3D BIOPRINTING OF SILK FOR CELLULAR APPLICATIONS

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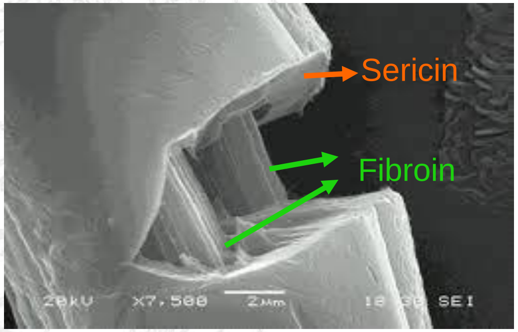
The biomaterial of the future: silk

- **Silk origins**
  - Natural protein fiber of animal origin *(Bombyx mori)*

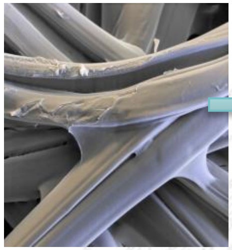
- **Silk structure**
  - Two filaments of fibroin (75%)
  - Coating of sericin (25%) which must be removed



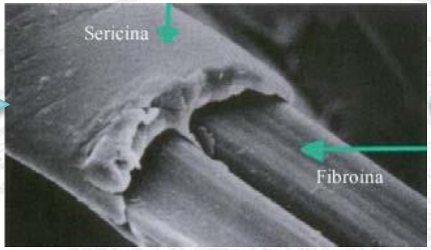
- **From cocoons to fibroin solution**

The biomaterial of the future: silk

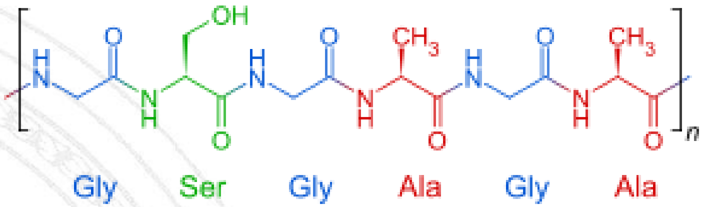
Cocoons



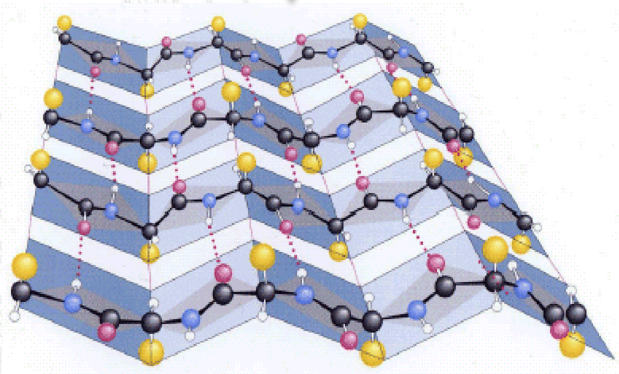
Silk fibers



Single silk fiber



Primary structure of fibroin



Secondary structure of fibroin (Beta – sheet)

- **Fibroin structure**
  - Primary structure: *hexapeptide*
  - Self-assembly of *Beta-sheet* which gives strength and flexibility

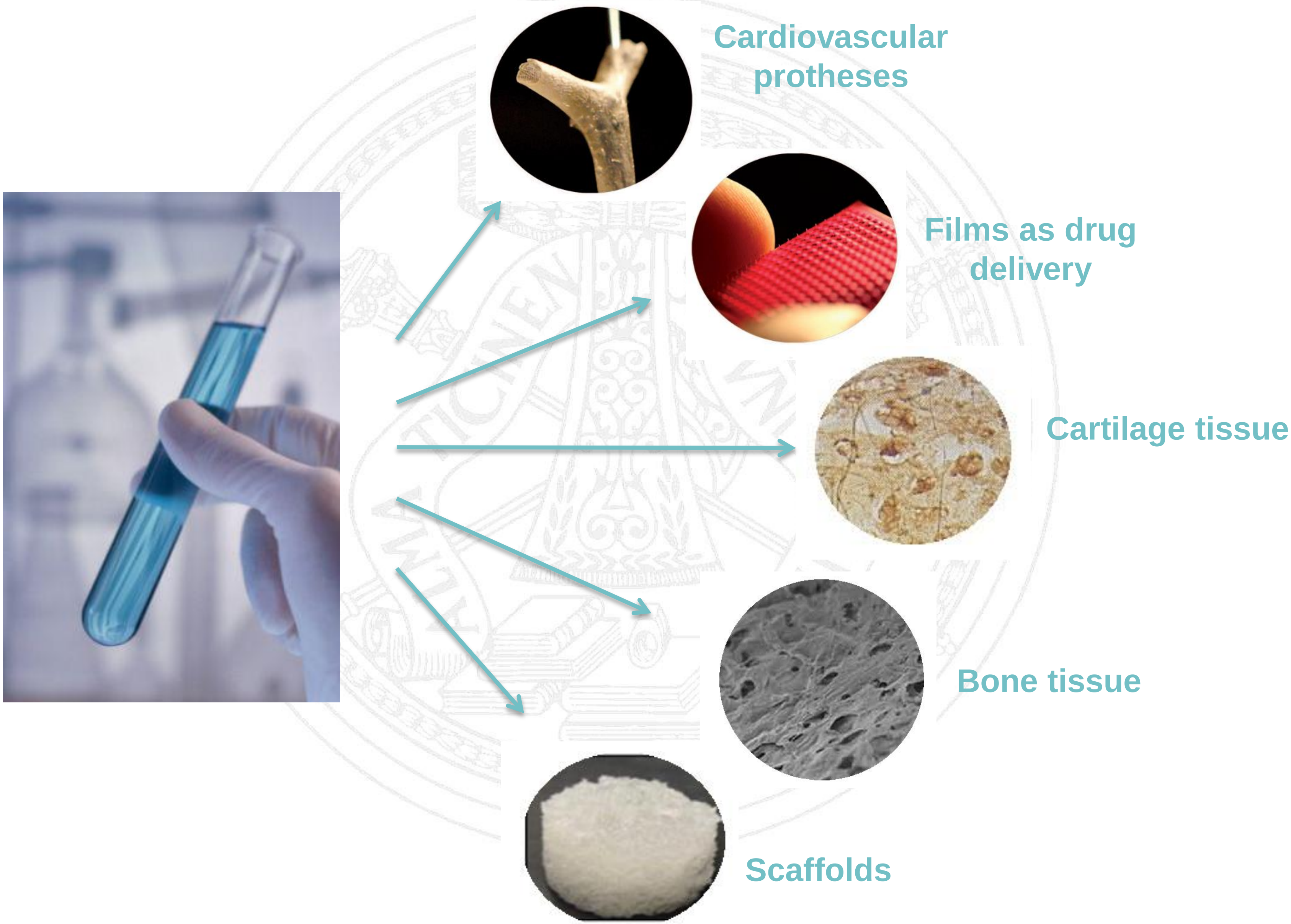
- **Property**
  - *Biocompatible*
  - *Biodegradable*
  - *Sterilizable* in autoclave (121° C, 2 atm, 15 min)

Purpose of activity

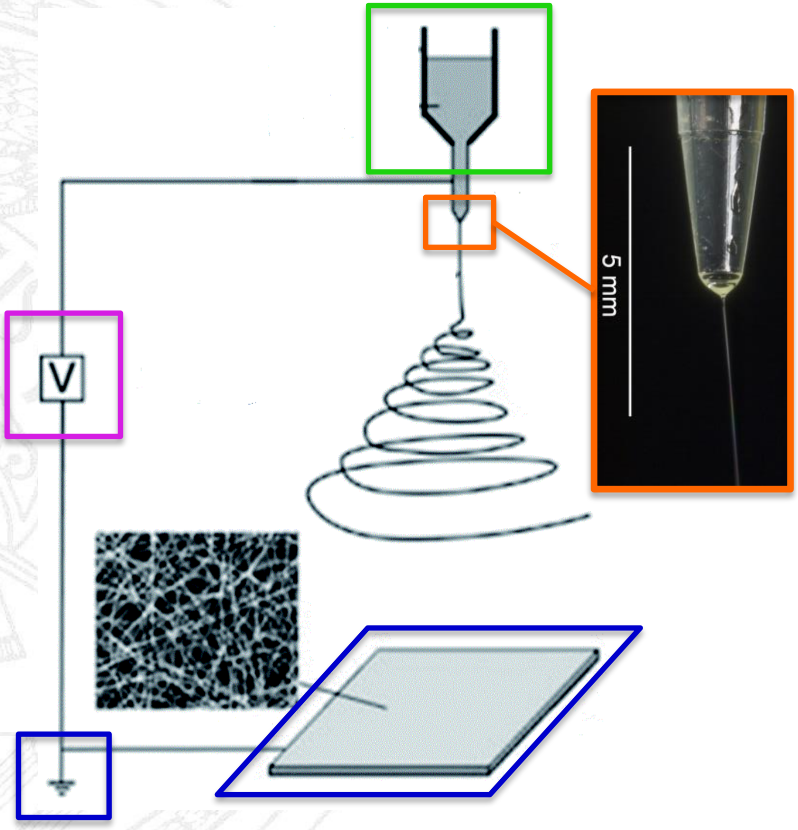
- **Silk as a biomaterial today**
  - Overview of current applications
  - Main technical manufacturing: *Electrospinning*
  - Example application: 3D silk bone marrow niche for platelets generation ex vivo - Prof. Alessandra Balduini *et al* (Università degli Studi di Pavia and Tufts University of Boston)

- **Silk as a biomaterial tomorrow**
  - 3D Bioprinting of silk-based bioink to produce scaffolds
  - First study in literature: bioink of silk and polyol
  - Our possible implementation

Current applications



- **Instrumentation**
  - Capillary needle or pipette
  - High voltage supplier
  - Grounded collector



- **Method**
  - Application of an *electric potential*
  - Overcome the surface tension and development of the *Taylor cone*
  - Ejecting of fiber jet

- **Post – treatment**
  - Crystallization by aqueous methanol at room temperature for 10-60 min

- '' Electrospun Silk Biomaterial Scaffolds for Regenerative Medicine'', Xiaohui Zhanga *et al*
  - ''Processing of Bombyx mori silk for biomedical applications'', B. D. Lawrence

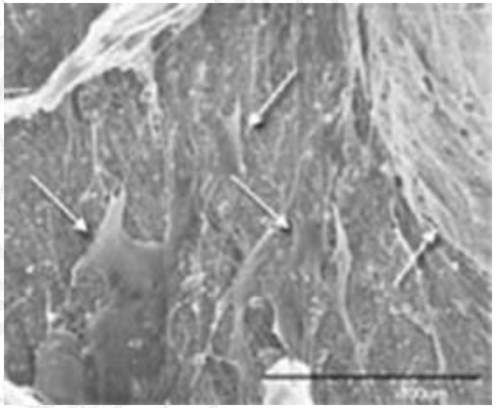
Electrospinning to produce 3D scaffolds

➢ Method

- Collector: *bath filled with methanol*
- *Porogen*: NaCl particles with a diameter of 300-500 µm
- Salt leaching

➢ Results

- Nano-sized pores at the interstices
- Pores formed by salt parcles with a
  diameter of 586-934 µm



''Electrospun Silk Biomaterial Scaffolds for Regenerative Medicine'', Xiaohui Zhanga *et al*

3D silk bone marrow niche – Prof. Balduini

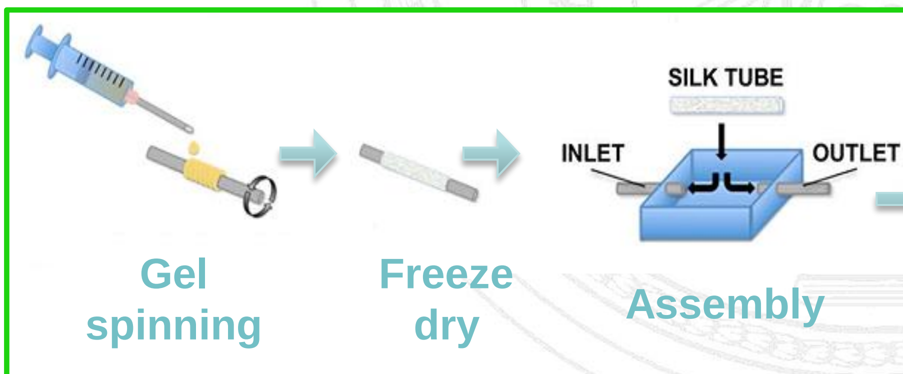
➢ Purpose

- Reproduce the physiology of the bone marrow
- Produce platelets *ex-vivo* (hematopoiesis)

Hematopoiesis

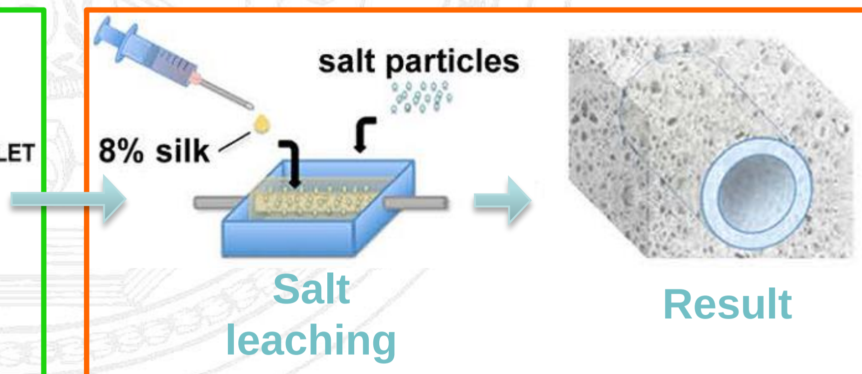
➢ Structure

Microtube



Microvasculature

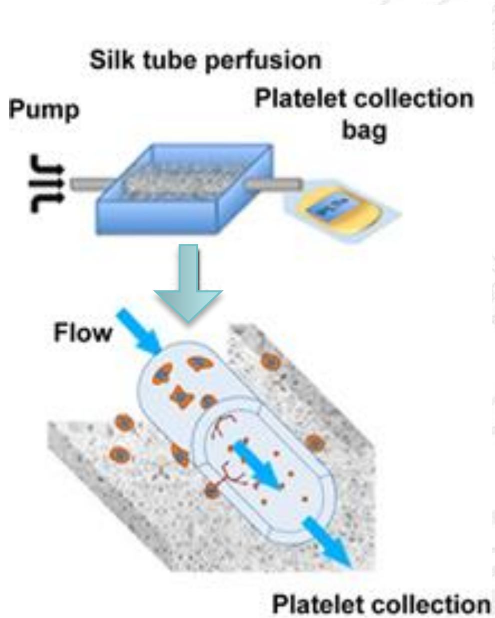
Sponge

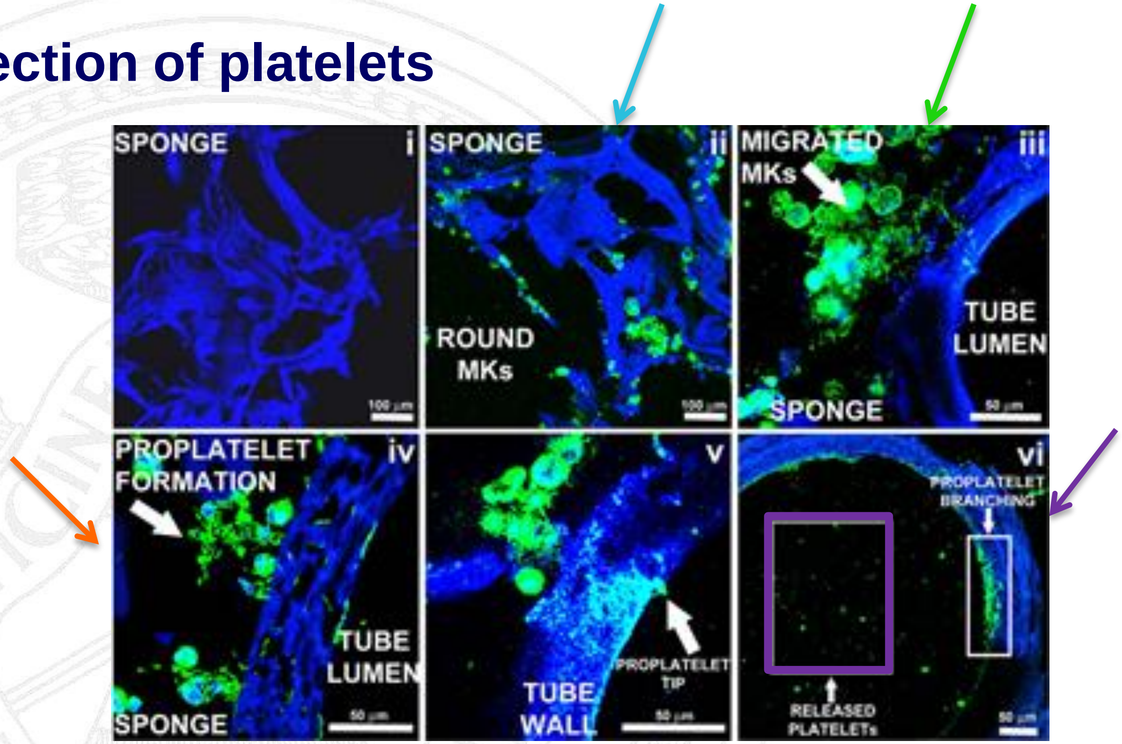


Spongy bone

''Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies'', Di Buduo *et al*

Production and collection of platelets



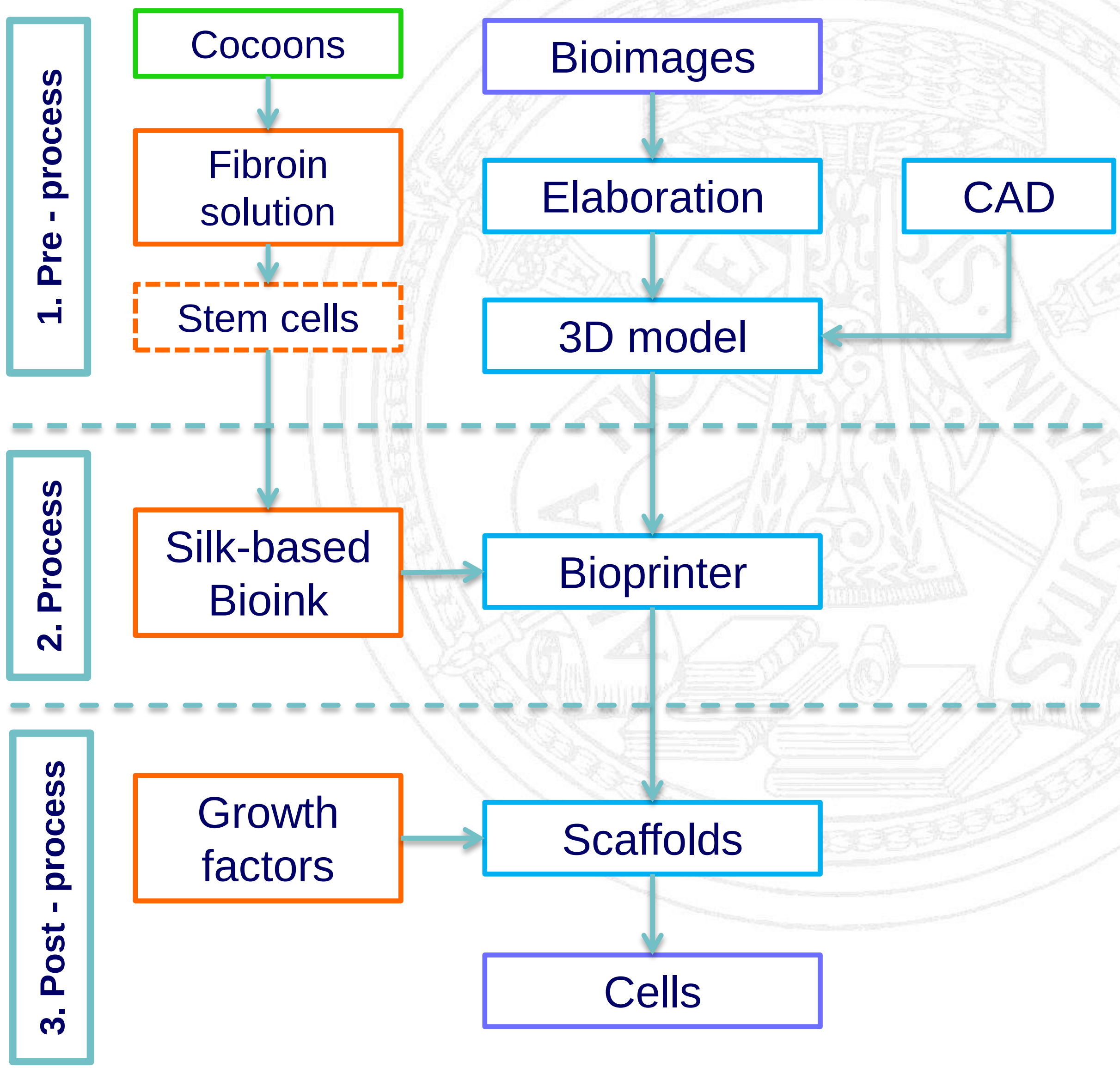


- Addition of the megakaryocytes and their migration after 16 h
- Extension of proplatelets after 24 h
- Blood perfusion for platelets collection into bags containing an anticoagulant (after 6 h)

''Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies'', Di Buduo *et al*

3D Bioprinting of silk to produce scaffolds: what we need

Process



Human resources

Farmes

- Provide cocoons

Medical Research (MD)

- Provide bioimages, stem cells and ECM components
- Evaluates the cells function in humans

Bioengineers

- Disegn models
- Run the printer with appropriate bionks

Biologist/Biotechnologist

- Provide bioik of silk
- Culture of stem cells
- Evaluates the cell funcion in rats

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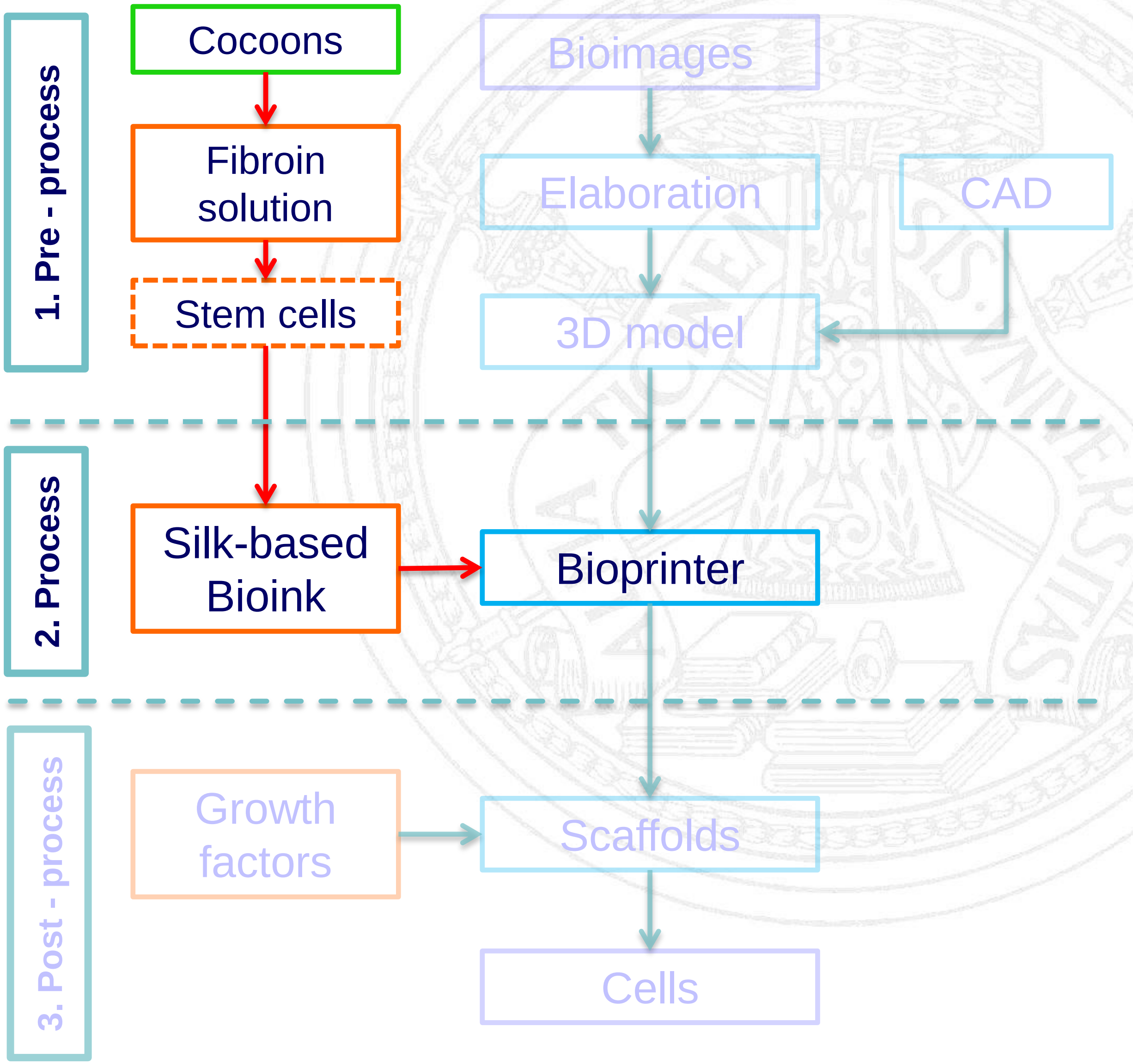

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Silk-based bioinks formulation - Tufts University of Boston

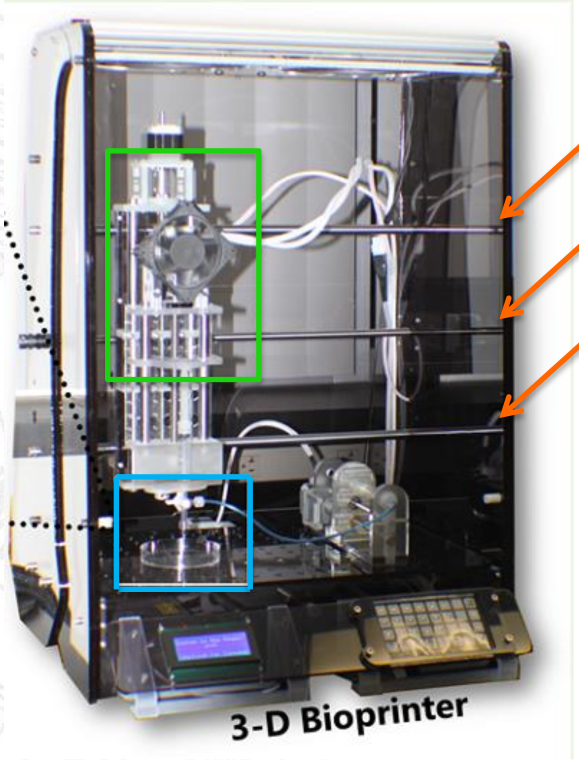
Pattern	Target of measure	Method	Results
Films	Solubility	• PBS at 26° C • Phase contrast microscop	• NS regardless of the printed volume (Glycerol) • S for volumes less than 5 µL (Erythritol) → Contemporary print for support strucutures
	Beta-sheet content	Spectrometry	Beta-sheet $\frac{1}{\propto}$ solubility
Discs	Profile	Interferometry	• Linear dependence between disc height and printed layers • Low presence of clots
Bioinks	Viscosity	• 15% silk, 5% polyol, 26° C • Viscometer	• Low without additives • Very high with propanediol → Influence of print resolution
Drops	Buckling height	• 80% silk, 20% polyol • 5 µL, 1 x 3 mm • Contact angle	• Rapid deformation without additives • Homogeneous deformation for glycerol and erythritol-based bioinks → Contemporary print for support structures

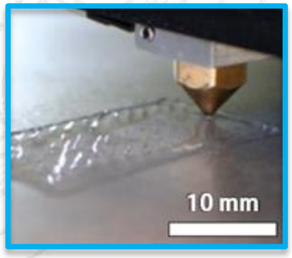
''Polyol-Silk Bioink Formulations as Two-Part Room-Temperature Curable Materials for 3D Printing'', Rod R. *et al*

3D Bioprinter at Tufts University of Boston

- Positioning of the printhead carriage and estrusion accomplished by 2-phase unipolar 1.8° steppers motor
- Lead screw: linear movement of 6.53 μm for step
- Temperature control: catrage heaters and thermistors

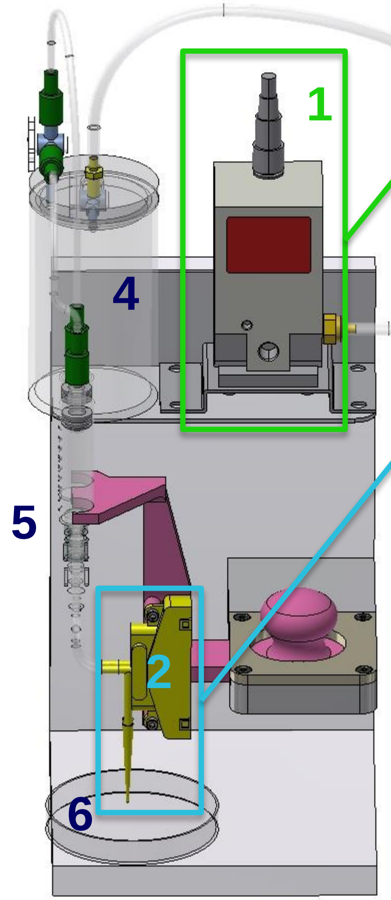
→ Layer-by-layer deposition of bioink to produce 3D structures

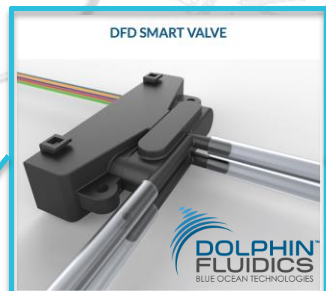




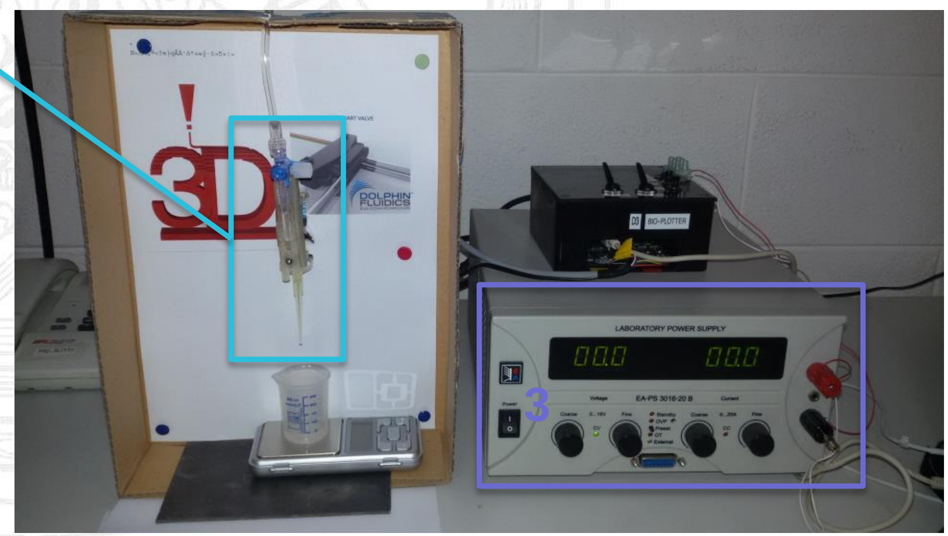
''Polyol-Silk Bioink Formulations as Two-Part Room-Temperature Curable Materials for 3D Printing'', Rod R. *et al*

Our possible implementation





1. Digital pressure regulator (ITV-2010; SMC, Japan)
2. Microelettrovalve (DFD SMART VALVE)
3. Power supply (0 – 3,3 V)
4. Pressurized tank
5. 5 or 10 mL disponsable syringes
6. Petri disc



Conclusions

- **Silk**
  - Excellent biomaterial because of robustness, elasticity, biocompatibility and programmable biodegradability

- **Electrospinning**
  - Pros: Nanoscale fibers that mimic the extracellular environment
  - Cons: Inability to control shape of structure and size of pores

- **3D Bioprinting**
  - Novel self-curing silk-polyol blended inks
  - Mechanically robust and insoluble layers for complex 3D geometries
  - Open problem: incorporate stem cells

Grazie per l’attenzione

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