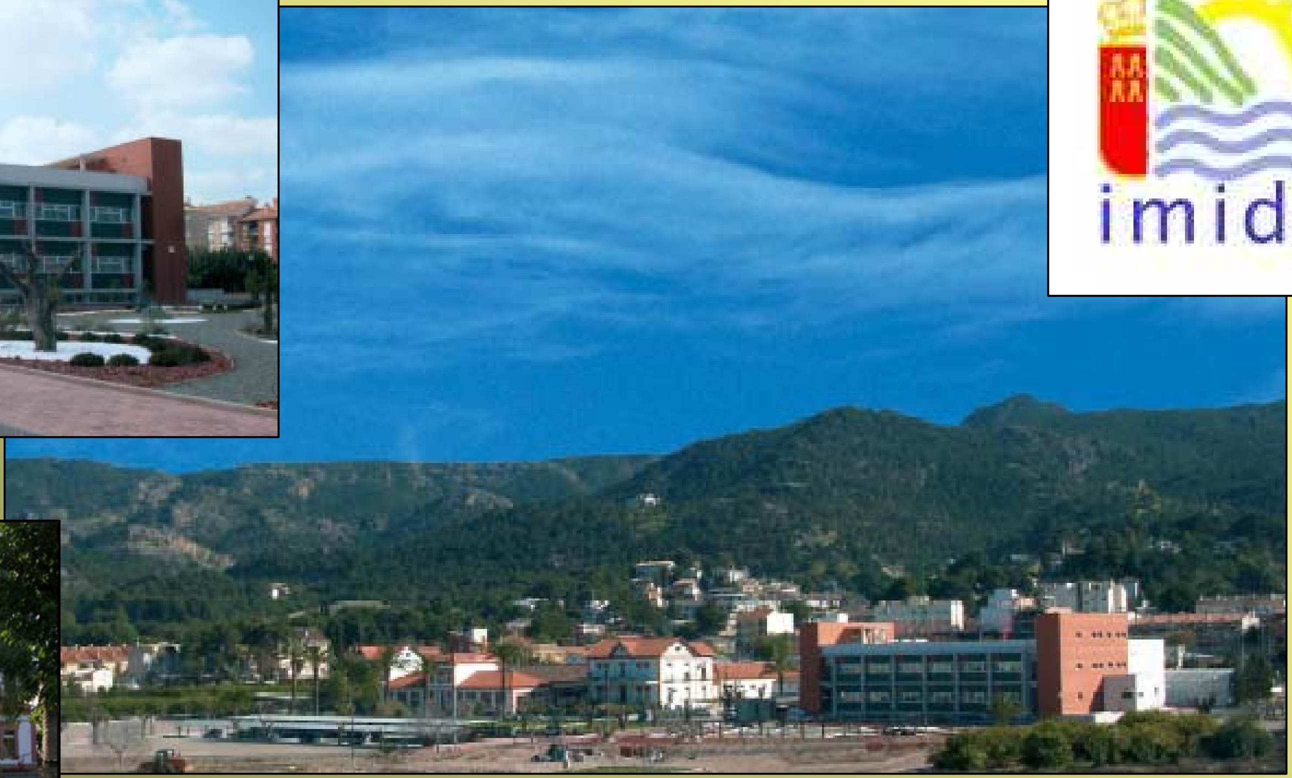
Development of silkworm (*Bombyx mori*) as a platform for producing biomaterials and growth factors for Tissue Engineering

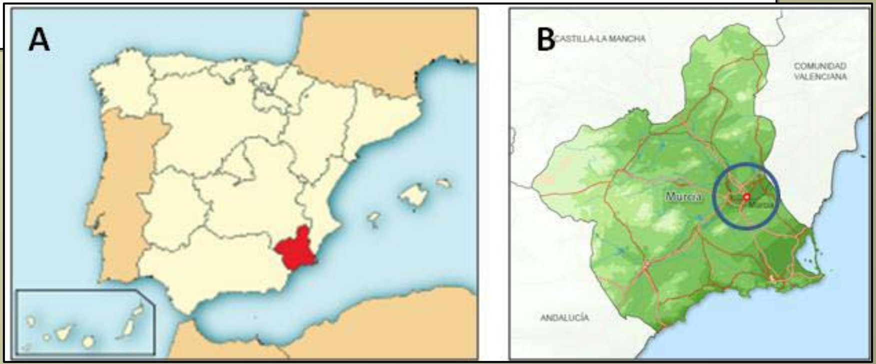


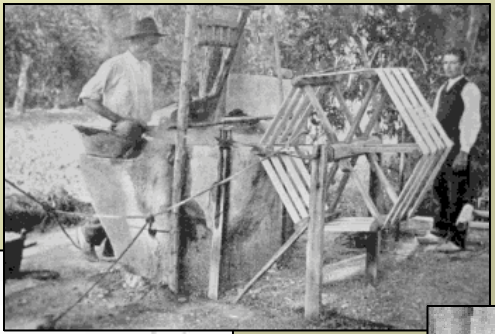
José Luis Cenis (IMIDA, La Alberca, Murcia, Spain)

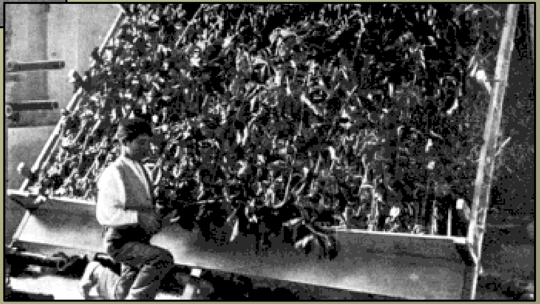
THE SILK IN SPAIN

- Introduced by the arabs
- Produced in all Spain, mainly in Valencia in the Mediterranean Coast
- Great economic importance from XV to XX centuries
- Historical peak of production: 12.400.000 Kg in 1850 (Valencia 50%)

- EPIDEMICS OF *Nosema bombycis* (1860s): Ruin of the activity in all Spain except in the Region of Murcia



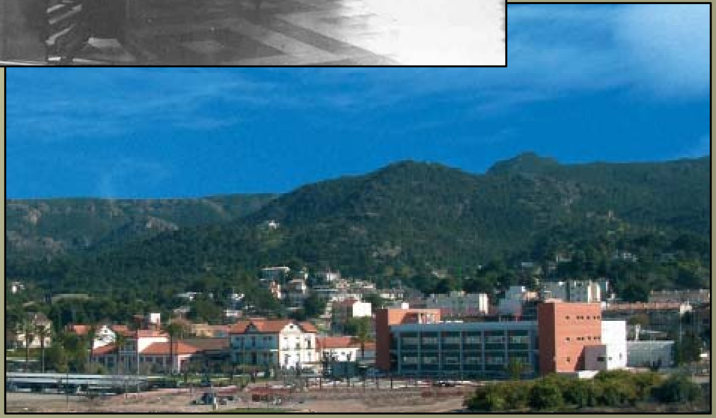


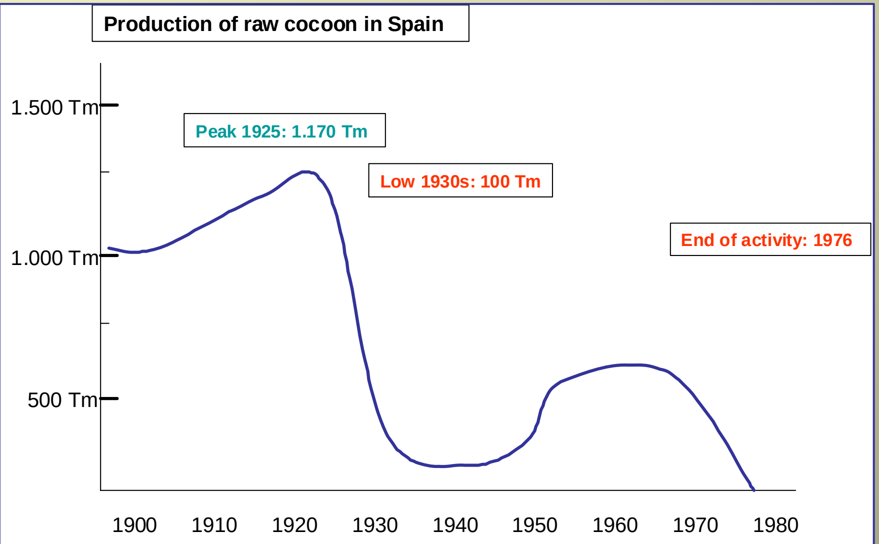


THE SILK IN SPAIN

SERICICULTURE ACTIVITY IN SPAIN IN XX CENTURY

- **Establishment of the Sericicultural Station of Murcia in 1892 to provide technology, on the model of the Padova Sericicultural Station**





NON TEXTILE USES OF SILK: BIOMATERIALS FOR BIOMEDICINE

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Review

Stem cell-based tissue engineering with silk biomaterials

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www.elsevier.com/locate/ppolysci

Silk as a biomaterial

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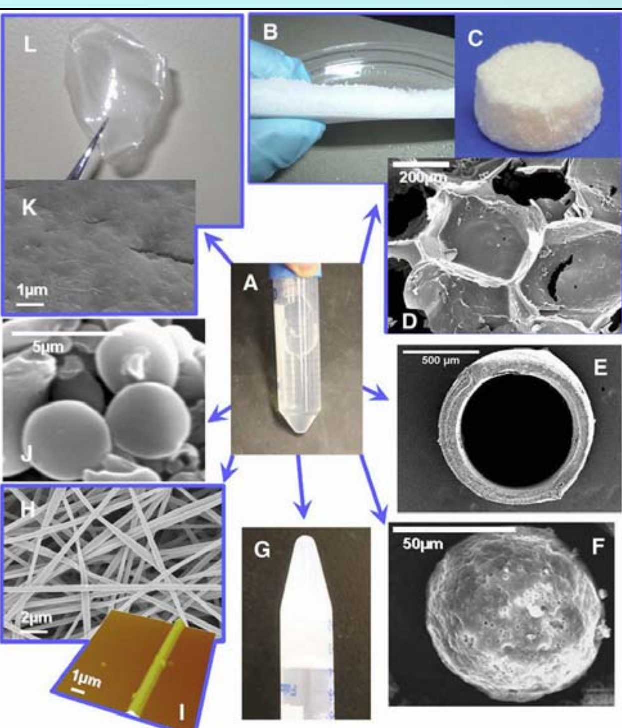

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Silk: A Potential Medium for Tissue Engineering

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The authors have no conflicts of interest.

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NON TEXTILE USES OF SILK: EXPRESSION OF RECOMBINANT PROTEINS WITH BACULOVIRUS VECTORS IN *BOMBYX MORI* BODY

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Journal of Biotechnology 111 (2004) 253–261
Journal of Biotechnology
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Expression of human VEGF165 in silkworm (*Bombyx mori* L.) by using a recombinant baculovirus and its bioactivity assay

Xiaofeng Wu[a,*], Zhaozheng Yin[a], Cuiping Cao[a], Ling Huang[b], Xingmeng Lu[a], Jianxin Liu[a], Weizheng Cui[c]

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Journal of Biotechnology 123 (2006) 236–247
Journal of BIOTECHNOLOGY
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Expression, purification and characterization of human GM-CSF using silkworm pupae (*Bombyx mori*) as a bioreactor

Jian Chen[a,b], Xiang-Fu Wu[c], Yao-Zhou Zhang[b,*]

[a] *College of Life Sciences, Zhejiang University, Hangzhou 310029, China*
[c] *Shanghai I*
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Appl Microbiol Biotechnol (2005) 69: 385–389
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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Yizhen Wang · Xiaofeng Wu · Guangfu Liu · Cuiping Cao · Haiqing Huang · Zirong Xu · Jianxin Liu

Expression of porcine lactoferrin by using recombinant baculovirus in silkworm, *Bombyx mori* L., and its purification and characterization

Through the innoculation of insect larvae with a recombinant baculovirus vector it is possible to get protein expression levels higher than in an insect cell culture at a lower cost

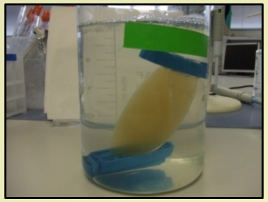
PREPARATION OF AQUEOUS SOLUTION OF FIBROIN

Rearing of Silkworms

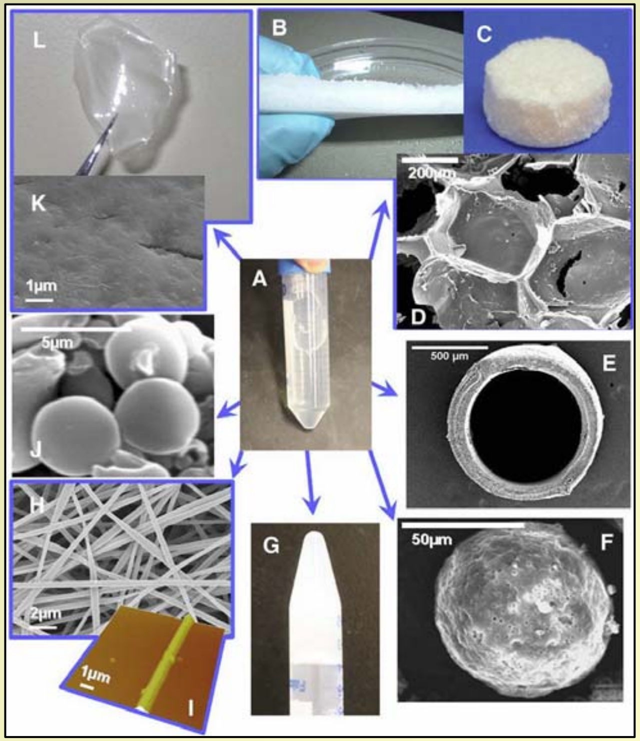
Washing of sericin

Disolution of fibroin

Dialysis in water.



Aqueous solution of fibroin



Presentations of fibroin:

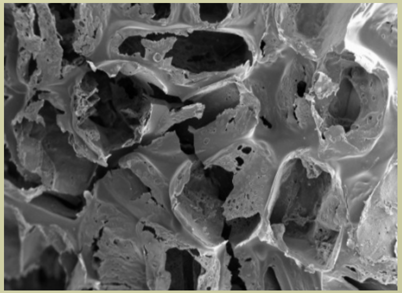
- *Films*
- *Porous 3D Sponges*
- *Electrospun nanofibers*
- *Hydrogels*
- *Micro and nanoparticles*
- *Tubular structures*
- *Extruded Fibers*

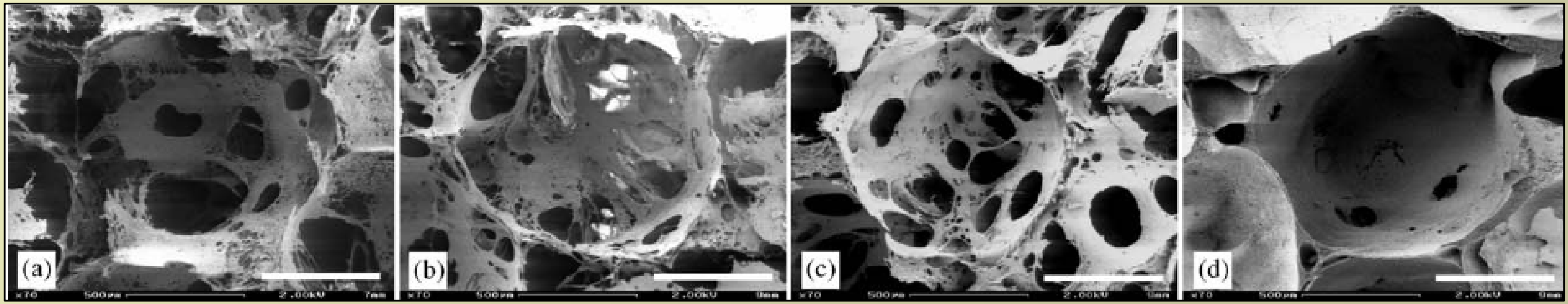
PRESENTATION OF FIBROIN BIOMATERIALS

POROUS SPONGES 3D

•Scaffold for growing mesenchymal stem cells for reparation of bone

Fibroin in 10% solution → Addition de ClNa, 800 micr., in a mold → Inmersion in methanol for insolubilizing the fibroin. Washing of salt with water

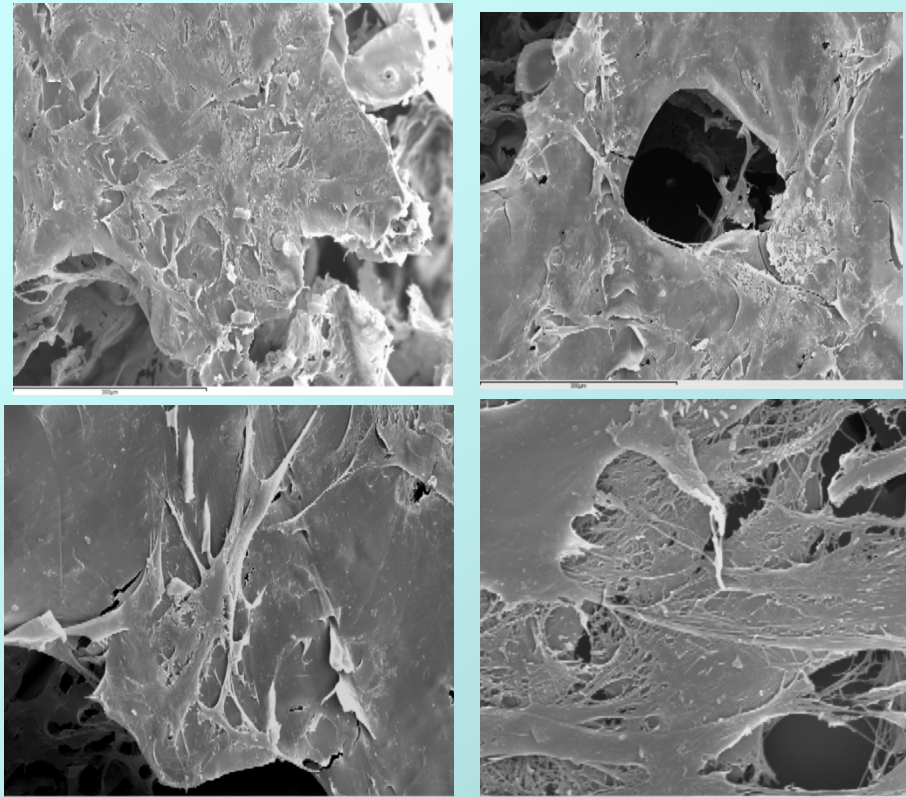


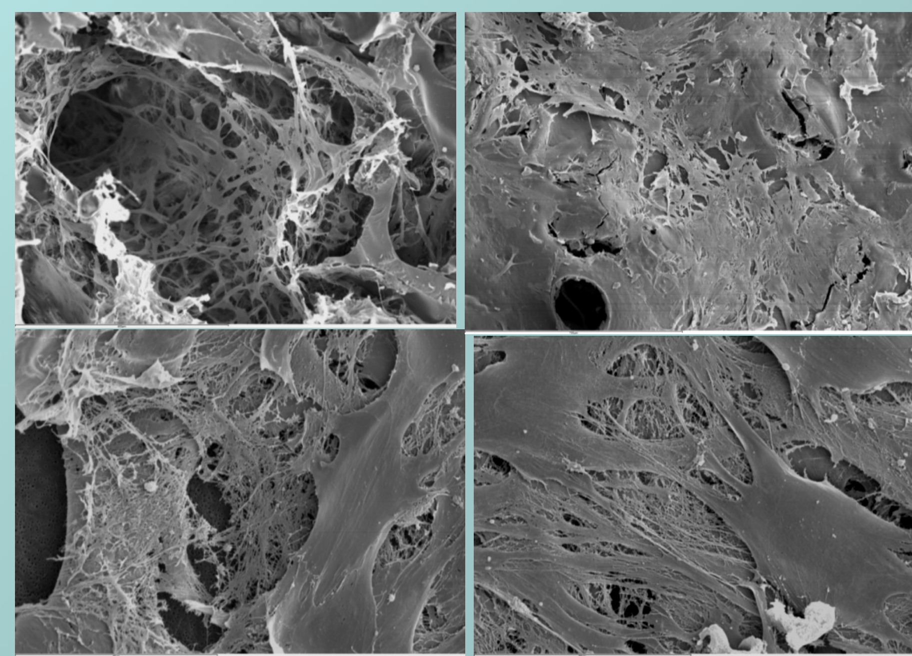


Bar: 500 Mμ.

1. PROJECT IN THE IMIDA WITH POROUS SPONGES 3D

Development of scaffolds of fibroin premineralized with tricalcium phosphate and seeded with mesenchymal cells for bone reparation



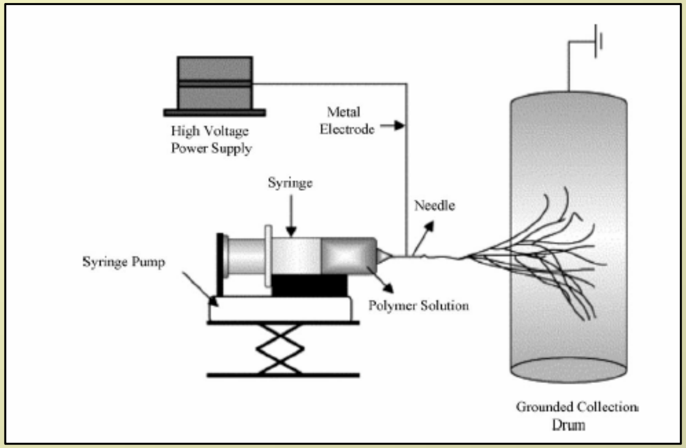


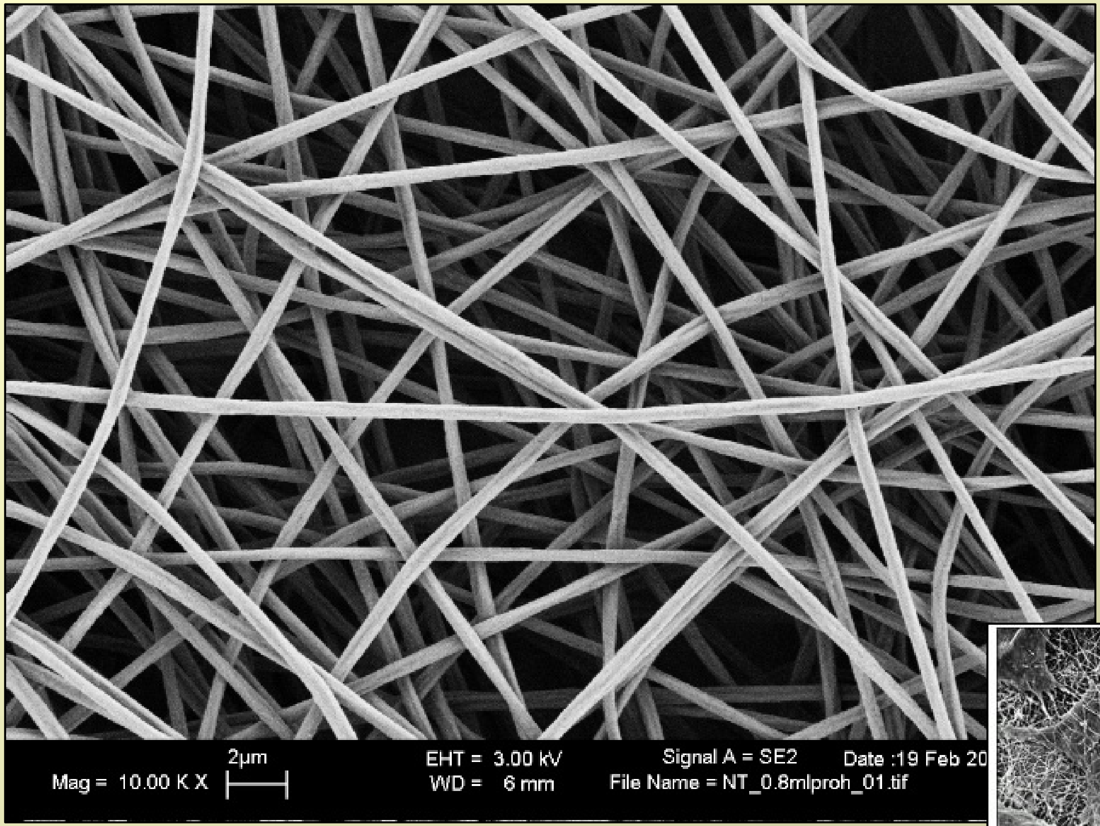
Seeding with adult mesenchymal cells. 72 hours/ 1 week

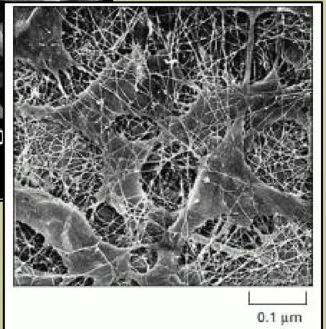
PRESENTATION OF FIBROIN BIOMATERIALS

NANOFIBER MATS OBTAINED BY ELECTROSPINNING

- **100 to 400 nanometers of diameter**
- **5 micrometers of pore**
- **Similar configuration than extracellular matrix**





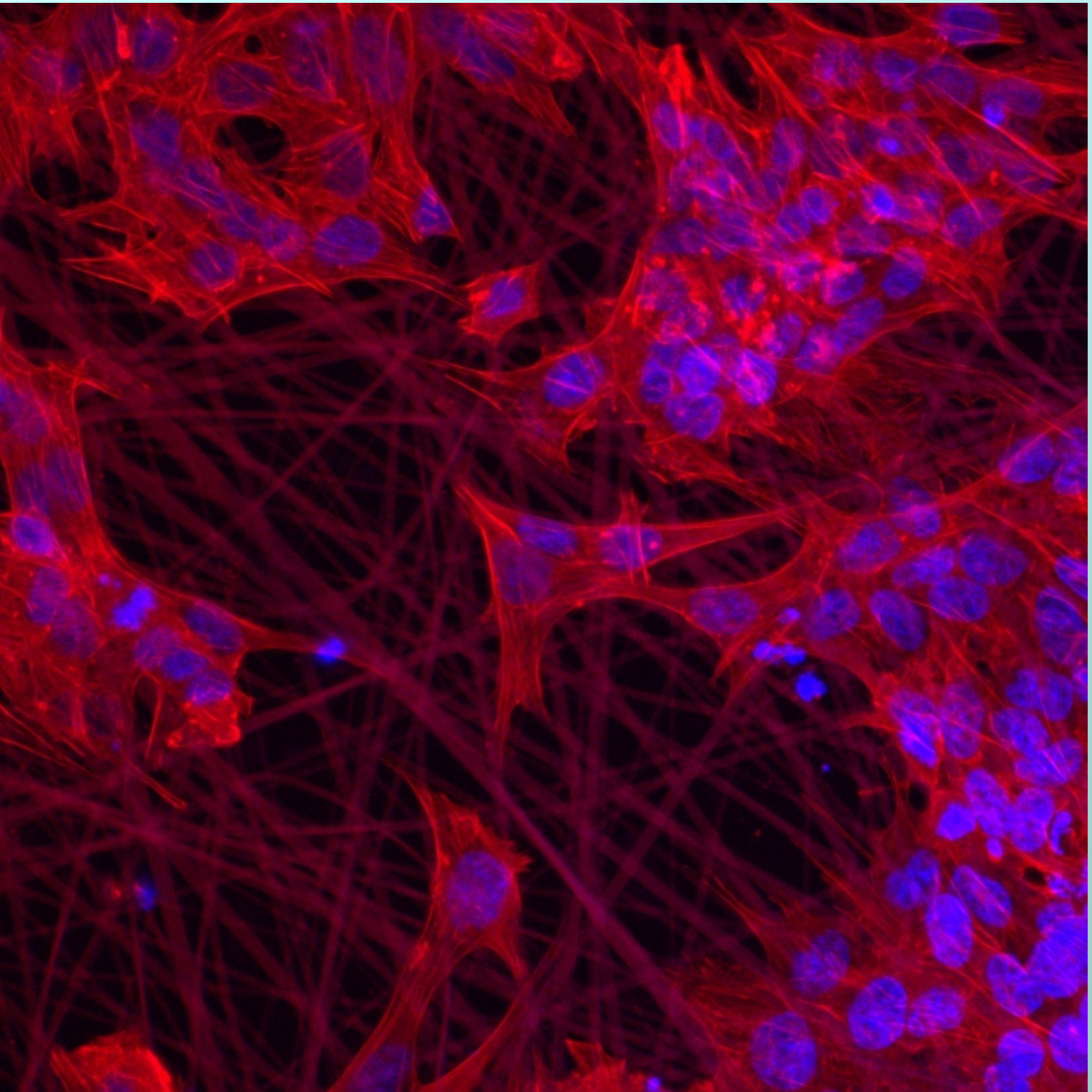


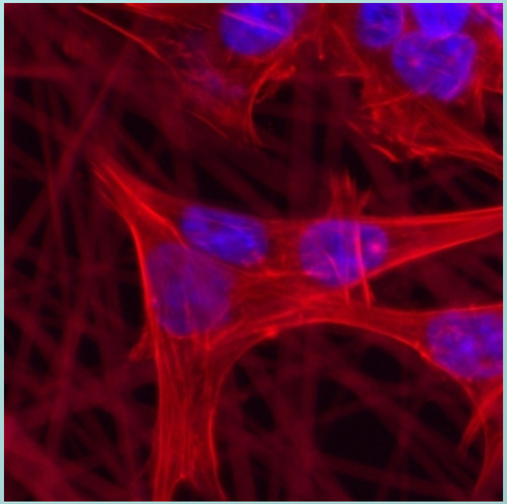
Growth of human cells on a non-woven silk fibroin net: a potential for use in tissue engineering. R. Unger et al. (2004). Biomaterials 25: 1069-1075.

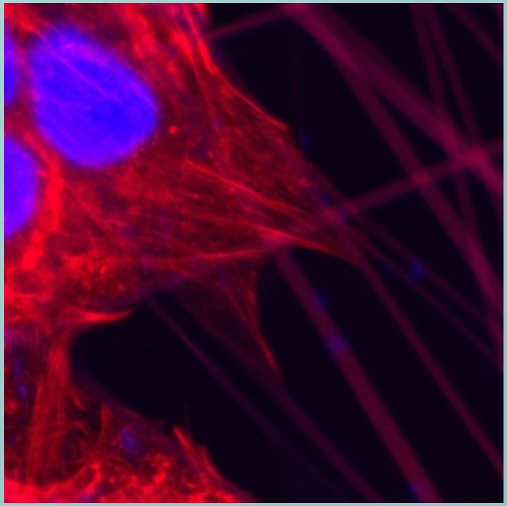
2. PROJECT IN THE IMIDA WITH ELECTROSPUN NANOFIBERS

Development of nanofiber mats for growing keratynocytes and fibroblasts for skin substitutes

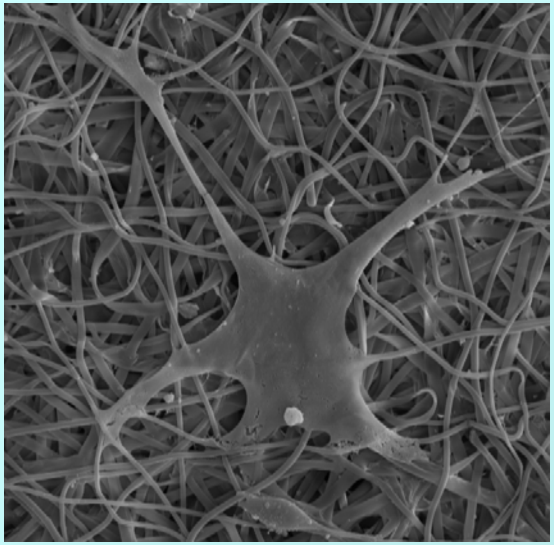
Seeding with cells CCMv1Lu

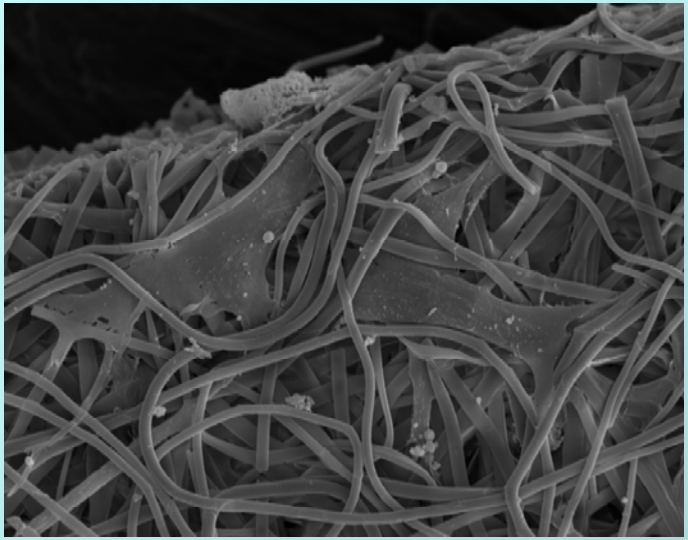




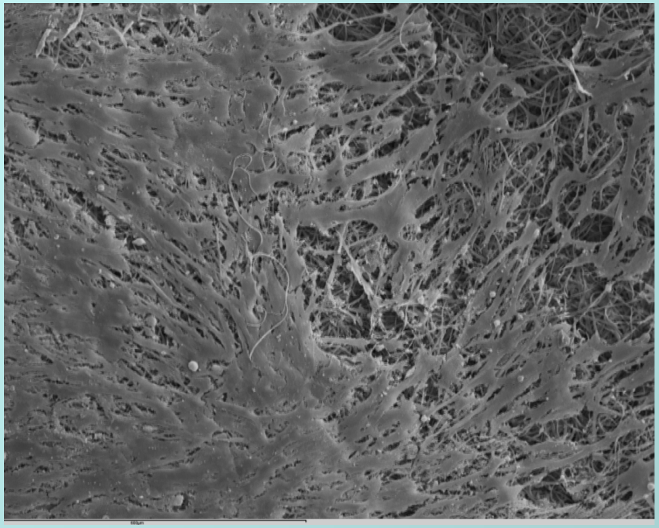


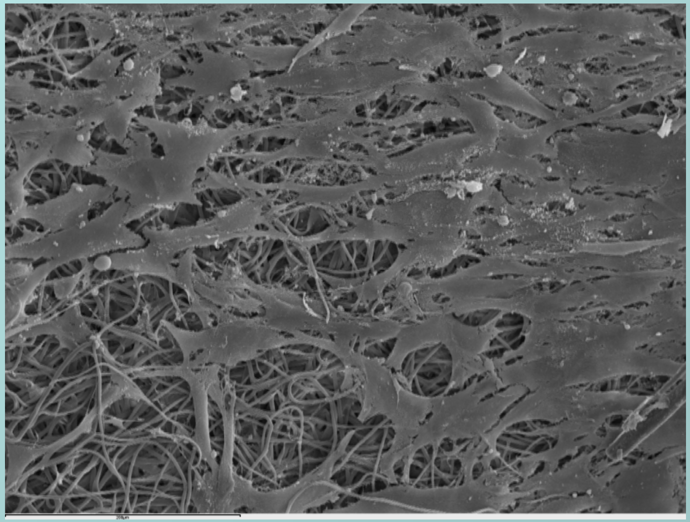
Seeding with adult mesenchymal cells. 72 hours



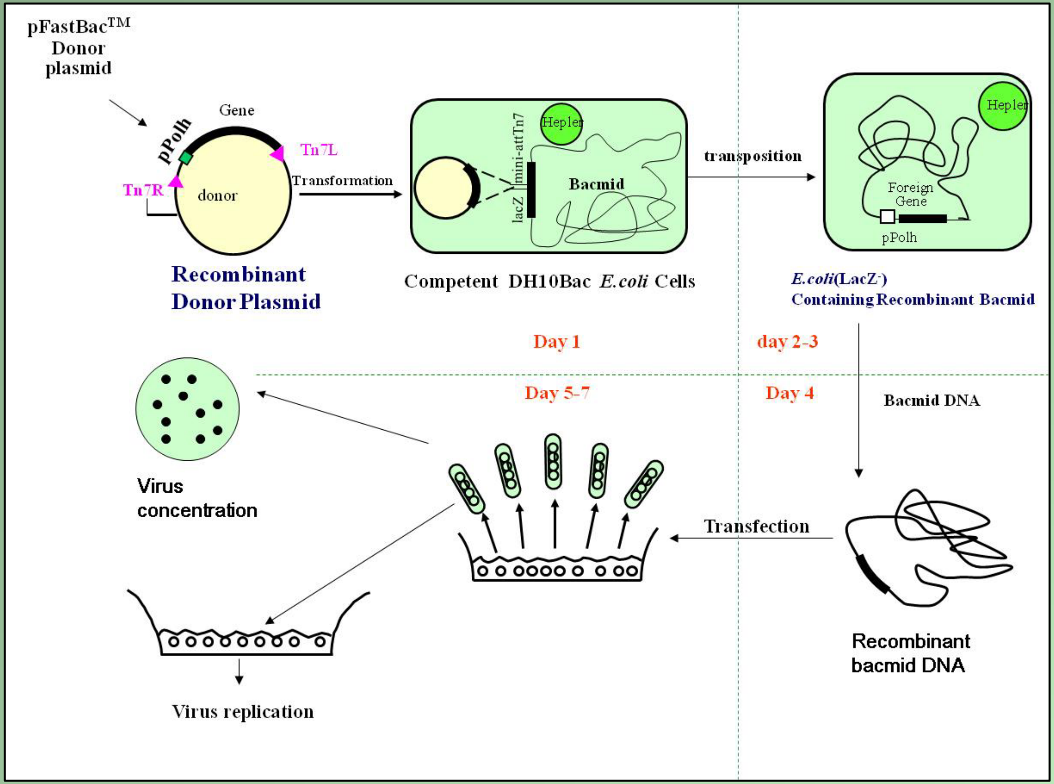


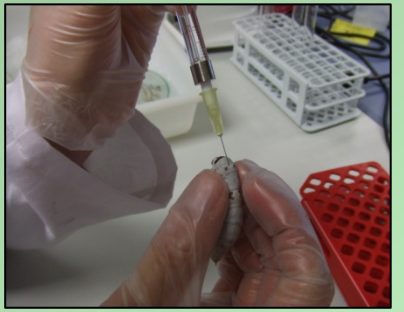
Seeding with adult mesenchymal cells. 1 week





PROTEIN EXPRESSION IN *BOMBYX MORI* BODY





3. PROJECT IN THE IMIDA WITH EXPRESSION OF PROTEIN IN BOMBYX MORI WITH BACULOVIRUS VECTORS

Four proteins are currently expressed with the silkworm/baculovirus platform:

- Canine Interferon-alpha
- Dihydrofolate Reductase

- Fibroblast Growth Factor basic (bFGF)
- Vascular Endothelial Growth Factor (VEGF)

DIHYDROFOLATE REDUCTASE

Appl Biochem Biotechnol
DOI 10.1007/s12010-010-8961-9

Purification and Kinetic Properties of Human Recombinant Dihydrofolate Reductase Produced in *Bombyx mori* Chrysalides

Soledad Chazarra · Salvador Aznar-Cervantes · Luis Sánchez-del-Campo · Juan Cabezas-Herrera · Wu Xiaofeng · José Luis Cenis · José Neptuno Rodríguez-López

Table I. hDHFR yield after infection of larvae and chrysalides of *B. mori* with the recombinant virus

	Number of individuals inoculated	Average group body weight (g)	Total hDHFR yield (mg)[a]	Average hDHFR yield per individuals (µg/per individual)	Average hDHFR yield per body weight (µg/g body weight)
Larva	50	216.4	29.1	582	134.6
Chysalide	50	74.4	9.5	190	127.8

[a]The yield of hDHFR (mg) in crude extracts was calculated based on DHFR activity (U) measurements. Calculated U in crude extracts were extrapolated to standard curves of U vs. mg of hDHFR obtained with a homogenised purified samples

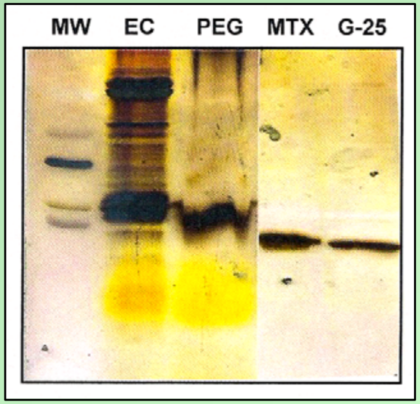
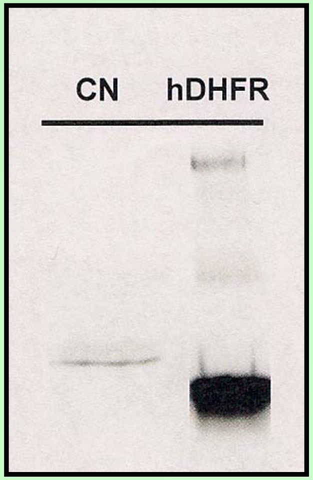


Table II. Summary of the purification procedure of hDHFR from 50 chrysalides of *B. mori*

	Protein (*mg*)	Activity (*U*)	Specific Activity (*U/mg*)	Yield (*%*)	Purification (*-fold*)
Crude extract	2025	18.3	9×10^{3}	100	1
14% PEG 6000	501	15.9	0.032	86.9	3.56
MTX Agarose[a]	1.88				
Sephadex G-25	1.49	2.9	1.95	15.8	217

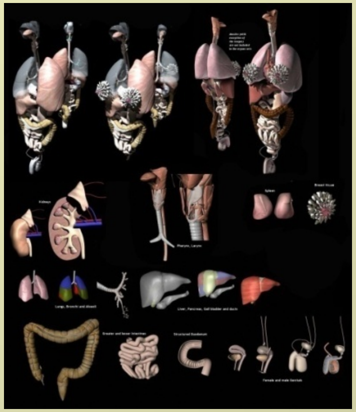
[a]The activity in this fraction could not be determined due to the presence of TMP.



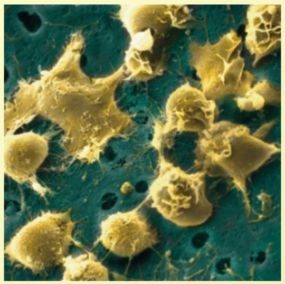
PACKAGE OF APPLICATIONS OF SILK PROTEINS IN TISSUE ENGINEERING

Factores de crecimiento

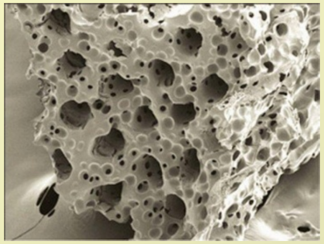
TISSUES AND ORGANS



STEM CELLS



SCAFFOLDS



SERICIN

•Used for the stimulation of the cellular proliferation in vitro. It is used as a component in cell culture and cryopreservation media as a substitute of FBS (fetal bovine serum) at 1/200 of the cost.

FIBROIN

•Used for the fabrication of at least three configurations of scaffolds: film, porous sponges and electrospun nanofiber mats.

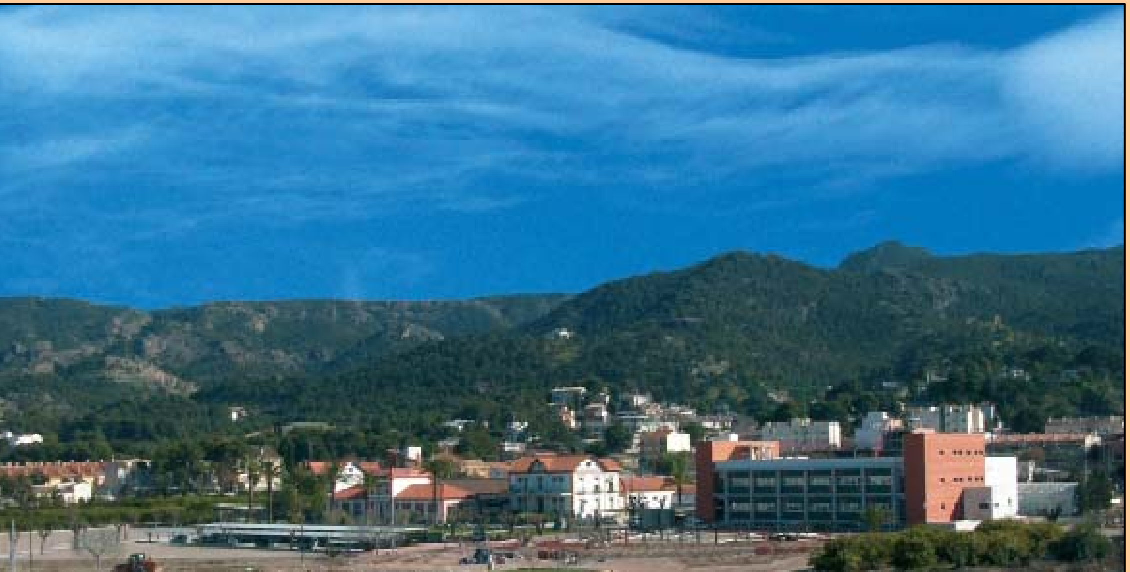
PUPA

•Used as a biorreactor for the expression of two growth factors: bFGF and VEGF

The production of proteins for Tissue Engineering provides a potential source of income for silkworm rearing

The proof of concept described is made in cooperation with a network of Institutes of Regenerative Medicine

A new facility has been built at the IMIDA for this specific development to produce silkworm, silk and scaffolds under GMPs conditions



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