MUNI

Biomaterial of the future

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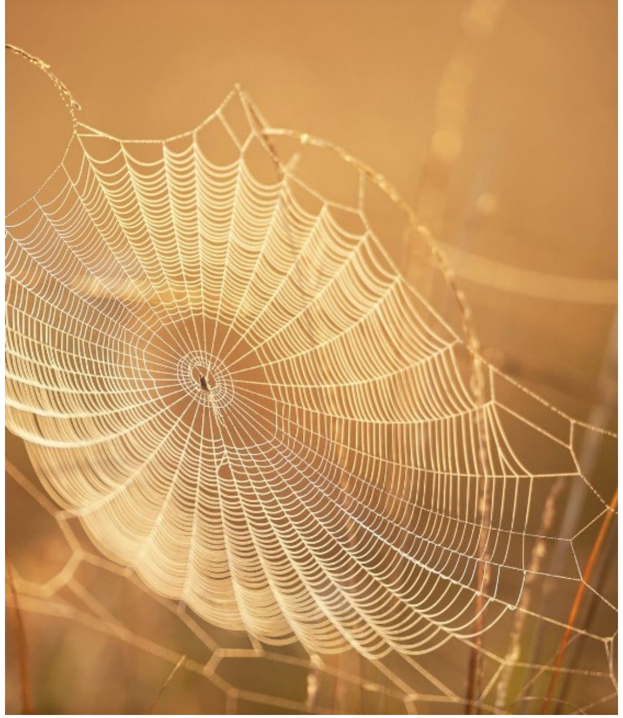
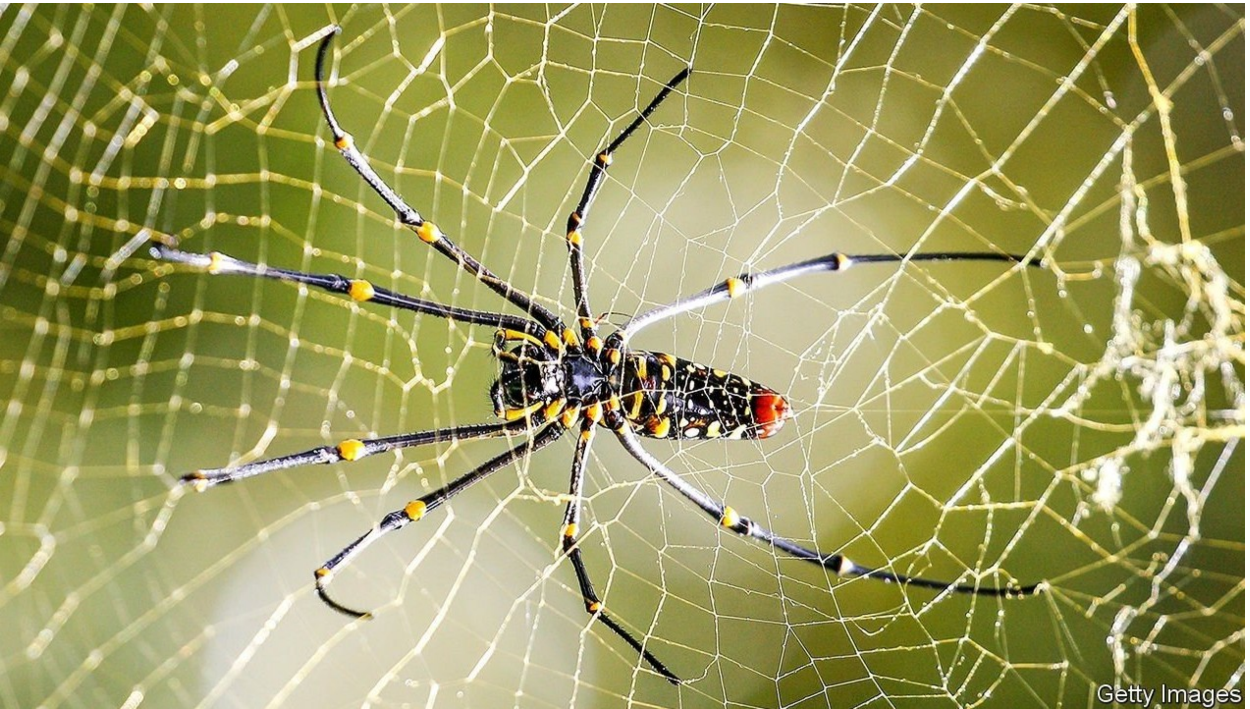
Introduction

Properties of spider silk protein

Engineering and host systems

Aplications





Spider silk

Protein fibre spun by spiders.

Spiders use their silk to make webs or other structures as a nests or cocoons to protect their offspring, or to wrap up prey. They can also use their silk to suspend themselves, in some cases, spiders may even use silk as a source of food

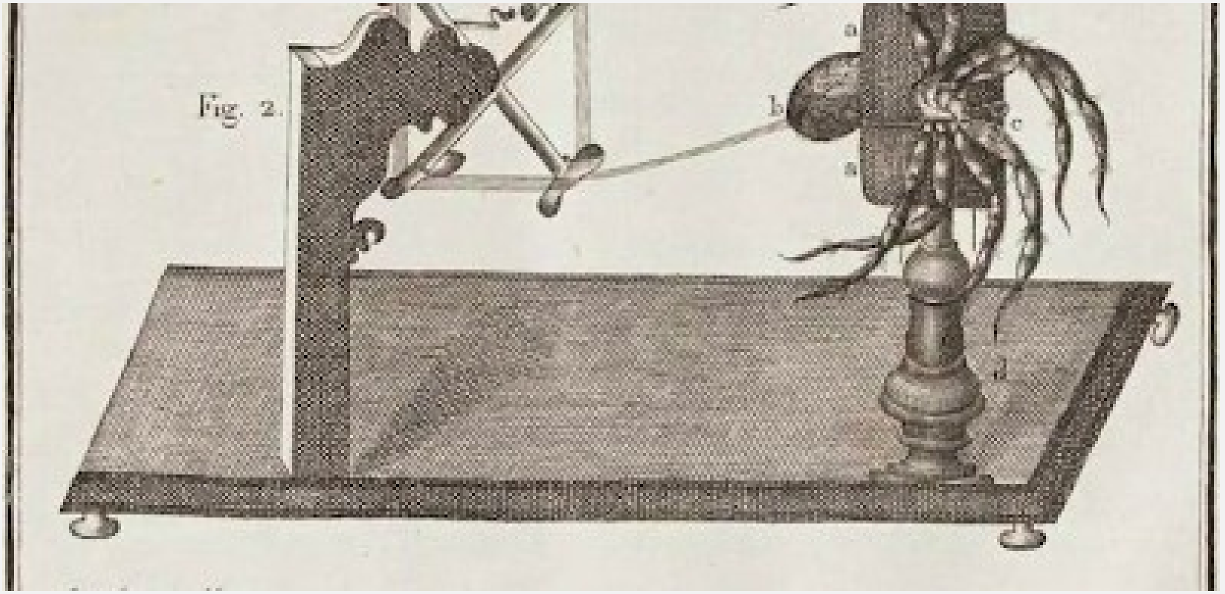


Fig. 2.

Properties

- Strength
- Density
- Extensibility
- Toughness
- Temperature

Material	Strength (N/m^2)	Elongation (%)	Energy to break (J/kg)
Dragline silk	4×10^9	35	4×10^5
Minor ampullate silk	1×10^9	5	3×10^4
Flagelliform silk	1×10^9	>200	4×10^5
Tubuliform silk	1×10^9	20	1×10^5
Aciniform silk	0.7×10^9	80	6×10^9
Kevlar	4×10^9	5	3×10^4
Rubber	1×10^6	600	8×10^4
Tendon	1×10^6	5	5×10^3

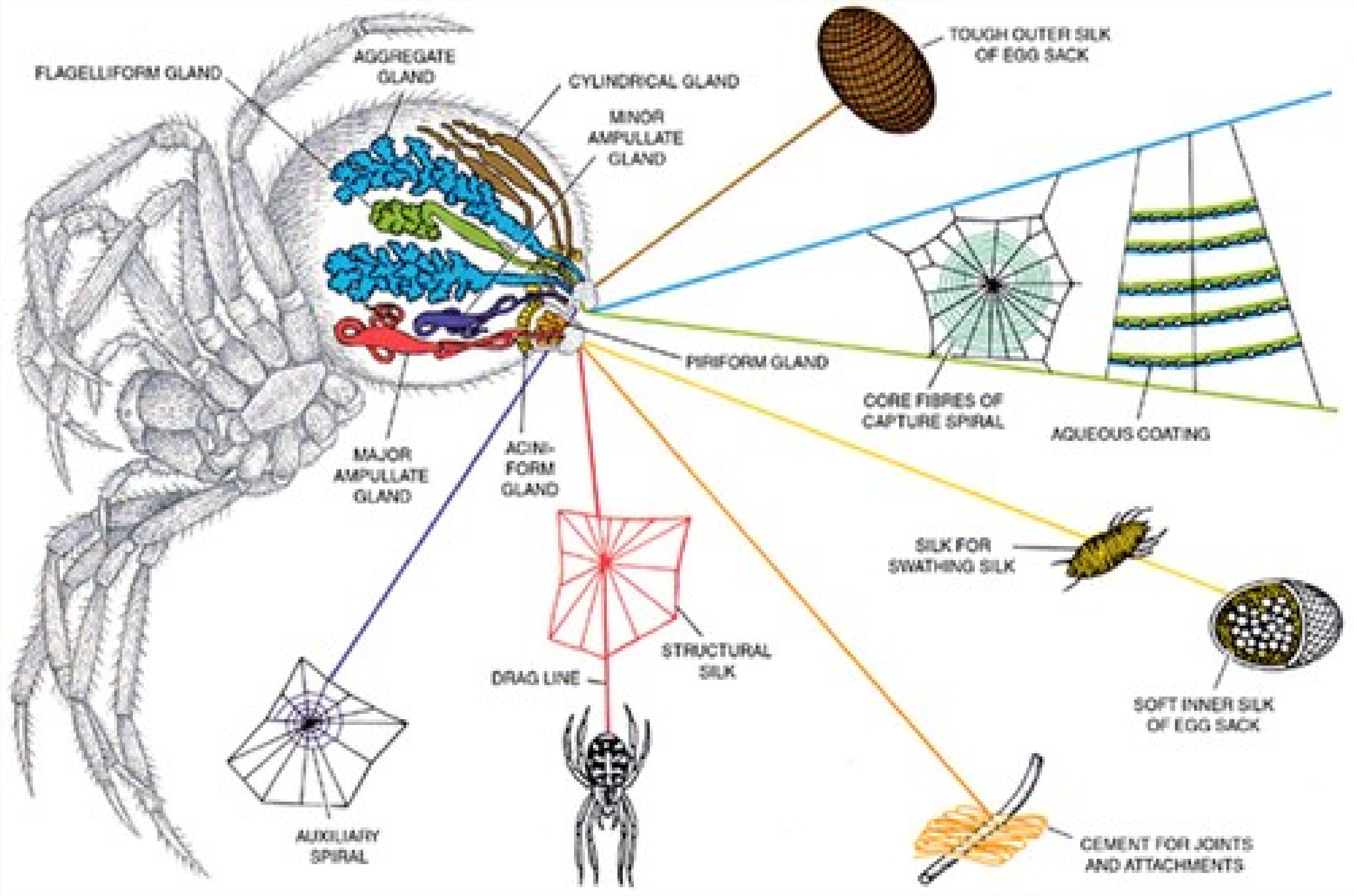
	tensile strength (MPa)		breaking strain (%)		Young's modulus (GPa)		toughness (MJ/m^3)	
specie	this work	literature[a]	this work	literature[a]	this work	literature[a]	this work	literature[a]
B. mori	635 ± 108	690 ± 20	21.9 ± 4.9	38.5 ± 6.4	11.7 ± 2.2	9.6 ± 0.6	101 ± 34	187 ± 33
		460 ± 80		24.1 ± 7.7		7.2 ± 1.7		81 ± 34
A. pernyi	426 ± 55	553 ± 64	50.8 ± 7.0	45.0 ± 3.7	5.0 ± 0.6	10.5 ± 0.8	128 ± 33	151 ± 25
		430 ± 80		29.6 ± 10		4.8 ± 1.1		79 ± 27
S. c. ricini	284 ± 88	560 ± 20	34.2 ± 12.5	47.4 ± 6.1	4.1 ± 0.6	6.3 ± 0.3	61 ± 33	156 ± 24
		470 ± 110		29.4 ± 8.0		4.7 ± 1.3		86 ± 30
A. assamensis	495 ± 48	490 ± 20	51.1 ± 6.4	55.9 ± 5.3	5.3 ± 0.4	4.5 ± 0.3	141 ± 28	148 ± 16
		360 ± 100		29.2 ± 10.7		4.3 ± 1.2		68 ± 31
N. clavipes[b]		1215 ± 60		20.0 ± 1.1		13.8 ± 0.8		111 ± 8
A. gemmoides[b]		1376 ± 40		22.0 ± 1.0		8.3 ± 0.5		141 ± 1
L. hesperus[b]		1441 ± 60		30.1 ± 1.8		10.2 ± 0.8		181 ± 10

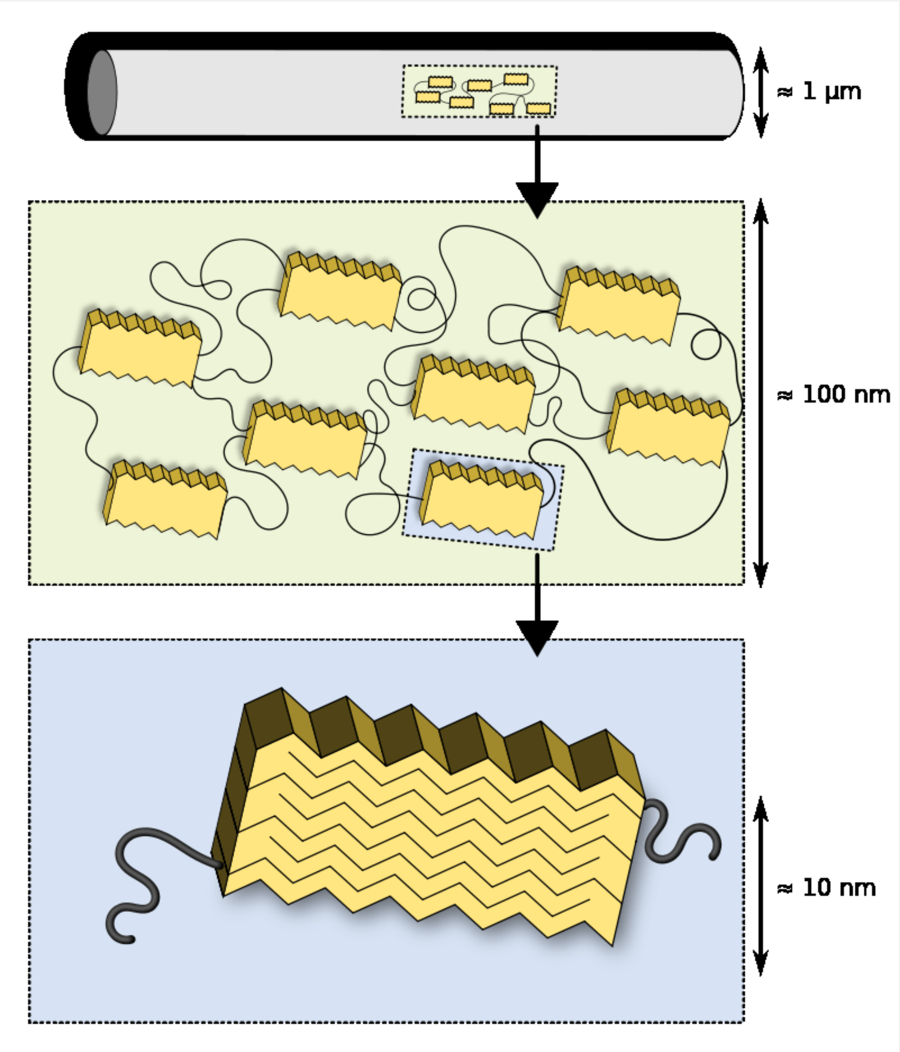
[a]Literature values are obtained from refs 18, 28, and 30. [b]Spider dragline silks.

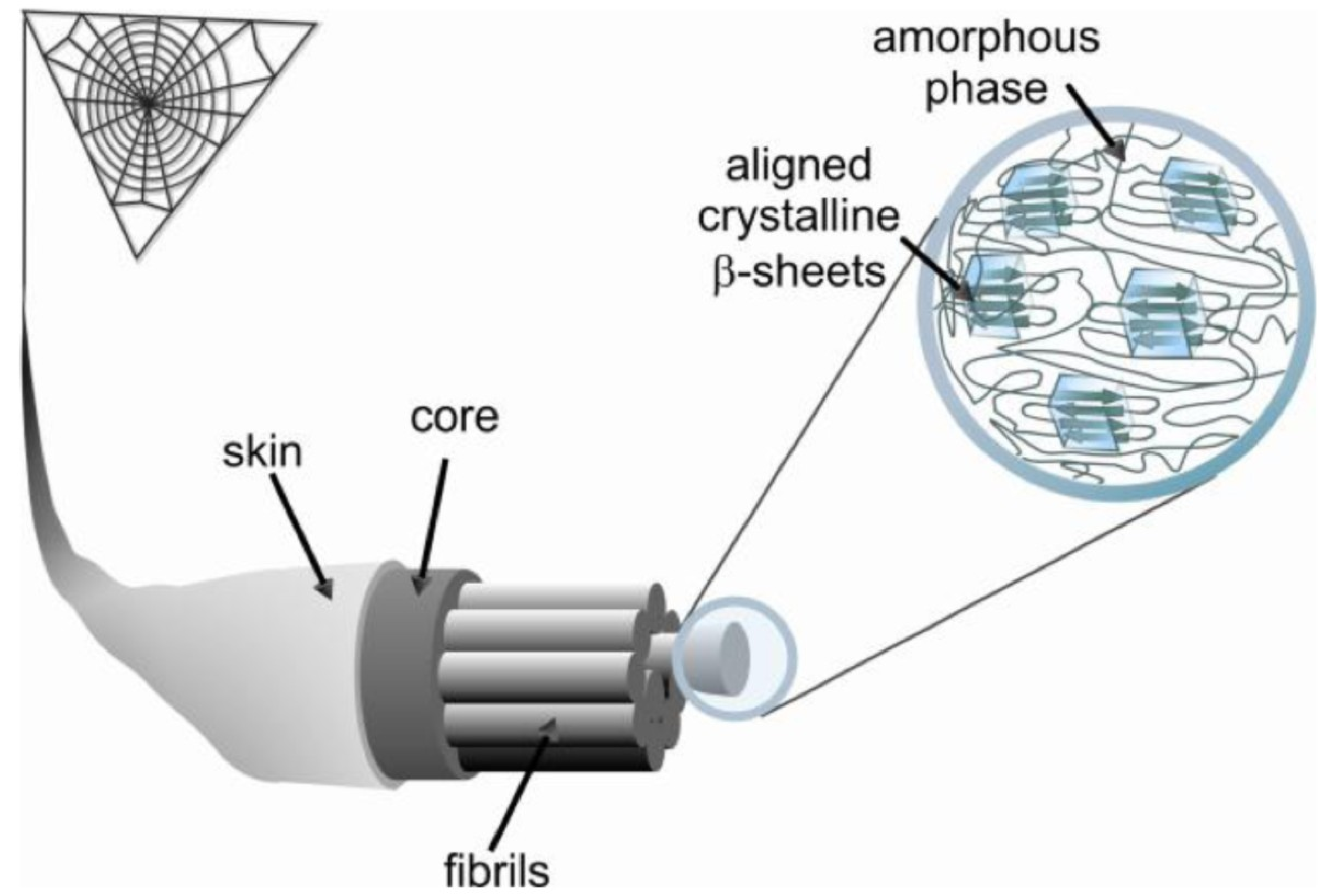
Stucture

- Spiders, can produce seven kinds of silk or glue from seven different types of glands.
- The structure-property-function relationship of these seven types of spider silk is significantly different.

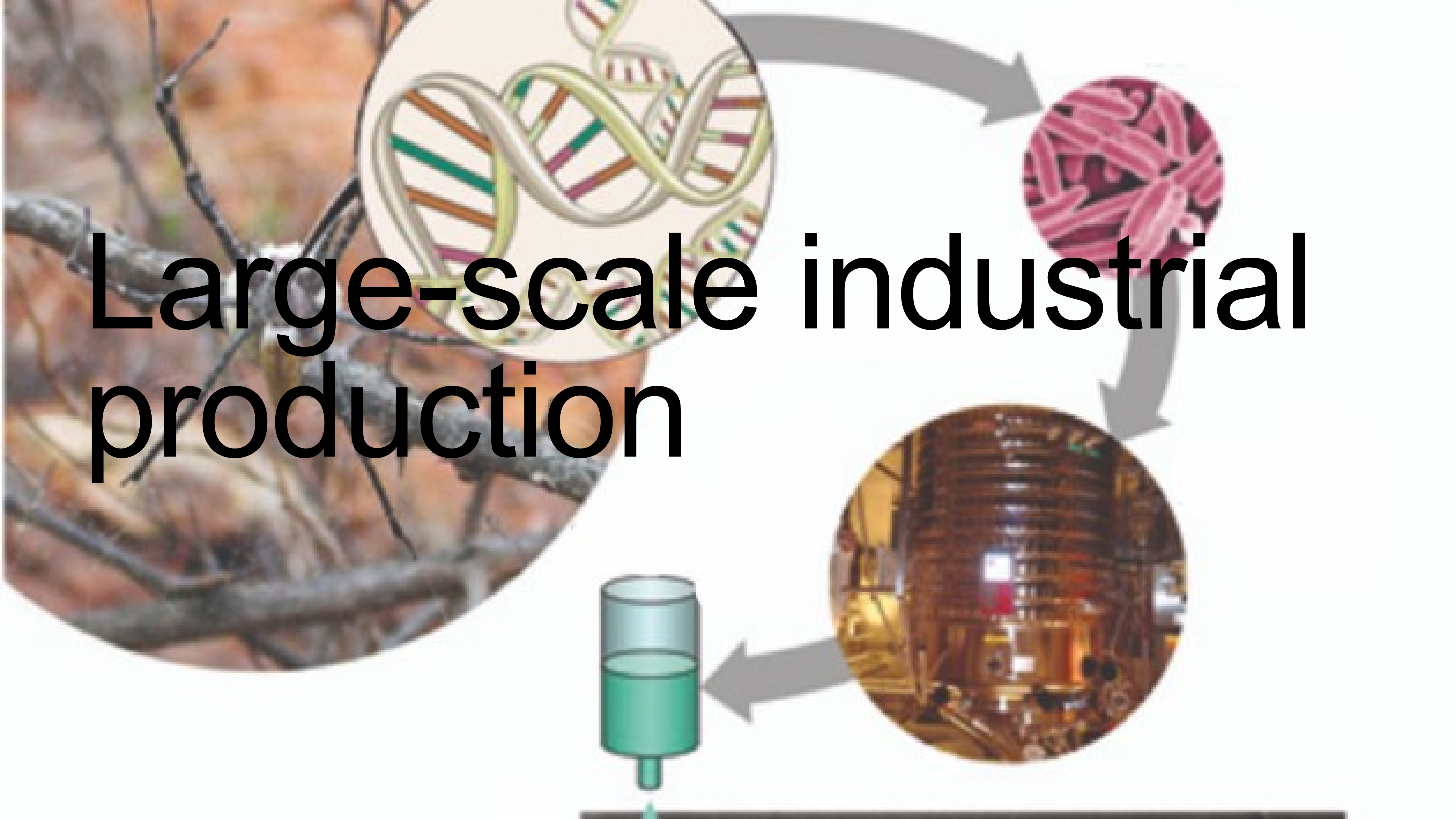
Silk	Use
major-ampullate (dragline) silk	Used for the web's outer rim and spokes and also for the lifeline. Can be as strong per unit weight as steel, but much tougher.
capture-spiral (flagelliform) silk	Used for the capturing lines of the web. Sticky, extremely stretchy and tough. The capture spiral is sticky due to droplets of aggregate (a spider glue) that is placed on the spiral. The elasticity of flagelliform allows for enough time for the aggregate to adhere to the aerial prey flying into the web.
tubiliform (a.k.a. cylindriform) silk	Used for protective egg sacs. Stiffest silk.
aciniform silk	Used to wrap and secure freshly captured prey. Two to three times as tough as the other silks, including dragline.
minor-ampullate silk	Used for temporary scaffolding during web construction.


FLAGELLIFORM GLAND
AGGREGATE GLAND
CYLINDRICAL GLAND
MINOR AMPULLATE GLAND
TOUGH OUTER SILK OF EGG SACK
PIRIFORM GLAND
CORE FIBRES OF CAPTURE SPIRAL
AQUEOUS COATING
MAJOR AMPULLATE GLAND
ACINI-FORM GLAND
SILK FOR SWATHING SILK
STRUCTURAL SILK
DRAG LINE
SOFT INNER SILK OF EGG SACK
AUXILIARY SPIRAL
CEMENT FOR JOINTS AND ATTACHMENTS


≈ 1 µm
≈ 100 nm
≈ 10 nm


amorphous
phase
aligned
crystalline
β-sheets
core
skin
fibrils

Large-scale industrial production



Bacterial and Yeast systems

E.coli stems

Easy for genetic manipulation

Short generation time

Comparative low cost

Potential for industrial scale-up

Issues

Low levels of production

Trascriptional errors attributable to highly repetitive amino acid sequence

(depletion of the alanyl- and glycyl-tRNA pool)

Salmonella typhimurium

Pichia pastoris

Saccharomyces cerevisiae


Plant systems

Gene silencing is common issue in trasgenic plants

Prevalently for genes containing highly repetetive sequences

More expensive and challenge compare to another host sytems

Bombyx mori and Insect cell lines

Transgenic silkworms containing spider silk gene

Baculovirusis - based expression system

piggyBac expression system

Engineered silkworms for expression chimeric silkworm/spider spidroin

Incorporation of spider silk gene by CRISPR/Cas9

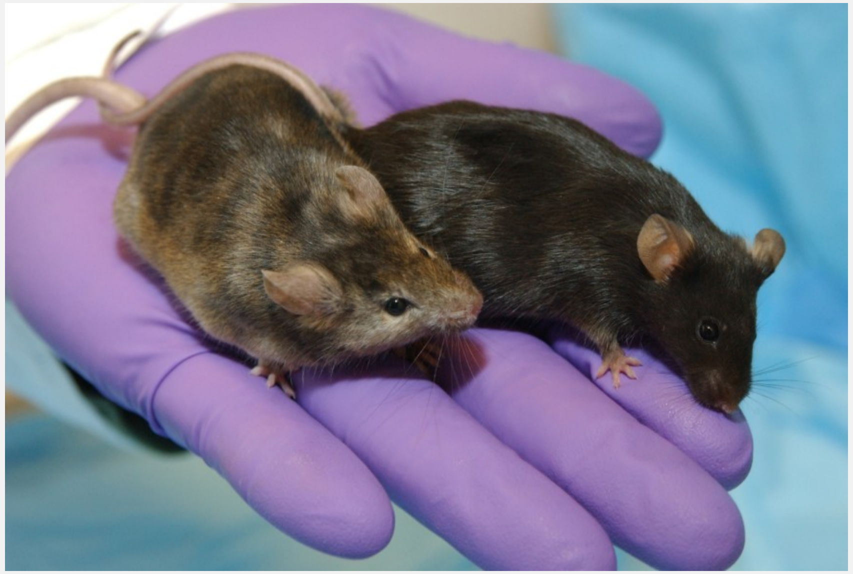
Mammalian cell lines and Transgenic animals

Mammalian cell lines …..

Baby hamster kidney cells = BHK

Bovine mammary epithelial alveolar cells = MAC-T

- Low production level
- 60-140 kDa



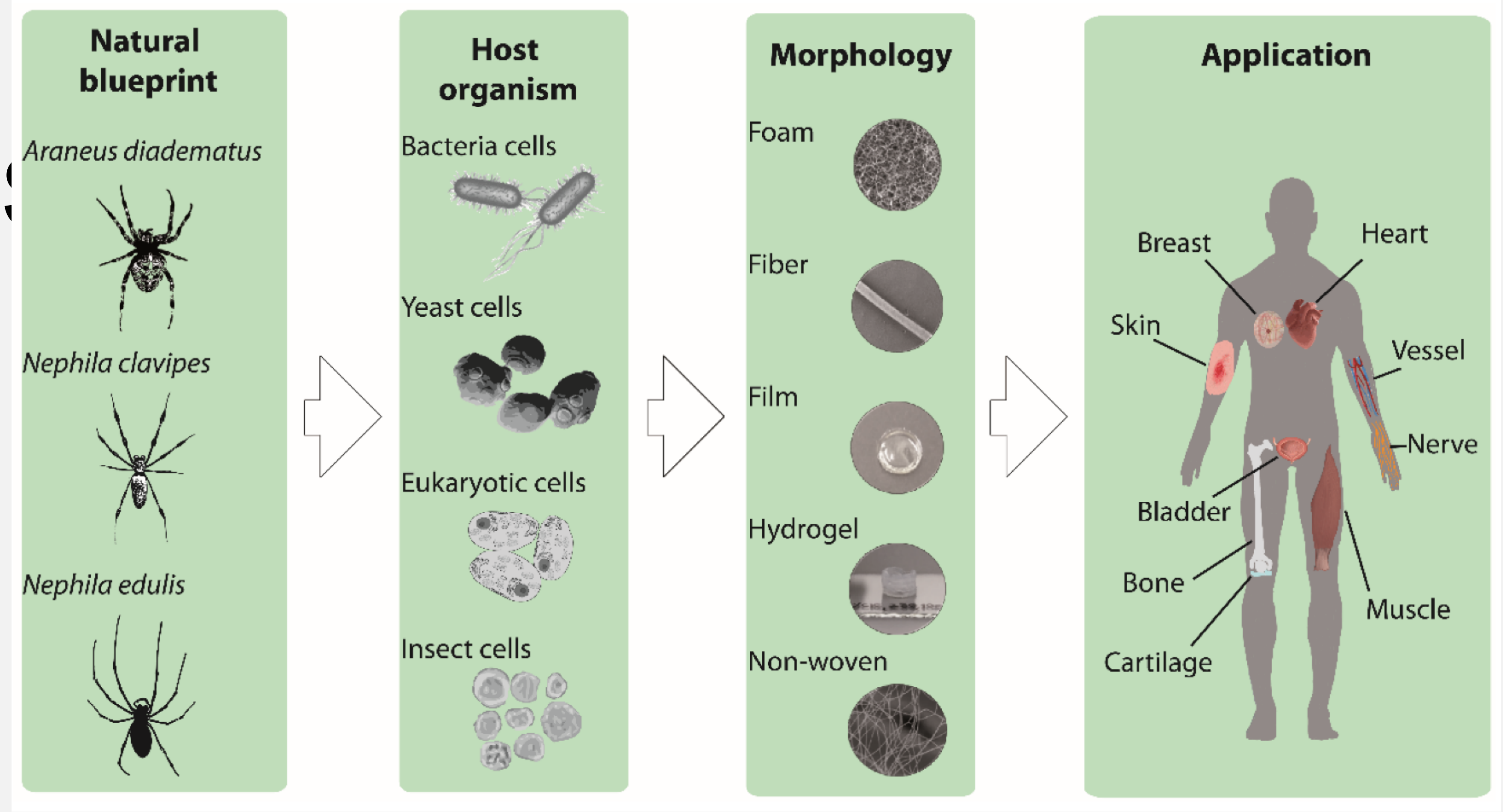
Summarize

E.coli and yeast – suitable for clothing and textile industry

Transgenic silkworms – biomedical applications

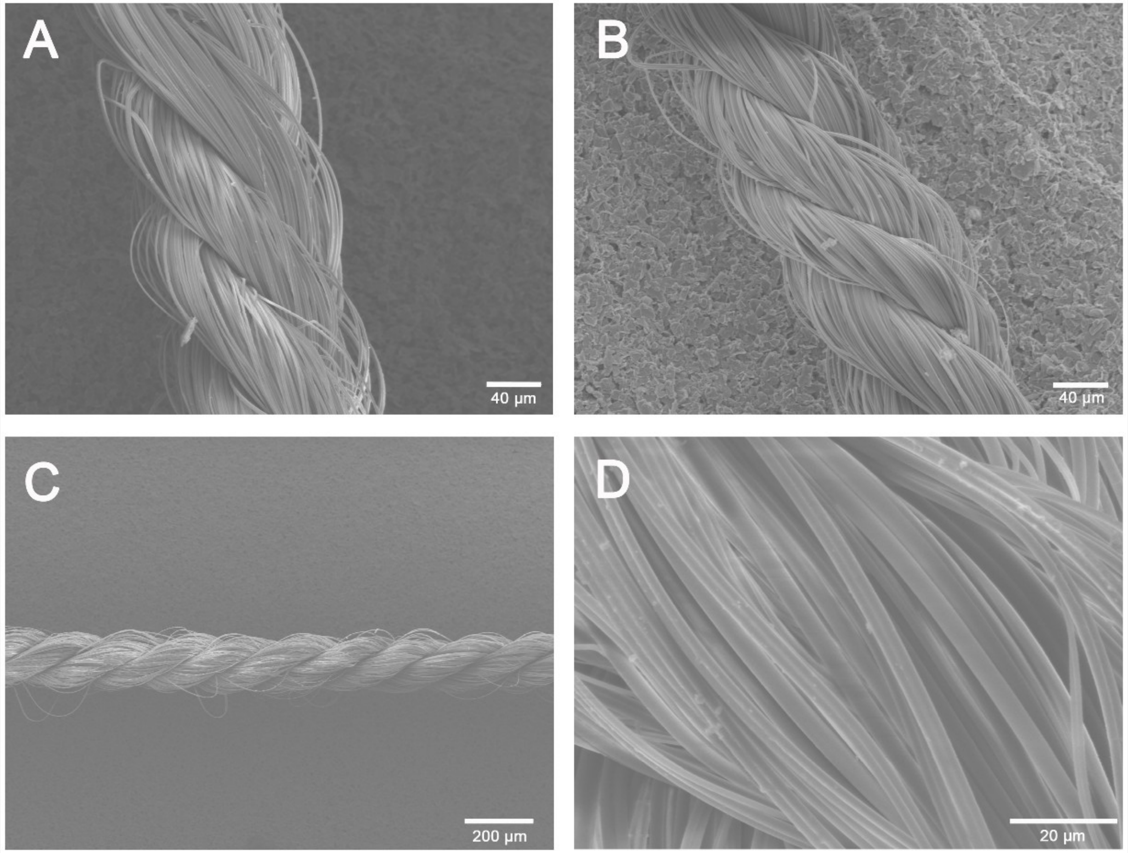
Mammalian systems – biomedical applications

Applications



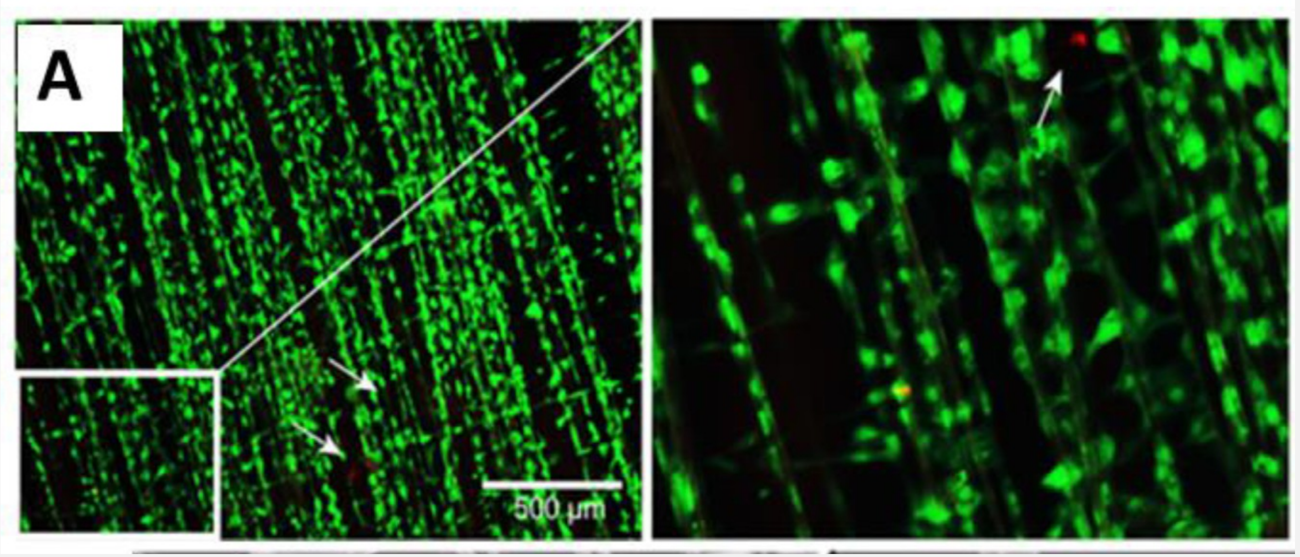
- Interesting in medical applications, due to their biocompatibility, environmental and mechanical stability as well as a high surface-to-volume ratio

TREATMENT OF TENDON RUPTURES



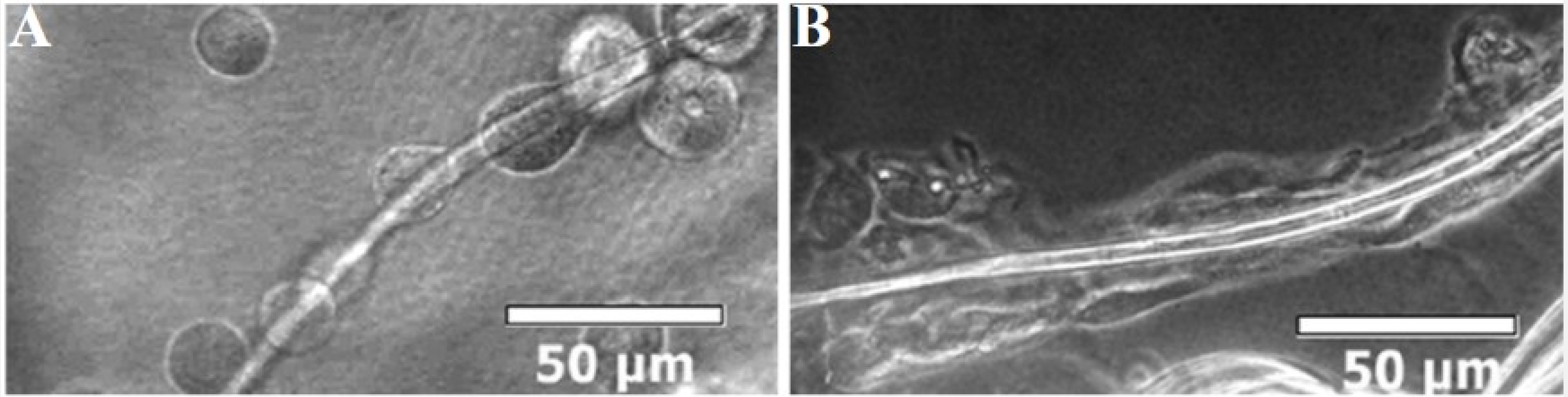
Spiderprotein fiber that are tied together and make suture.

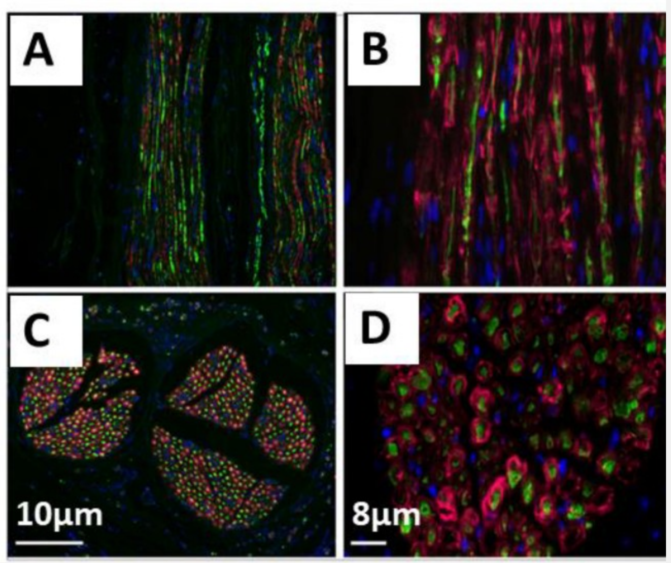
BLADDER RECONSTRUCTION



Spider silk fibres collected on stainless-steel frame as a cross wave mesh

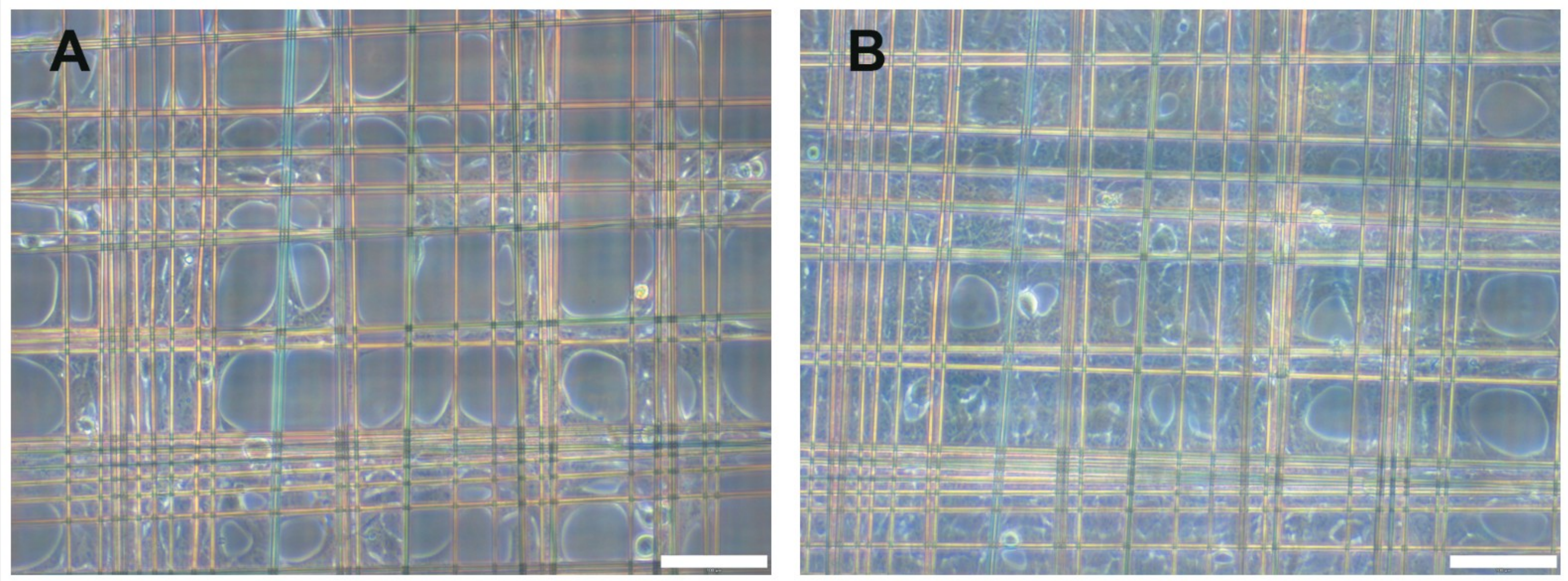
HUMAN NEURON MODEL

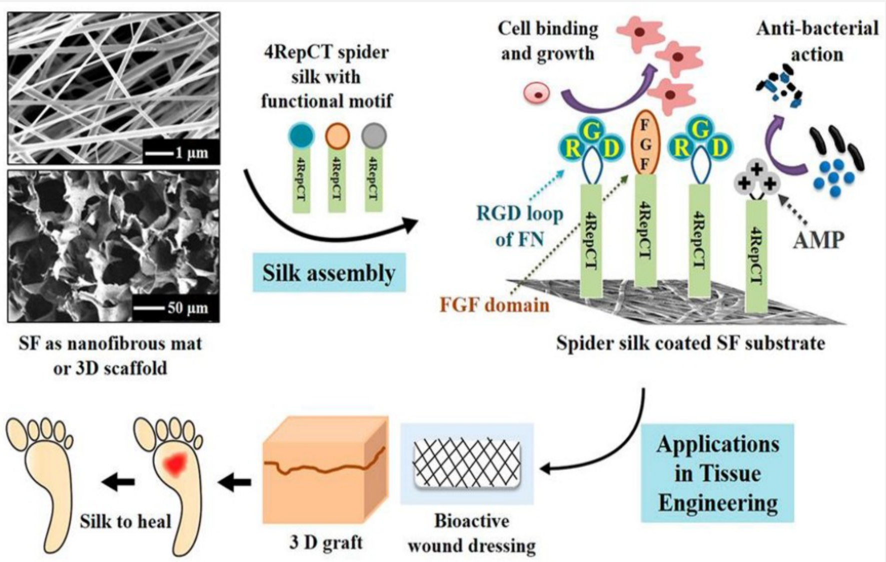




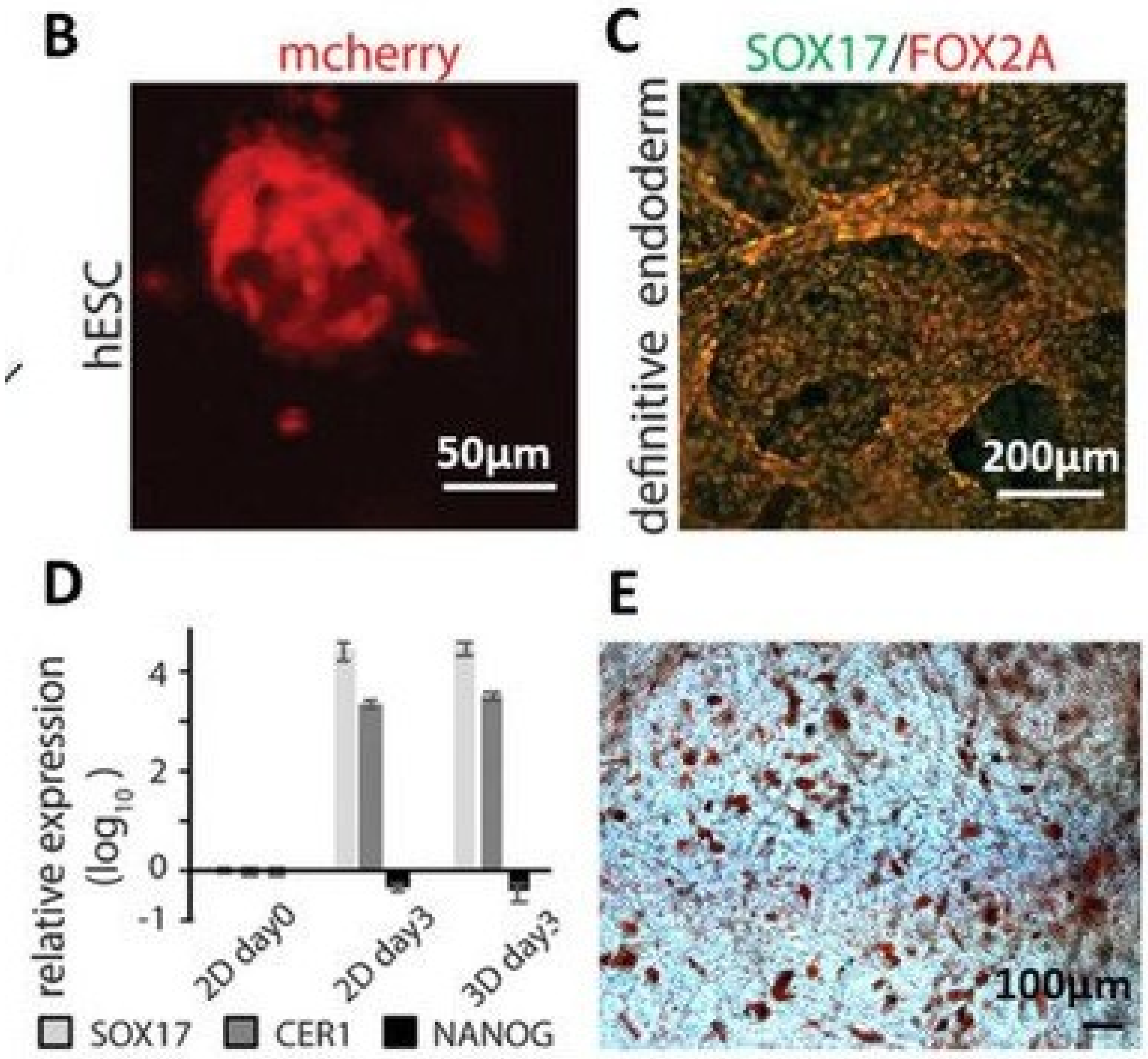
Spider silk protein as a guidance conduit for human neurons

SKIN REPAIR IN PLASTIC RECONSTRUCTIVE SURGERY





Spider silk fibres woven on steel frame used for cultivating cells.



Foams

- Can be used as 3D cell culture scaffolds.
- In artificial scaffolds, the inner free volume and degradation behavior, as well as the mechanical properties of the employed materials, can be tuned to compare with the natural ECM by controlling the porosity and pore sizes.

Development of methods to mass-produce spider silk has led to manufacturing of military, medical and consumer goods, such as ballistics armour, athletic footwear, personal care products, breast implant (coating materials for implants and other biomedical products), mechanical insulin pumps, fashion clothing, and outerwear.

Golden orb spider silk

In Madagascar 2009

Largest know piece of cloth made of spider silk.

Resources

Spider Silk for Tissue Engineering Applications

Host Systems for the Production of Recombinant Spider Silk

De Novo Design of Recombinant Spider Silk Proteins for Material Applications

Functionalized DNA-spider silk nanohydrogels for controlled protein binding and release

Recombinant Spider Silk-Silica Hybrid Scaffolds with Drug-Releasing Properties for Tissue Engineering Applications

Interfacial Behavior of Recombinant Spider Silk Protein Parts Reveals Cues on the Silk Assembly

Thank you for your attention