Technological solutions for Spinal Cord Injuries

Dr. M. Teresa González

Department of Nanomedicine

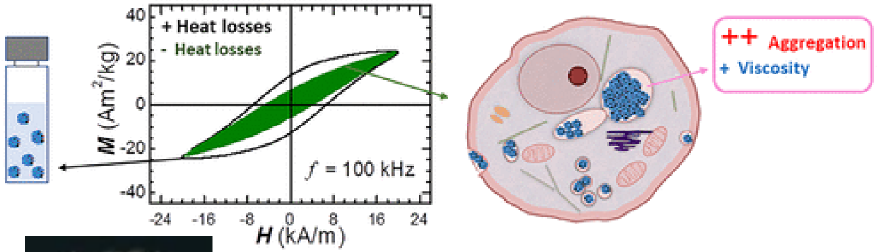
Fundación IMDEA Nanociencia – Madrid (Spain)

Presentation Outline

- Spinal Cord Injury (SCI) – some history
- How to cure Spinal Cord Injury?
  - Neural regeneration
  - Neuroprostheses
  - Brain-computer interfaces
- Nanotechnology for Spinal Cord Injury repair
  - Nanomaterials at IMDEA nanociencia

Nanomedicine at IMDEA Nanociencia

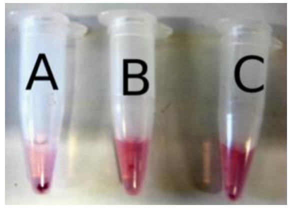
Magnetic nanoparticles for hyperthermia treatment to cancer

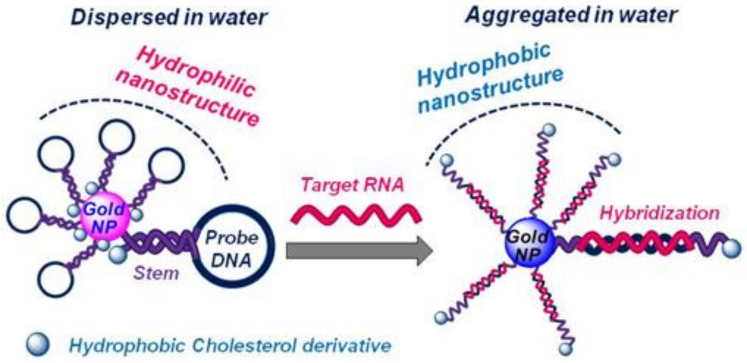


Fran J. Terán

[*ACS Nano* **12**, 3, 2741–2752 (2019)]

Sensors and Drug delivery based on oligonucleotides /Au nanoparticles

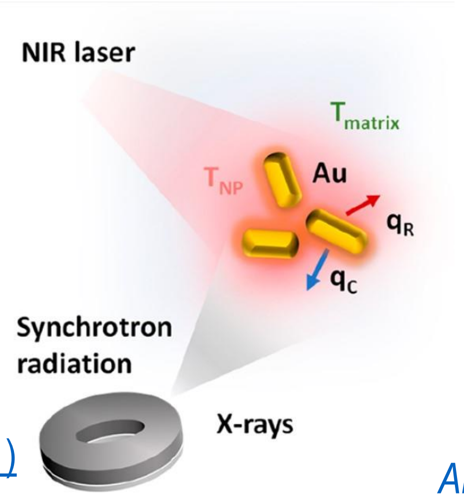




Álvaro Somoza including Covid-19

[*Chem. Commun* **50**, 3018-3020 (2014)]

Nanothermometry to improve anticancer strategies

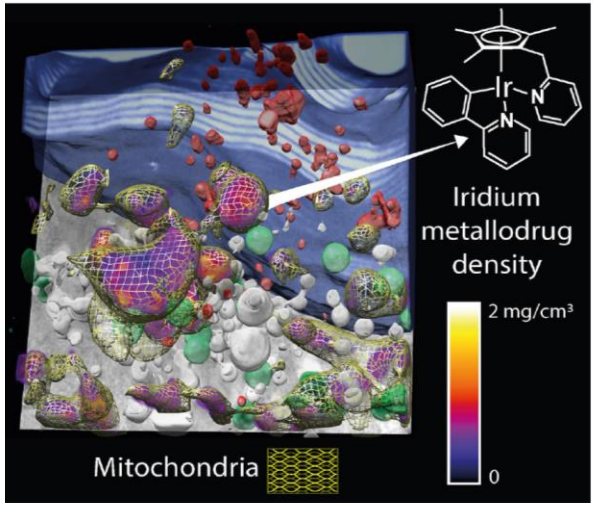


Ana Espinosa

[*Nano Lett.* **21**, 769–777 (2021)]

Single cell localization of metallodrugs in cryopreserved cancer cell

Ana M. Pizarro



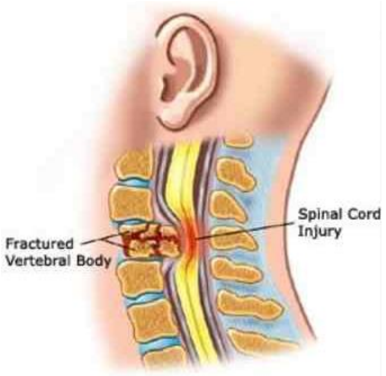
[*Angew. Chem. Int. Ed.* **59**, 1270–1278 (2020)]

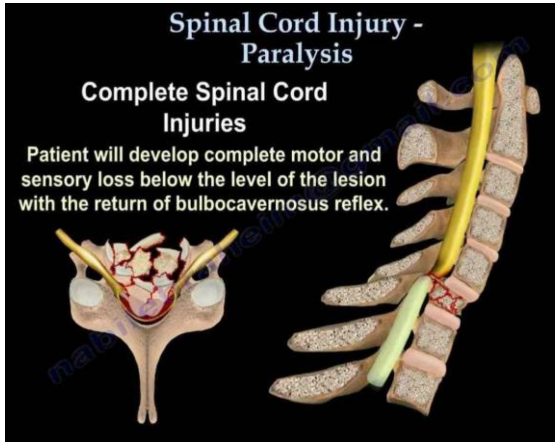
Spinal Cord Injury (SCI)

After a **Spinal Cord Injury** (SCI), communications between the brain and the neural circuits that dictate movement are interrupted.

Brain, muscles and receptors are working, but **the connection is lost!**

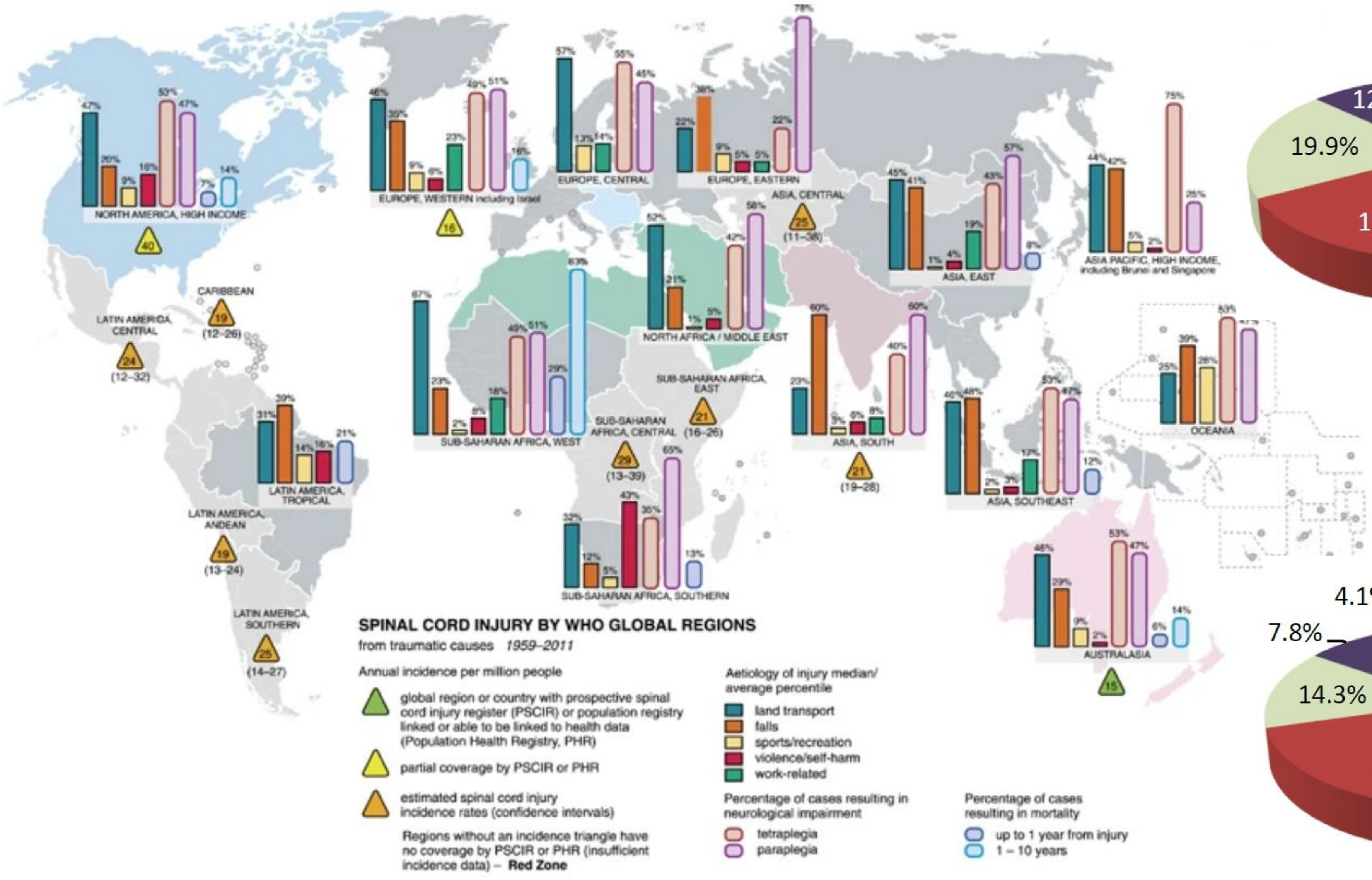
paralysis: paraplegia / tetraplegia

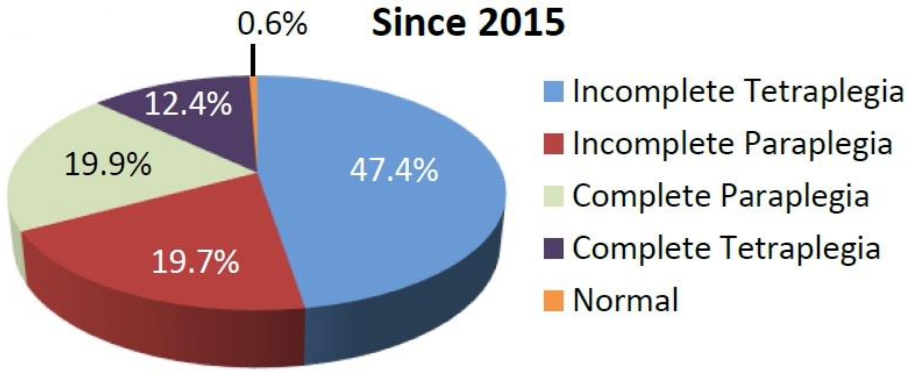


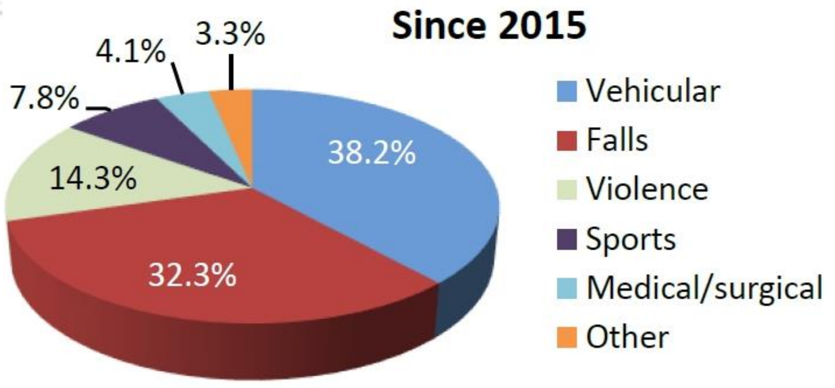


Between 250 000 and 500 000 people suffer a SCI every year (WHO)
In European countries 10 – 20 per million inhabitants-year
Estimated lifetime cost in US for complete tetraplegia occurring at 25 years old is over 5 million dollars

Spinal Cord Injury (SCI)





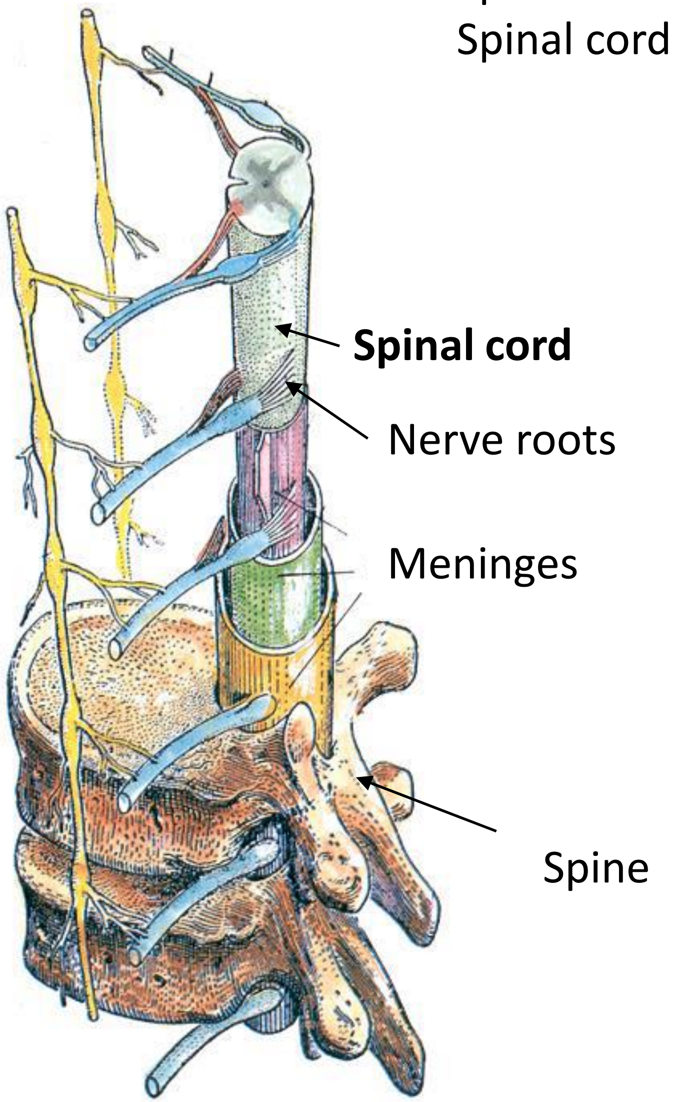


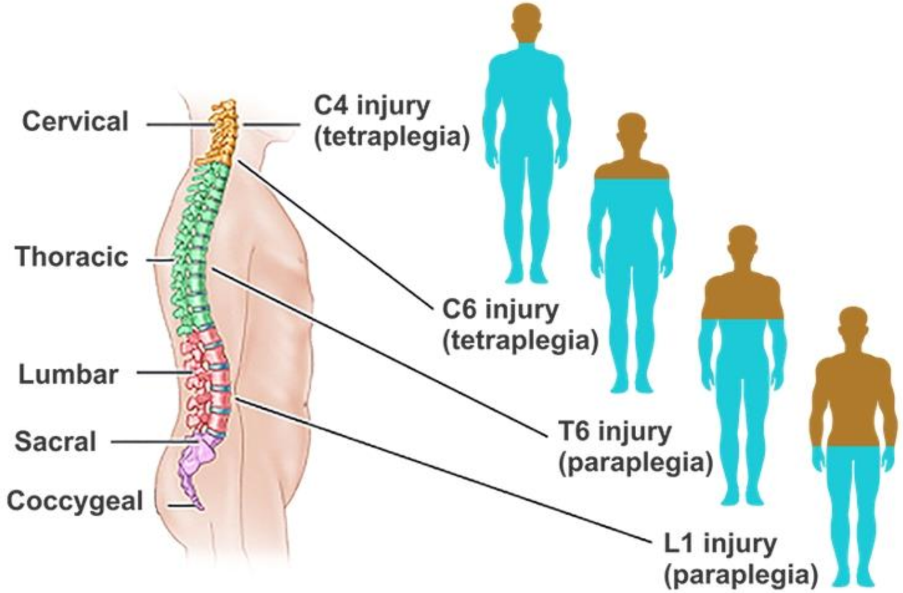
Lee et al. Spinal Cord 2014, 52, 110–116

US National SCI Statistical Center Facts and Figures 2021

Spinal cord anatomy

Spine: 73 cm long
Spinal cord: 40-45 cm long
1 cm diameter

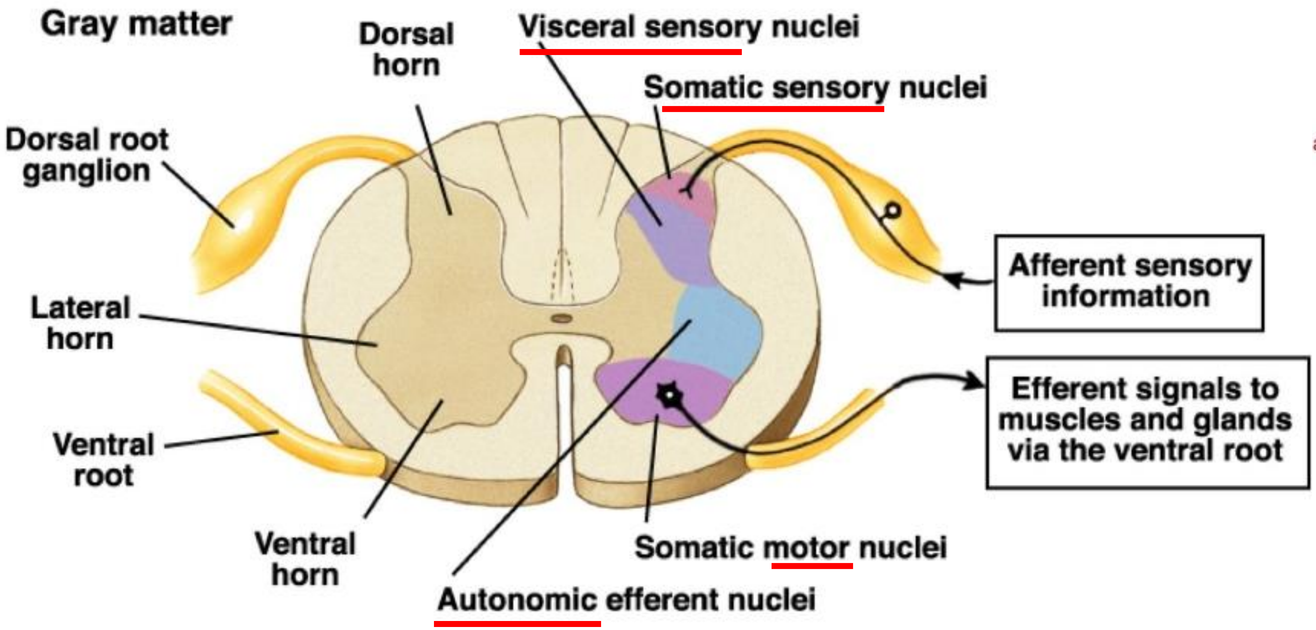


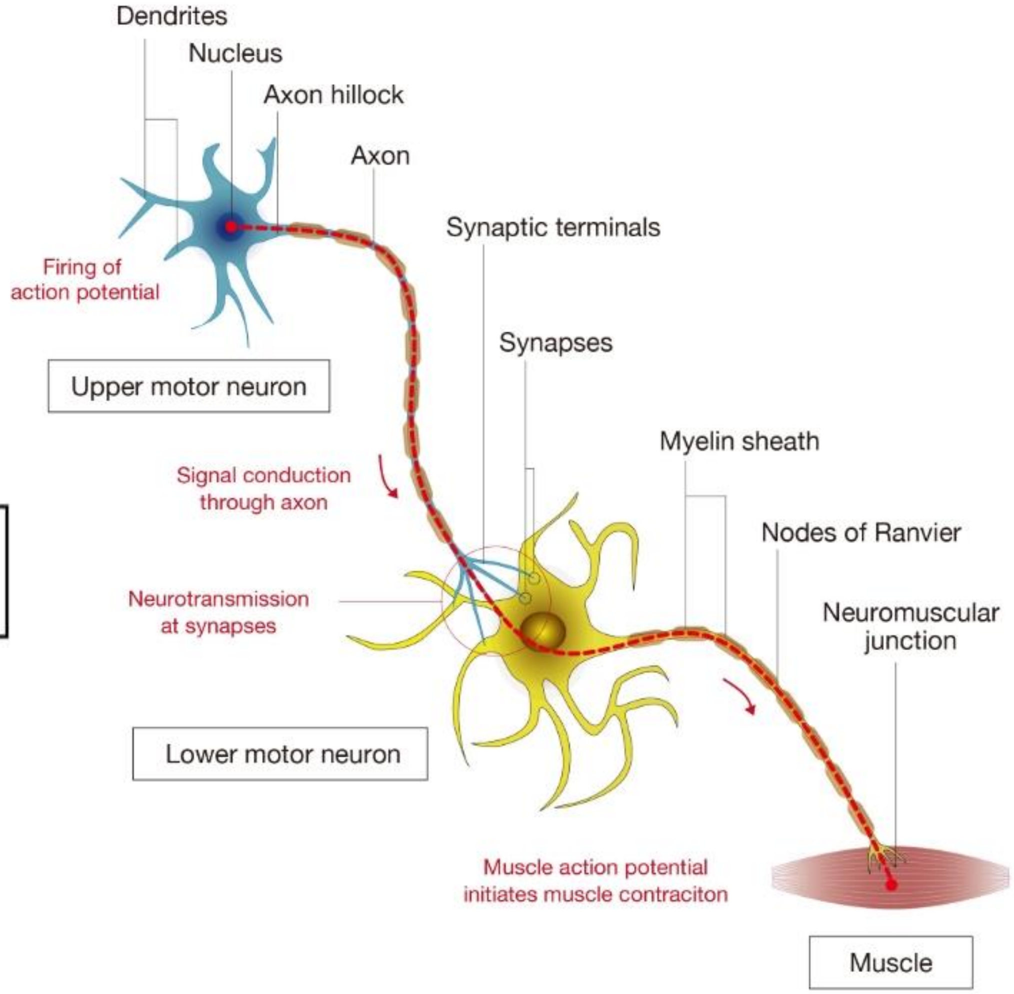


European SCI Federation webpage

R.P.Bungue et al., Advances in Neurology 1997, 72, 305-313

Spinal cord anatomy





- white matter: bundles of axons
- gray matter: neuron cell bodies

- Motor information
- Sensory information
- Automatic nervous system

H. Katoh et al. Front. Cell. Neurosci. 2019, 13, 248

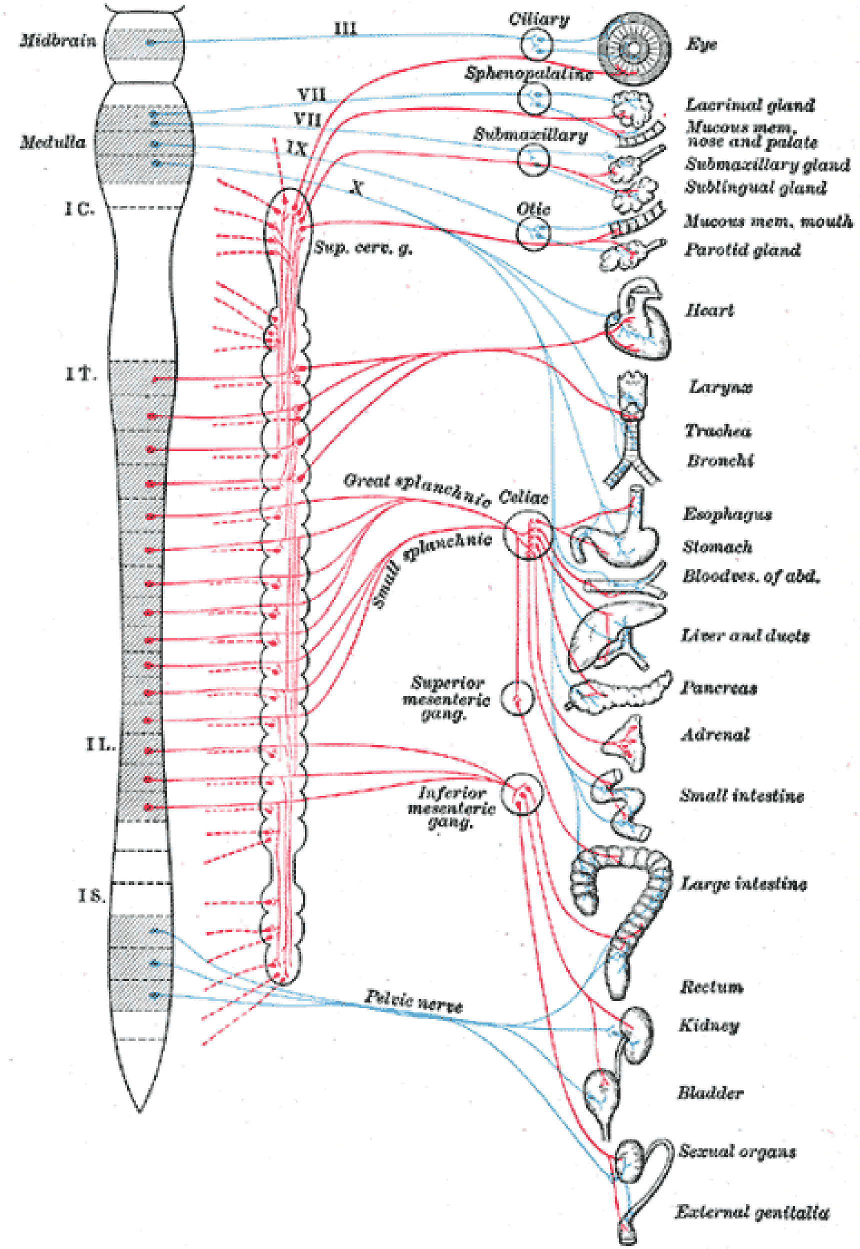
Spinal cord anatomy

Autonomic nervous system (**ANS**) regulates:

- Heart rate
- Digestion
- Respiratory rate
- Urination
- Sexual arousal

It acts mostly unconsciously

It is not only motion that is lost in SCI

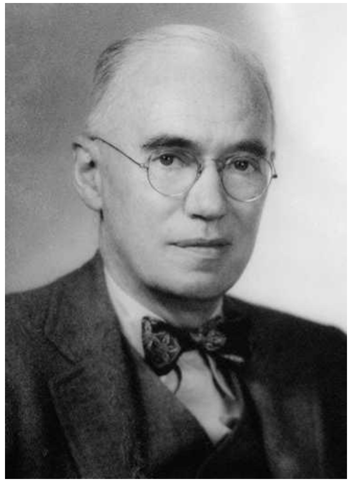


SCI historical progress

- … – 19th century: An ailment not to be treated
- 19th – early 20th century: Desperate treatments
- Thirties of the 20th century: **stabilization** with pharmacological treatment

  Essential the discovery of penicillin 1928 (**Alexander Fleming**) – Antibiotics
- Thirties – seventies : How to survive? Stabilization and **rehabilitation**
- Eighties – :

How to cure SCI?



Donald Munro (1898–1978)
Father of paraplegia
First SCI unit in 1936

John Young (1919–1990)
establishment of global rehabilitation

W. H. Donovan, J Spinal Cord Med. 2007,30,85–100

SCI in Spain: Hospital Nacional de Parapléjicos

- Inaugurated in 1974
- Extended in 2013
- Global rehabilitation
- Leading research
- Awards received each year

https://hnparaplejicos.sanidad.castillalamancha.es

How to cure (palliate) SCI?

Two strategies:

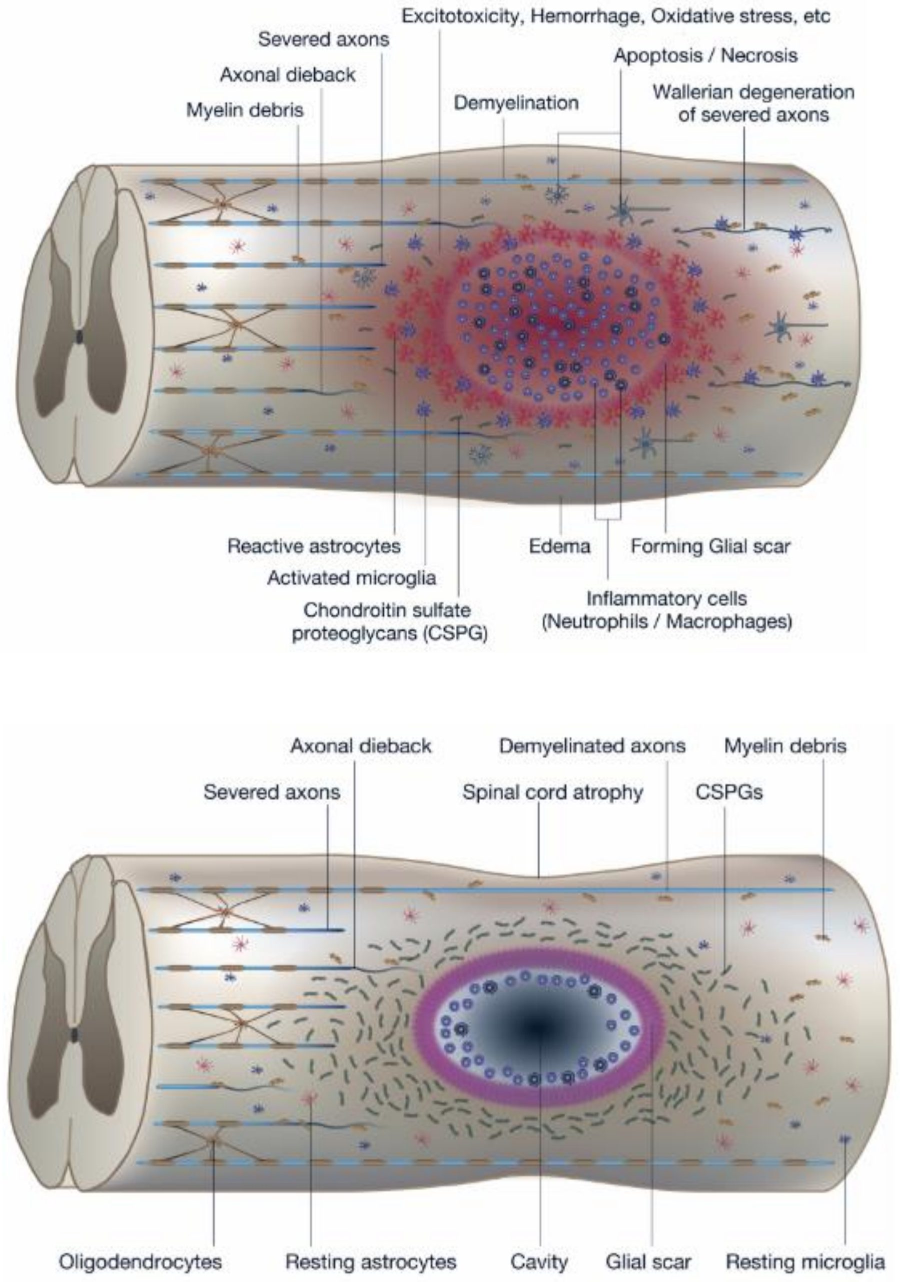
1. **Biological regeneration**: take advantage of the regenerative capability of central nervous system neurons in the proper environment
   - cell-based therapies
   - Scaffolds

2. **Neuroprosthetics**: use of devices that interface with the nervous system with aim to restore lost functions
   - Stimulation therapies
   - Brain-Computer Interfaces

Training rehabilitation is always essential

Biological regeneration

1. Neural tissue death (neurons and maintenance cell), demyelination and axonal degeneration
2. Intensive local inflammatory response leads to:
   - activation of resident microglia: scar-forming astrocytes
   - infiltration of macrophages
3. Formation of **glial and fibrotic scars**
   - Main impediment to CNS axonal regeneration
   - Prevent the spread of injury
4. Tissue degeneration: formation of **cystic cavities** full of cerebral spinal fluid



H. Katoh et al. Front. Cell. Neurosci. 2019, 13, 248

Biological regeneration

1. Cell-based therapies

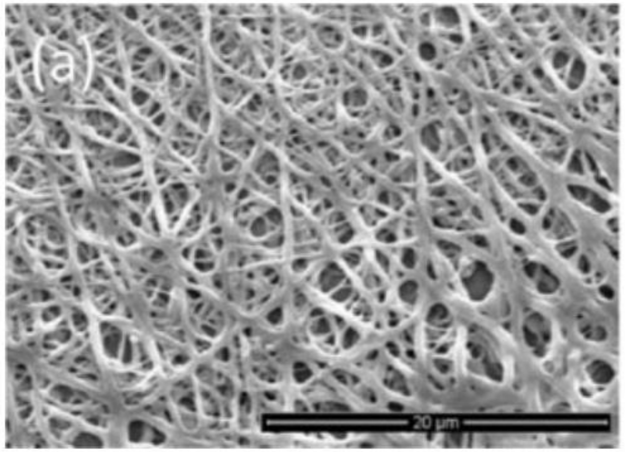
Transplantation of neural stem/progenitor cells (NSPCs) into the lesion site to make it a hospitable environment.

- replace damaged cells
- provide local neurotrophic factors
- promote axonal sprouting, regeneration, and remyelination
- promote neural plasticity

2. Biomaterial scaffolds

Implantation of a ‘scaffold’ into the cavity that provides axons with a substrate on which to grow and to restore tissue continuity.

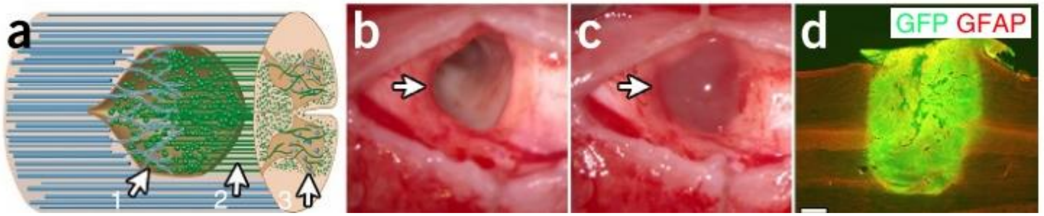
- Support for axonal sprouting and growth
- Deliver growth/vascularization factors and drugs

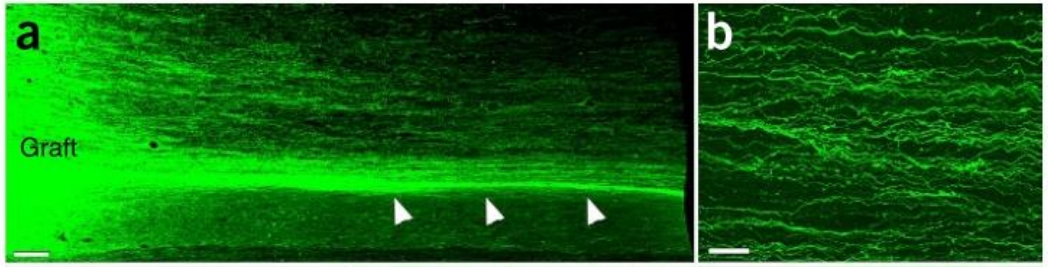


Fibrin matrix

rhesus monkeys
right-side C7 hemisection

NPCs plus
fibrin gel





host white matter caudal
to the lesion site

6 mm

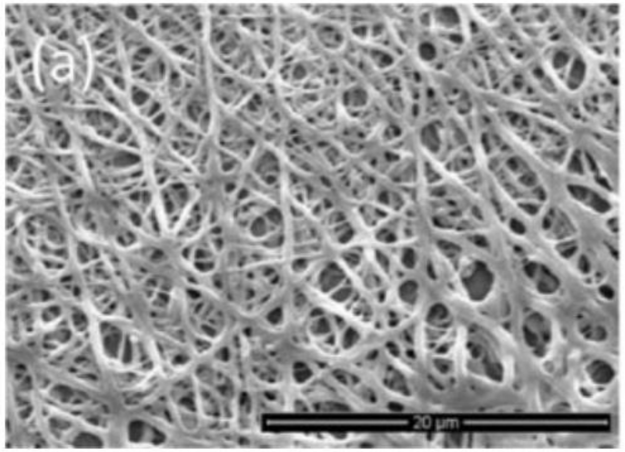
Rosenzweig et al., Nature Medicine 2018, 24, 484–490

Biological regeneration

1. Cell-based therapies

2. Biomaterial scaffolds

Not an easy task: poor translation of successful strategies in animal models of SCI to human patients
Need to determine the **regeneration of specific pathways**
(chemical neural tracers)



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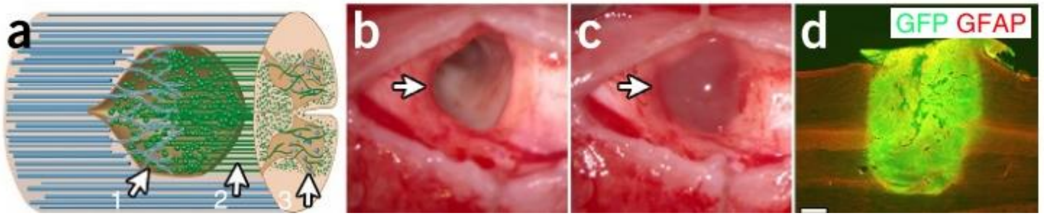

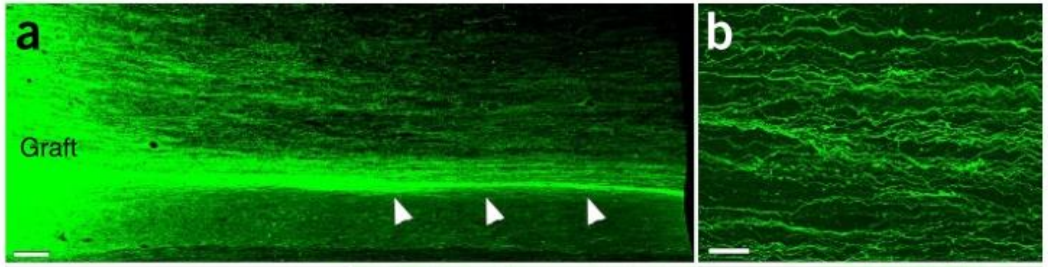

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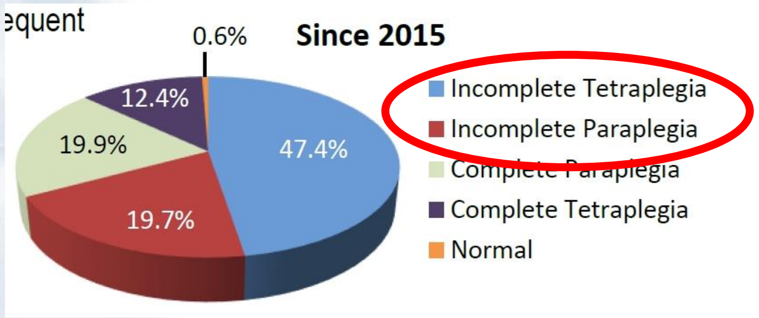
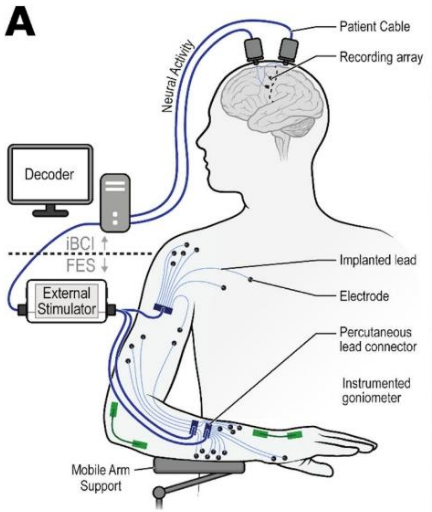
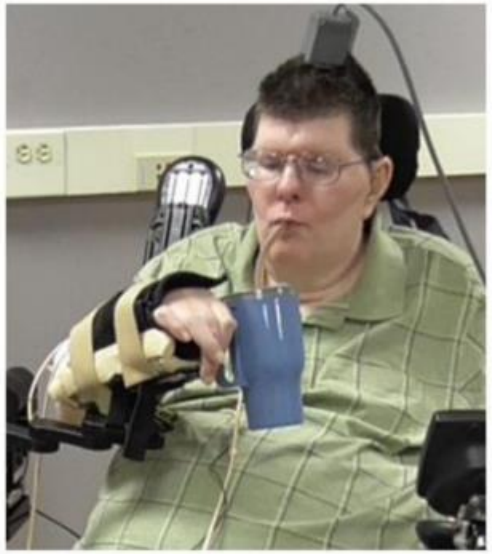
Neuroprostheses

Neuroprostheses are devices that use electrodes to stimulate and sense the nervous system including muscles, nerves, spinal cord, or the brain.

More invasive than biological regeneration but presently more advanced in human application.







Neuroprostheses

Stimulation therapies

- FES: Functional Electrical Stimulation. Electrical **nerve** stimulation
- TENS: Transcutaneous Electrical Nerve Stimulation, with electrodes are positioned on the surface of the skin
    - Sensorimotor improvement using FES for generating movement in paralyzed limbs (better than passive action alone)

Parastep I™ System

The Parastep I™ is a microcomputer controlled functional neuromuscular stimulation (FNS) system that enables independent, unbraced ambulation (i.e., standing and walking) by people with a spinal cord injury

https://www.sigmedics.com/

M. van Bloemendaal et al., Trials 2016, 17, 477

D. R. Gater et al., NeuroRehabilitation 2011, 28, 231–248

J. L. Collinger et al., J. Spinal Cord Med. 2013, 36, 258–272

Neuroprostheses

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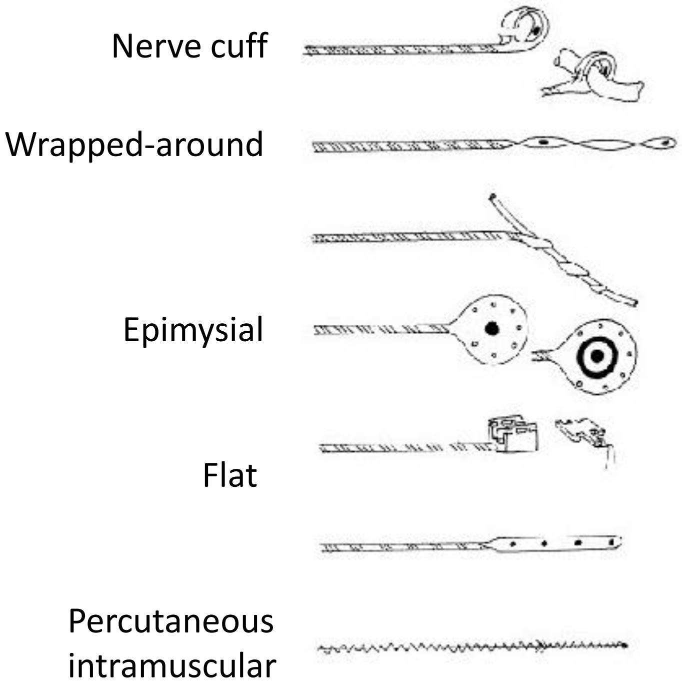
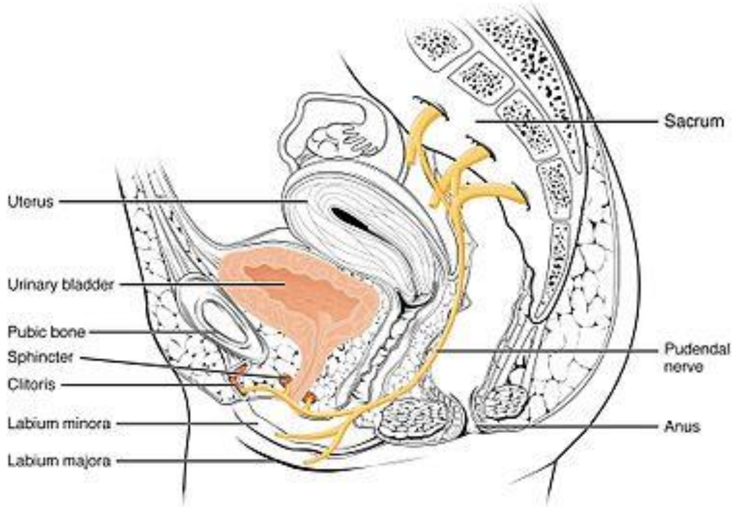
- TENS: Transcutaneous Electrical Nerve Stimulation, with electrodes are positioned on the surface of the skin
    - Sensorimotor improvement using FES for generating movement in paralyzed limbs (better than passive action alone)
    - Bladder control (FES at the pudental nerve)
    - Cardiac and diaphragmatic pacing
    - Pain management
    - Truncal stability





D. R. Gater et al., NeuroRehabilitation 2011, 28, 231–248

Neuroprostheses

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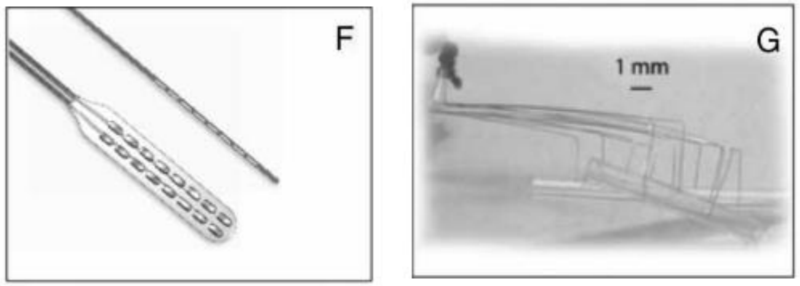
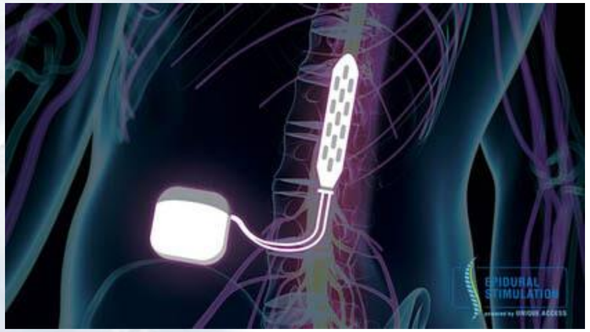


- EES: epidural electrical stimulation. Electrodes placed on the dura mater of the spinal cord
- ISMS: intra-spinal micro-stimulation.

Central pattern generators (CPGs) produce rhythmic activity of the limbs without requiring any sensory feedback

- **Chronic paralysis patients regain voluntary control of paralysed muscles**
- Use of spared circuit: connections silent due to damage of myelin or ionic channels

C. A. Angeli et al., Brain 2014, 137, 1394–1409
University of Louisville / University of California

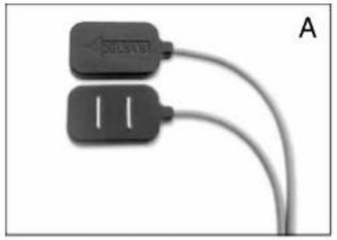


J. L. Collinger et al., J. Spinal Cord Med. 2013, 36, 258–272

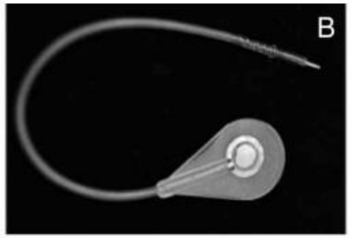
Close-loop Neuroprostheses

Using information of muscles motion or organ state as input for FES/EES

EMG: Electromyography: electrical activity in **muscles**

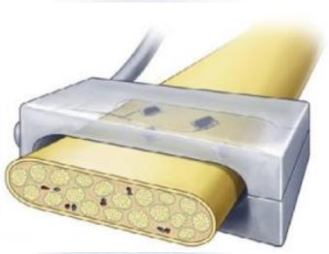


skin electrode

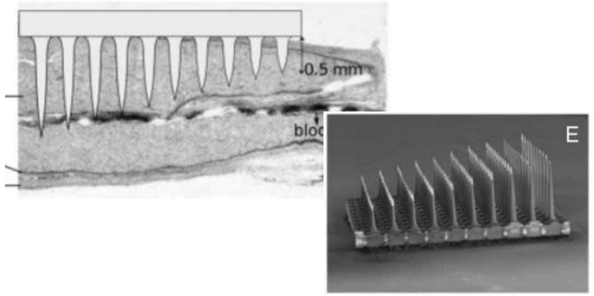


epimysial (attached directly to the muscle)

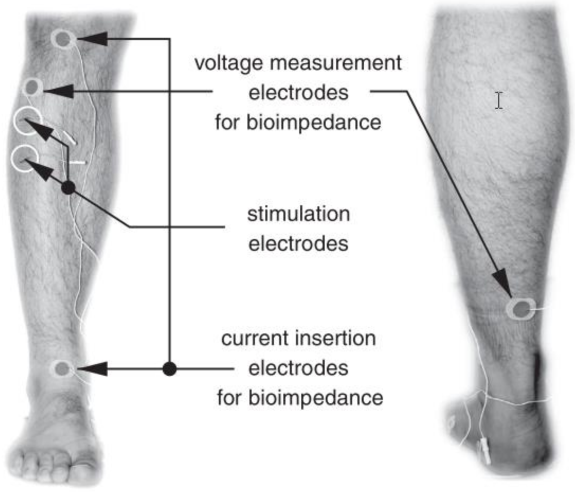
ENG: Electroneurography: electrical activity in **nerves**



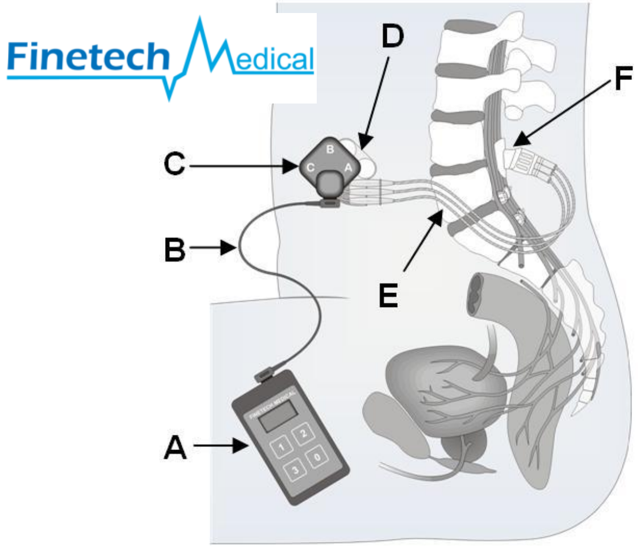
flat interface nerve electrode (FINE)



Utah Slanted Electrode Array (USEA)



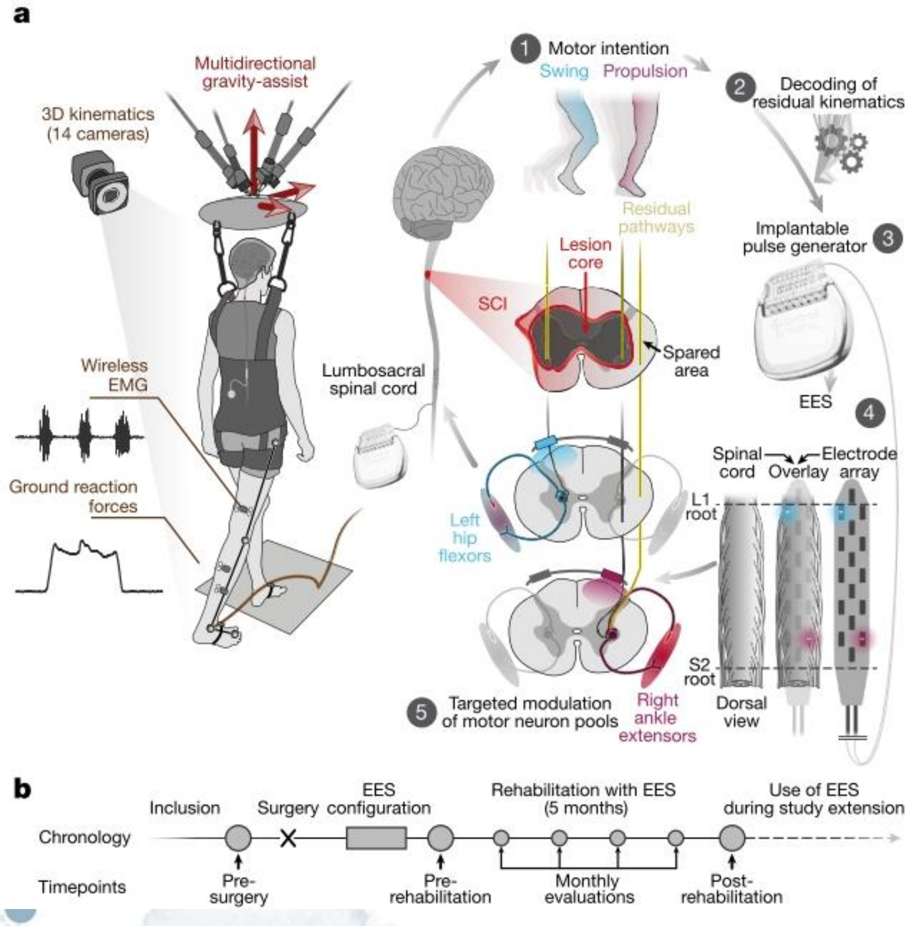
T. Schauer, Annual Reviews in Control 2017, 44, 355–374



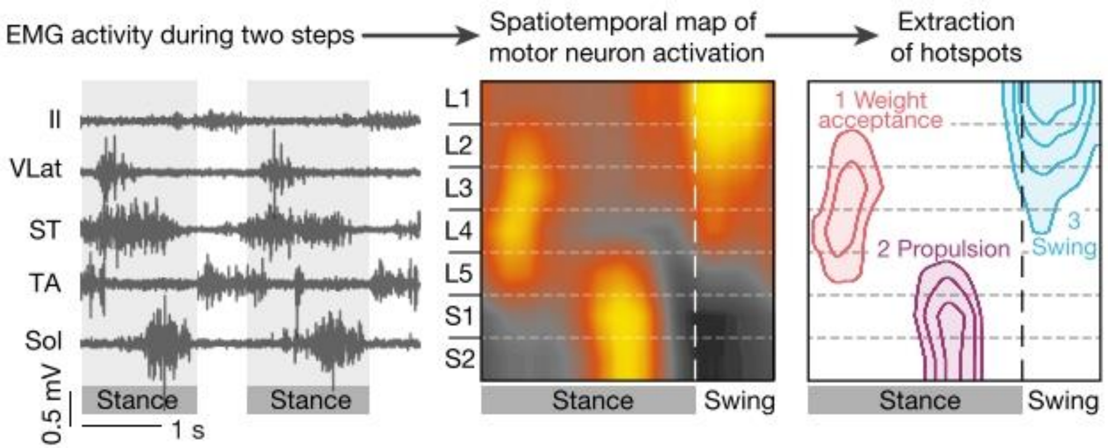
E. H. Rijnbeek et al., Neuroprosthetics. Front. Neurosci. 2018, 12, 350

Close-loop Neuroprostheses

Restoration of walking with spatiotemporal selective EES



EES activates motor neurons by recruiting **proprioceptive circuits** within the posterior roots of the spinal cord



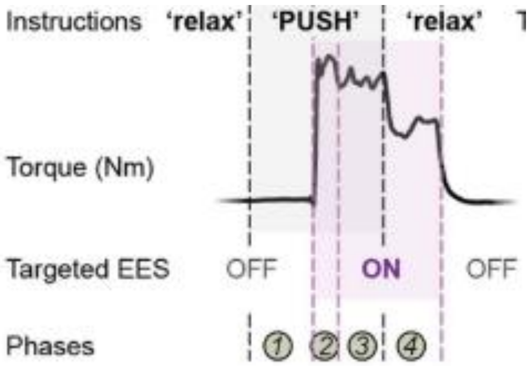
fine-tuned the timing of each spatially selective **stimulation train** using a closed-loop controller that triggered EES on the basis of foot trajectory

Targets: posterior roots associated with muscles that mobilize hip, knee and ankle joins

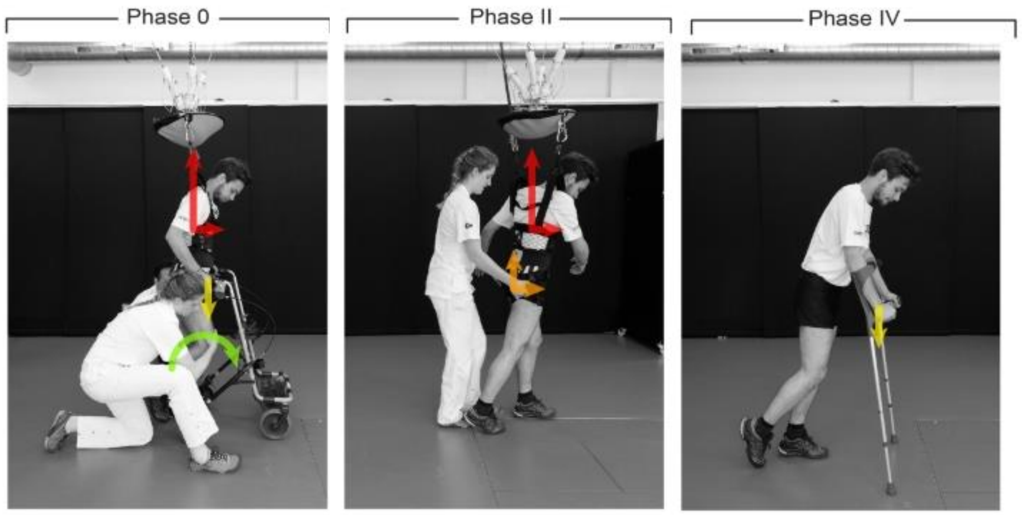
F. Wagner et al., Nature 2018, 563, 65–71
E. Formento et al., Nature Neuroscience 2018, 21, 1728–1741
EPFL (Lausanne), Grégoire Courtine team

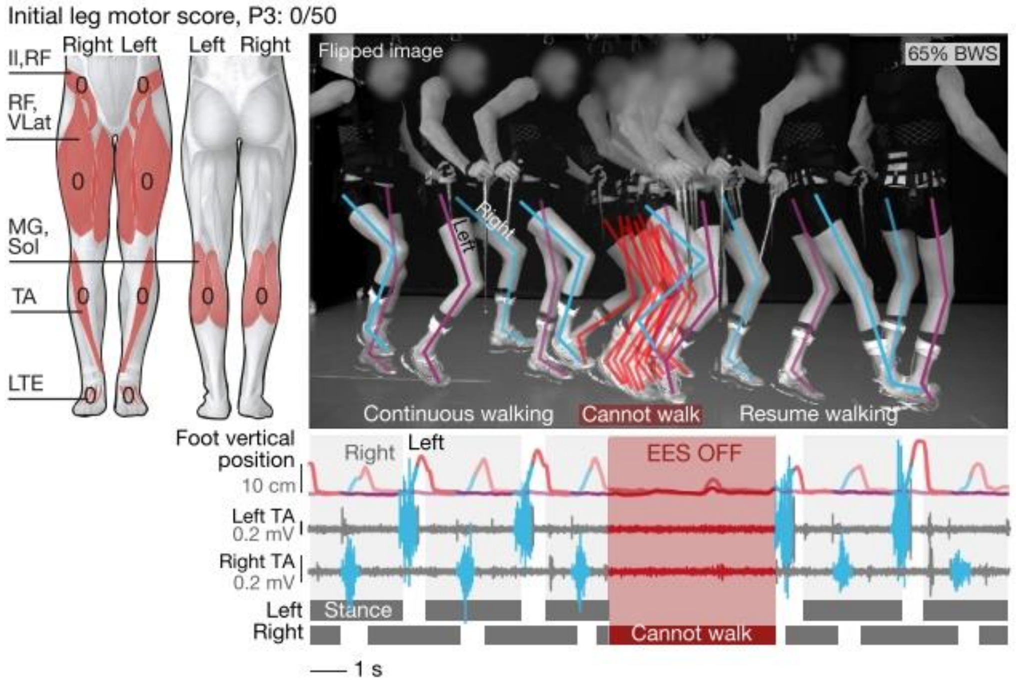
Close-loop Neuroprostheses

Restoration of walking with spatiotemporal selective EES

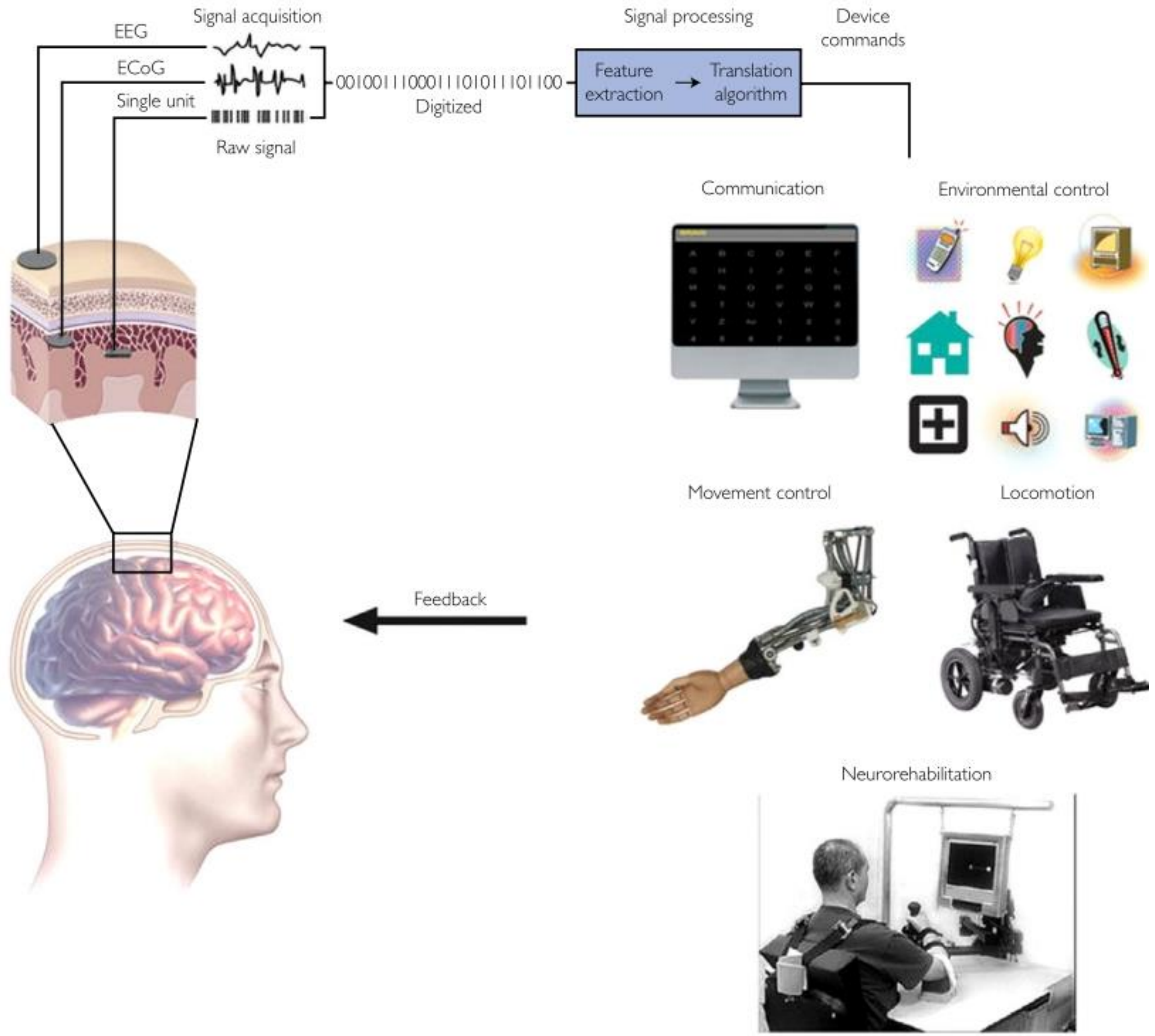


1. **Voluntary** motion: Without any voluntary contribution, EES induced minimal muscle contraction
2. Restoration of walking **without stimulus** after 5 months (initial spare connections must exist)





Brain-Computer Interfaces (BCI)



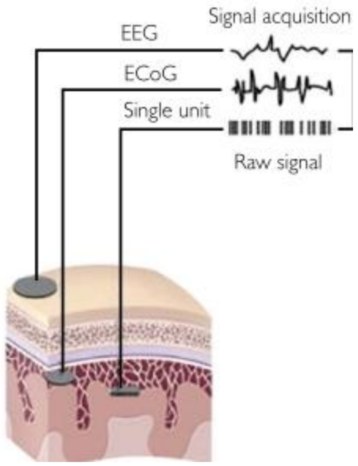
1. Acquire brain signals
2. analyze them and translate them into commands
3. output to devices or FES/EES

The act of **thinking** about performing a movement is transformed into a command and a **real action**

Artificial intelligence algorithms are essential

Shih J.J. et al. Mayo Clin. Proc. 2012, 87, 268–279
C. Pizzolato et al., Exper. Neurology 2021, 339, 113612

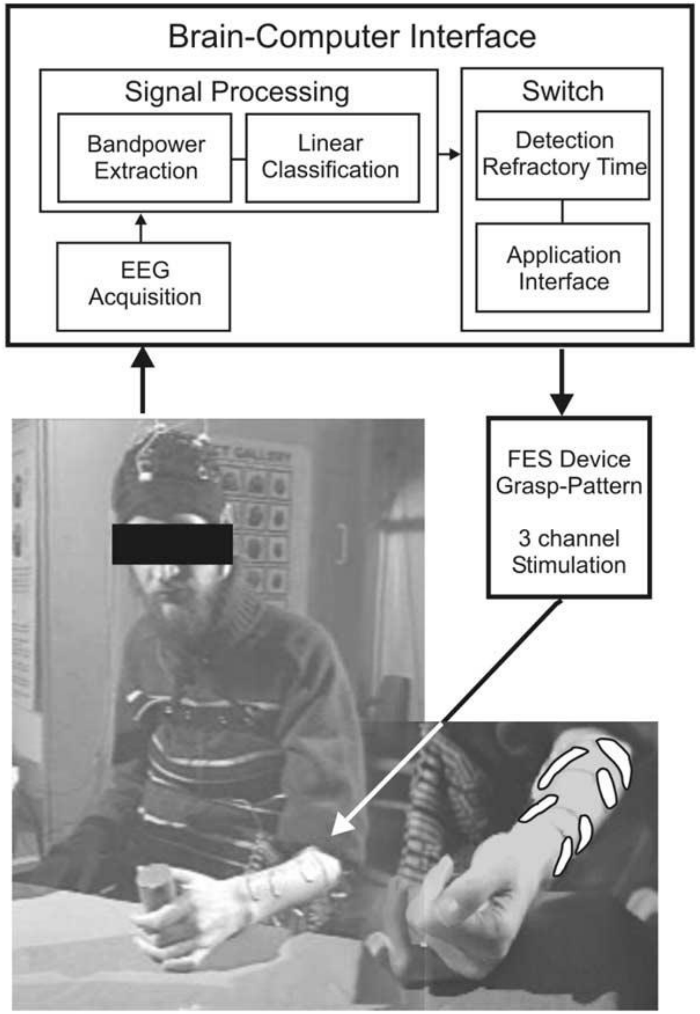
Brain-Computer Interfaces (BCI)



- **EEG: electroencephalography**
- ECoG: electrocorticography
- Intracortical Microelectrode

intendiX
USER-READY BCI APPLICATIONS

http://www.intelimed.es



G. Pfurtscheller et al., Neurosci Lett 2003, 351,33–36

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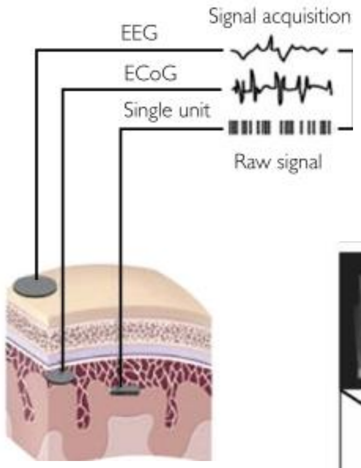
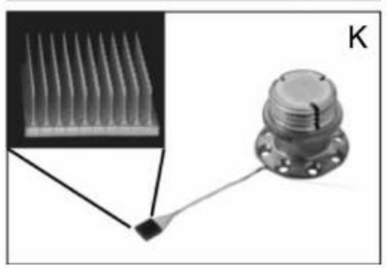


Intracortical Microelectrode

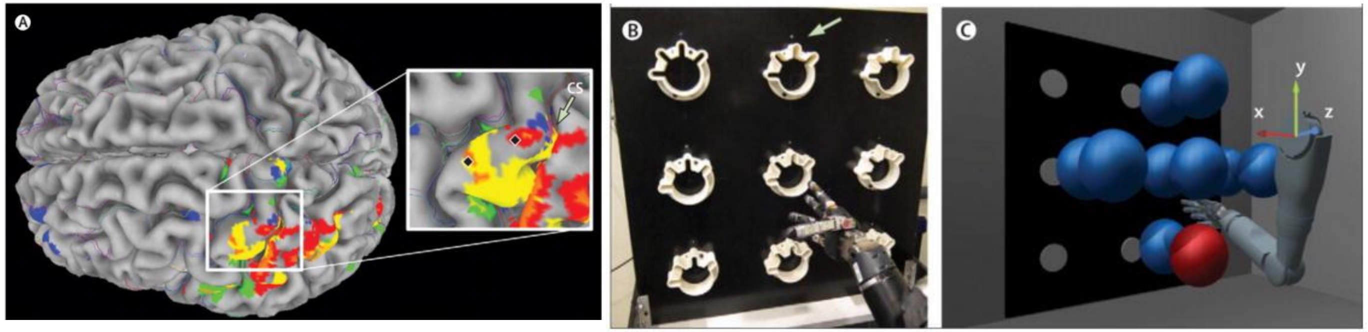


NeuroPort intracortical microelectrode array (Blackrock Microsystems)

approved by the FDA

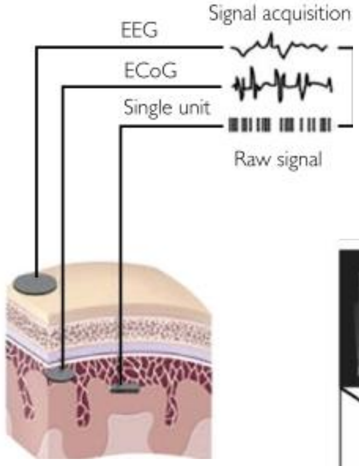
96 microelectrodes in the motor cortex of a human
control of the arm and hand over a broad space without explicit training

L. R. Hochberg et al., Nature 2006, 442, 164–171
L. R. Hochberg et al., Nature 2012, 485, 372–375
Rhode Island



J. Collinger et al., Lancet. 2013, 381, 557–564
University of Pittsburgh

Brain-Computer Interfaces (BCI)



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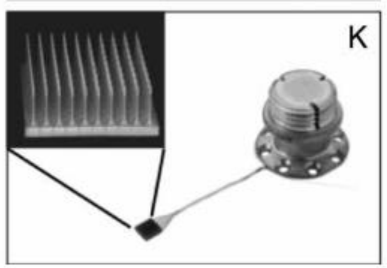

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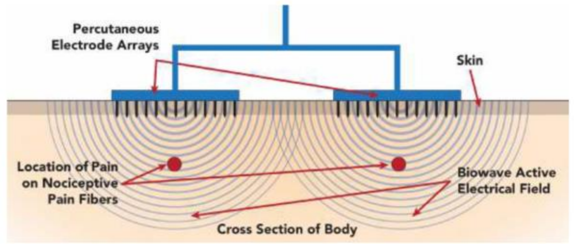
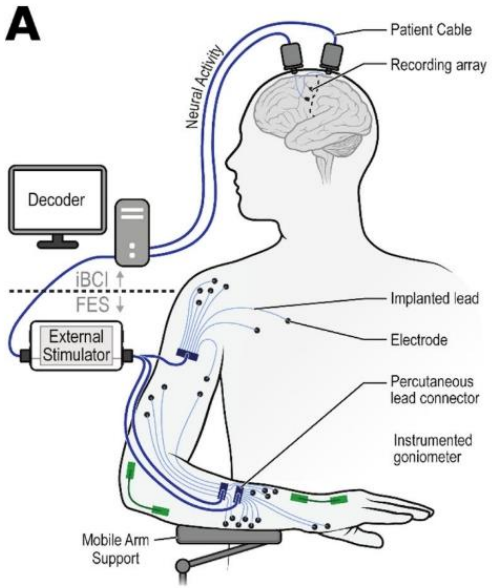
C.E. Bouton et al., Nature 2016, 533, 247-250
Ohio State University



percutaneous muscle stimulating electrodes (Synapse Biomedical, Oberlin, OH)



A. Ajiboye et al., Lancet. 2017, 389, 1821–1830
Cleveland / Rhode Island / Boston

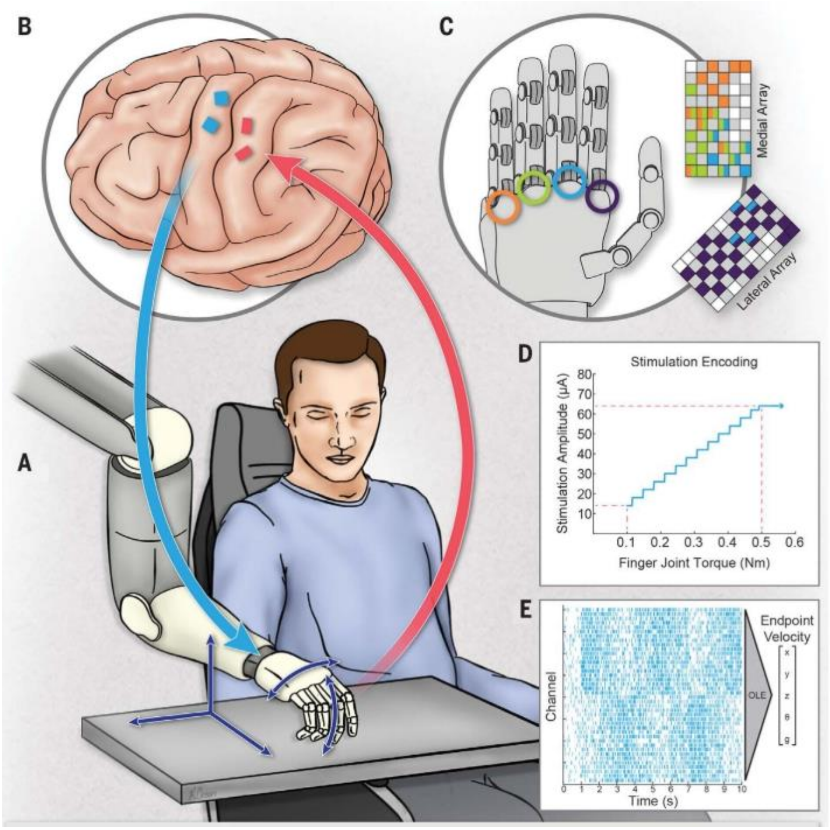
Brain-Computer Interfaces (BCI)

Bidirectional brain-computer interface with two intracortical microelectrode arrays:

- Records neural activity from the motor cortex
- Generates tactile sensations by stimulation of the somatosensory cortex

Stimulation driven in real time by sensors in a robotic hand

- Patients improve performance on functional tasks
- Patients ‘felt’ a sensation as originating from their own hand

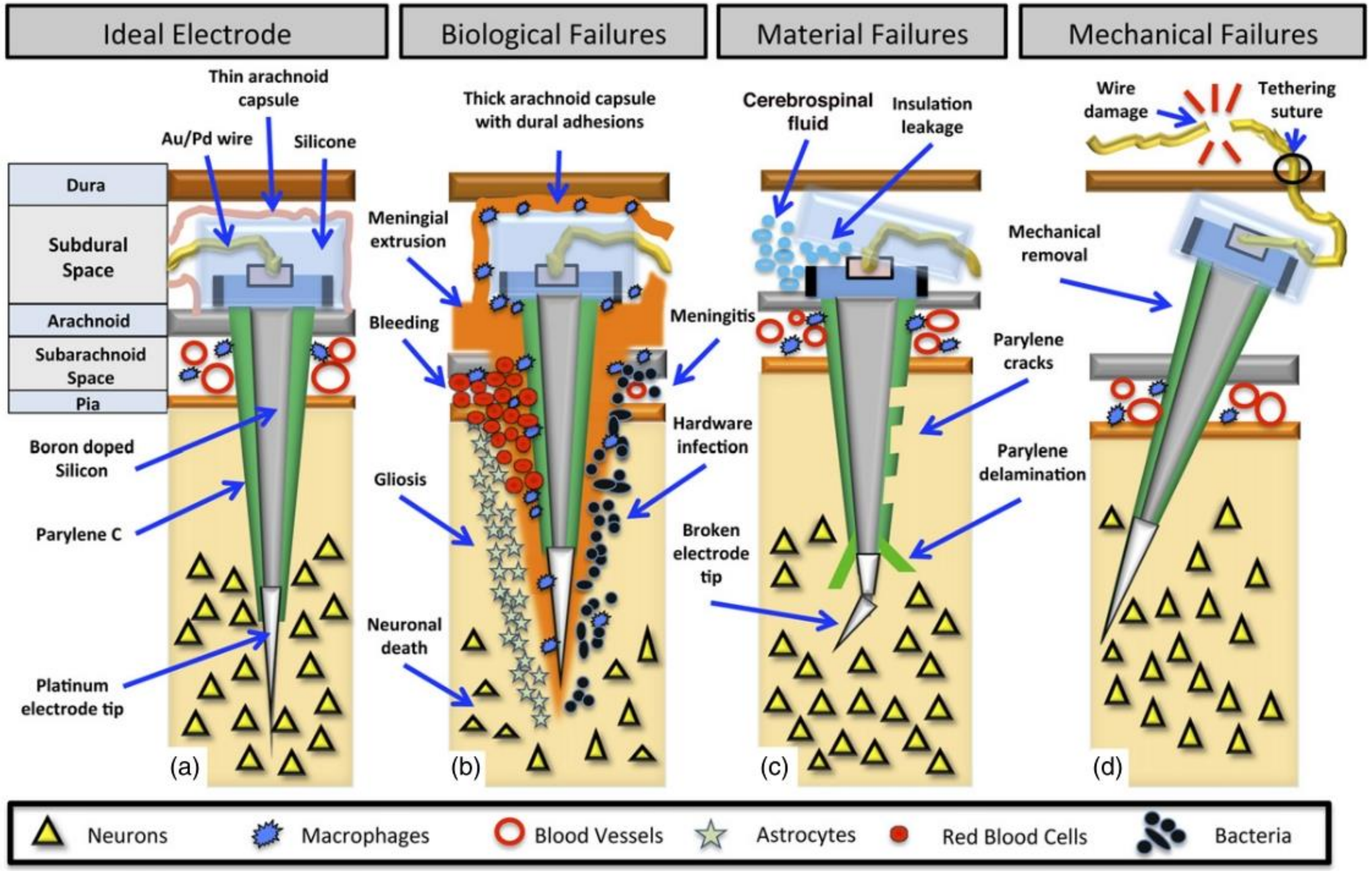


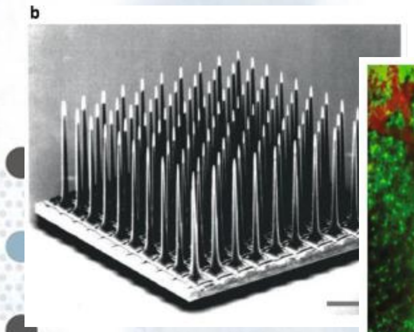
S. N. Flesher et al., Science 2021, 372, 831-836
University of Pittsburgh

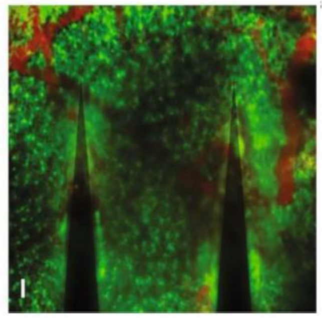
Brain-Computer Interfaces (BCI)

Limitation:
BCI requires electrodes permanently implanted

Application of intracortical microelectrodes is currently limited because they are **not able to reliably record** neuronal signals chronically



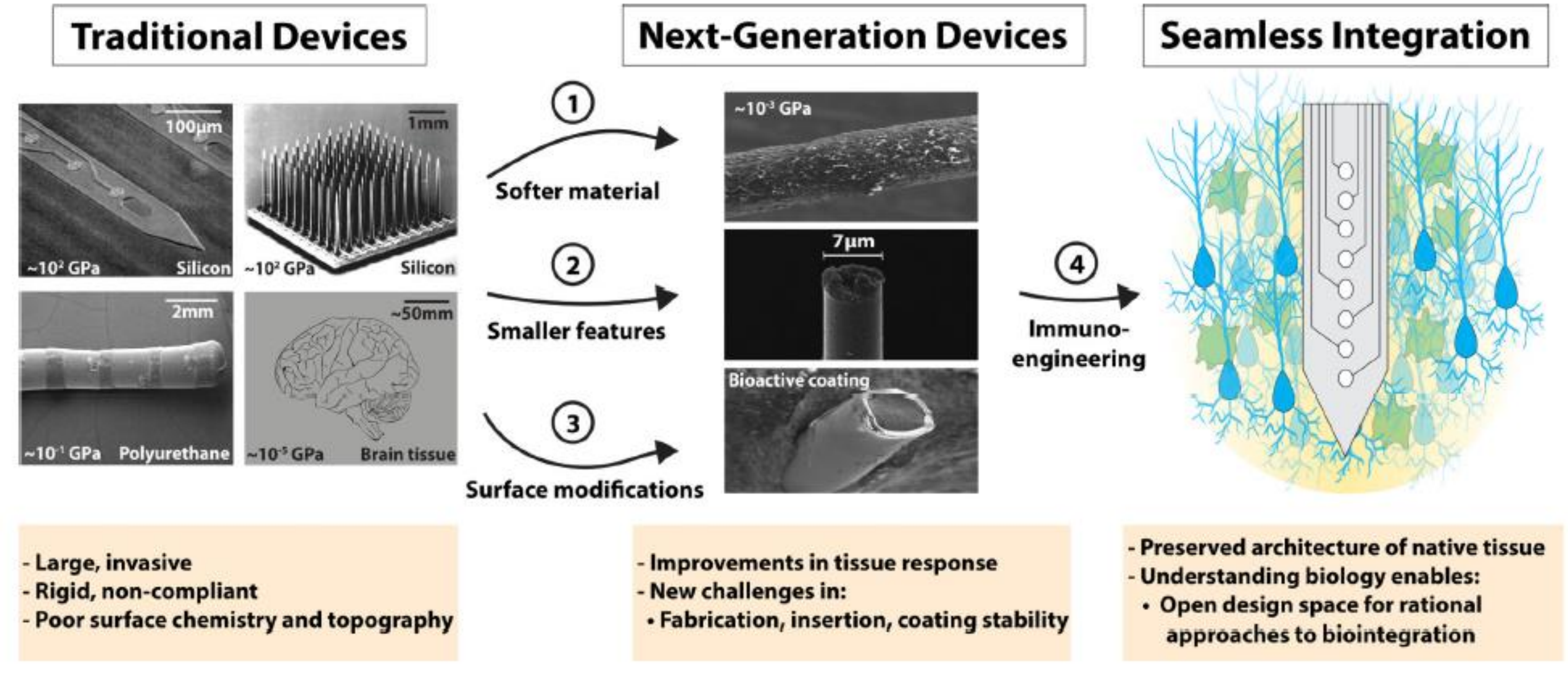




Microglia (green) form a compact scar

J. Barrese et al., J. Neural Eng. 2013, 10, 066014

Glial response to implanted electrodes



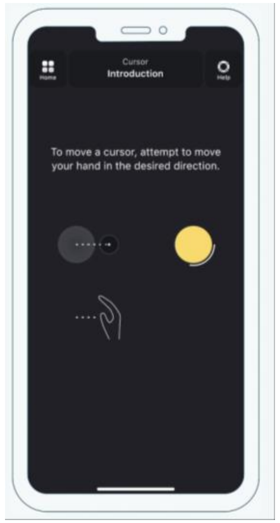
J. W. Salatino et al., Nat Biomed Eng. 2017, 1, 862–877

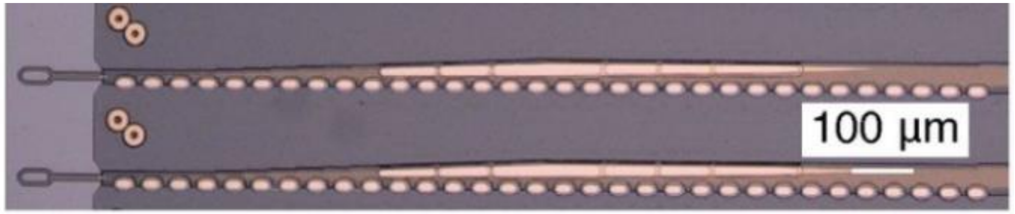
Ultra-fine polymer probes

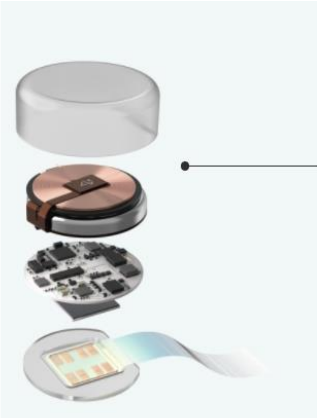
A total of 3072 electrodes in 96 threads

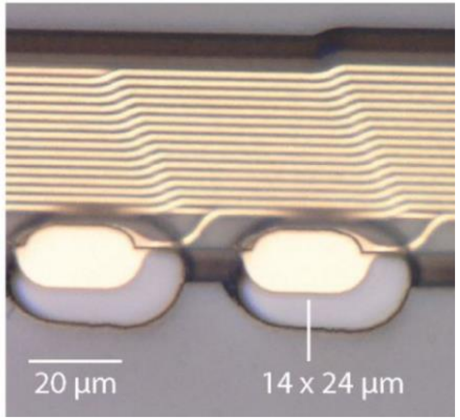
Together with a neurosurgical robot for implantation

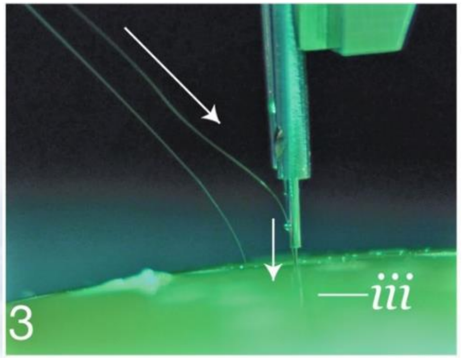
No long-term implantation performance provided

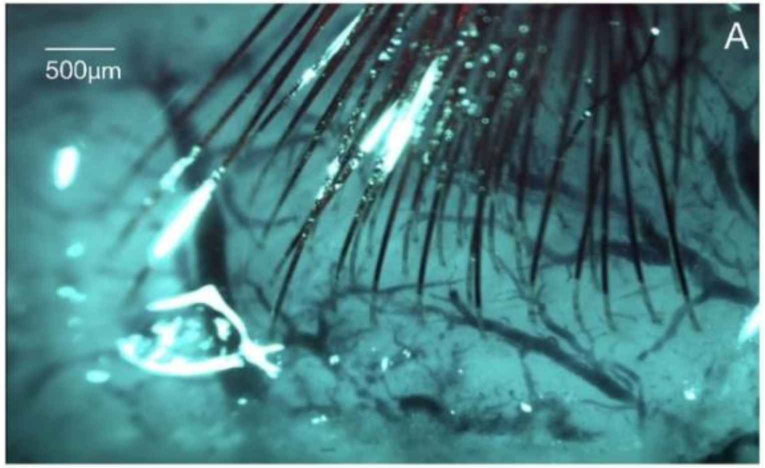












E. Musk, Neuralink, J Med Internet Res 2019, 21, e16194
https://neuralink.com/approach/
https://www.youtube.com/watch?v=DVvmgjBL74w

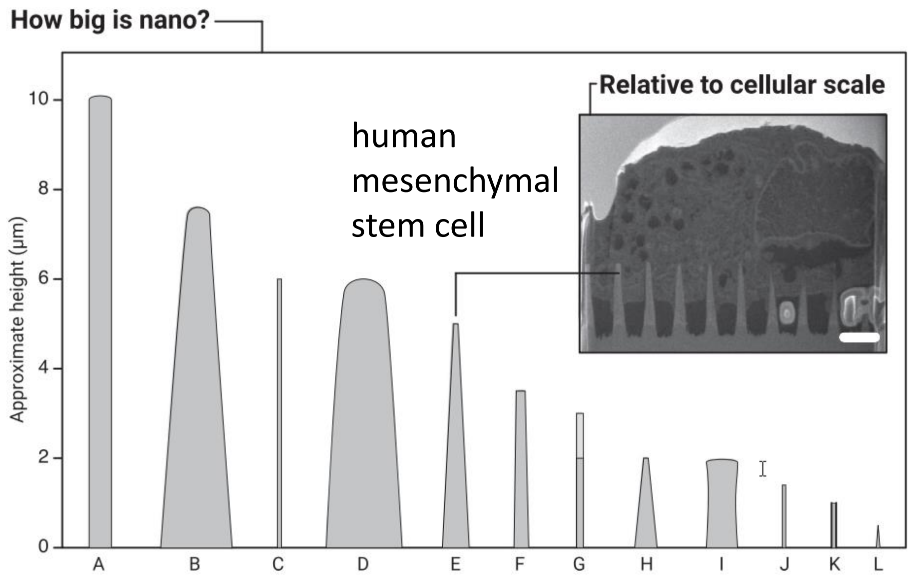
Presentation Outline

- Spinal Cord Injury (SCI) – some history
- How to cure Spinal Cord Injury?
  - Neural regeneration
  - Neuroprostheses
  - Brain-computer interfaces
- Nanotechnology for Spinal Cord Injury repair
  - Nanomaterials at IMDEA nanociencia

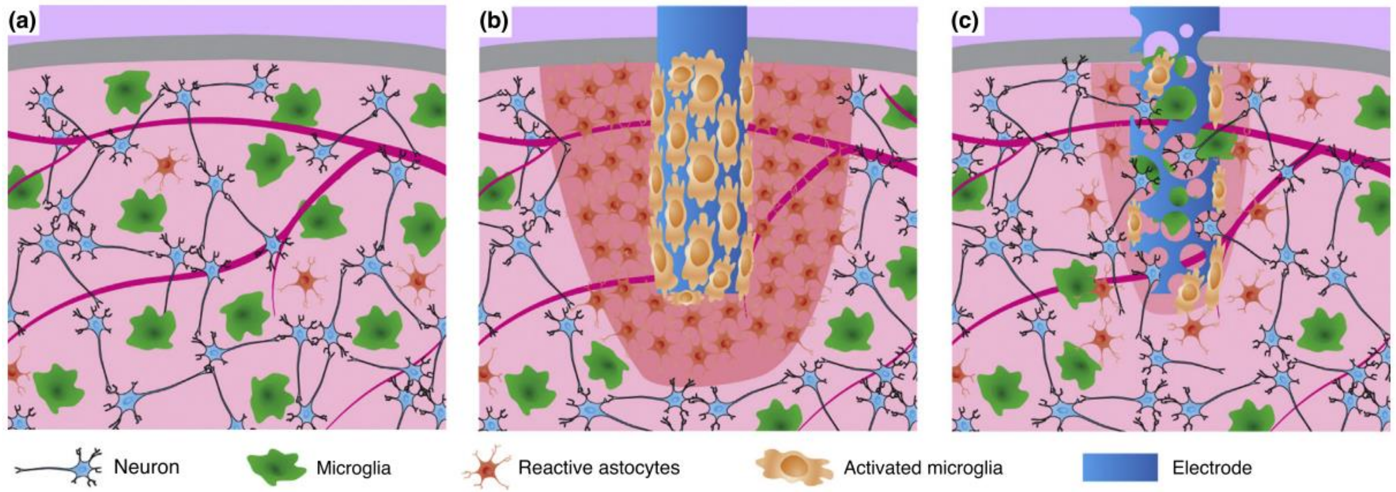
Nanotechnology for SCI repair

Nanometric motifs are of similar length scale to that of the biological entities

- Intimate interaction with the cellular features
- Mimic more precisely the structure of human tissues (better mechanical matching)
- Reduce inflammatory glial cells / foreign-body response
- Improved charge transfer / reduced impedance - favor stimulation or sensing of cellular activities
- Promote preferential stem cell differentiation into specific lineage



S. G. Higgins et al., Adv. Mater. 2020, 32, 1903862

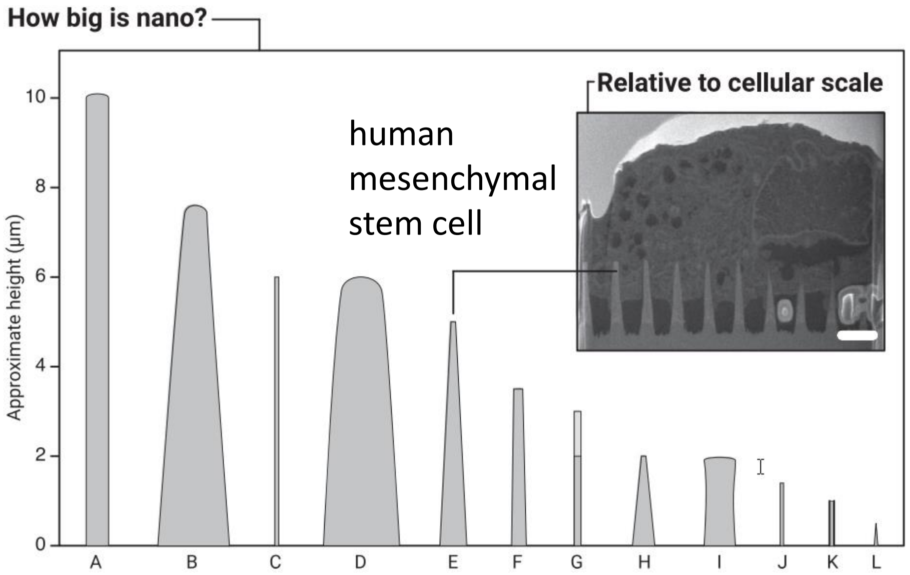


D. Scaini and L. Ballerini, Current Opinion in Neurobiology 2018, 50,50–55

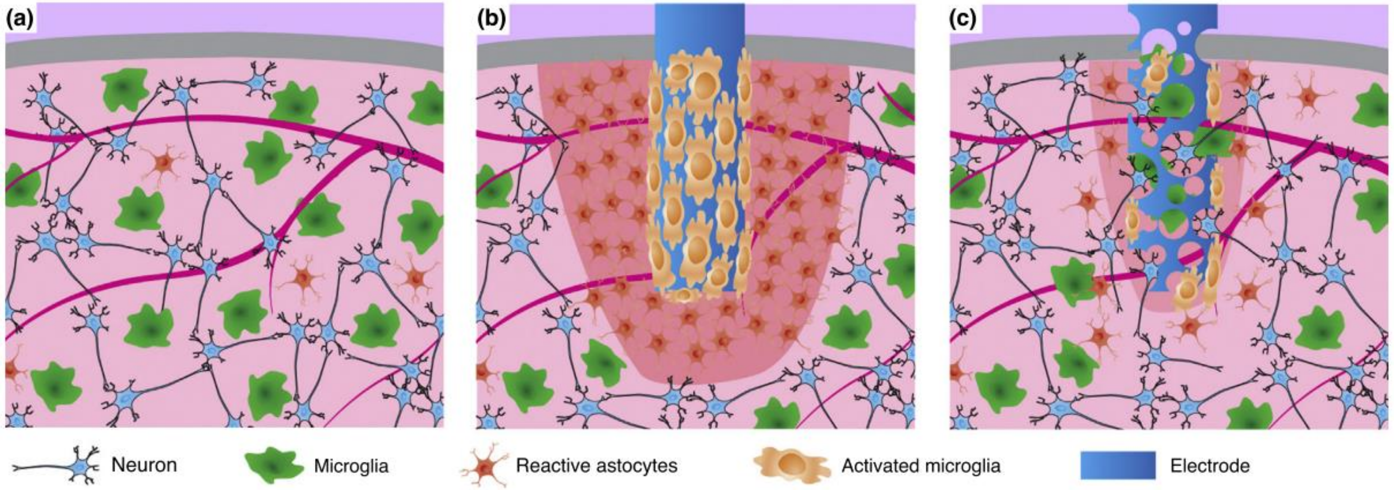
Nanotechnology for SCI repair

Nanometric motifs are of similar length scale to that of the biological entities

1. Neuroprostheses
2. Scaffolds

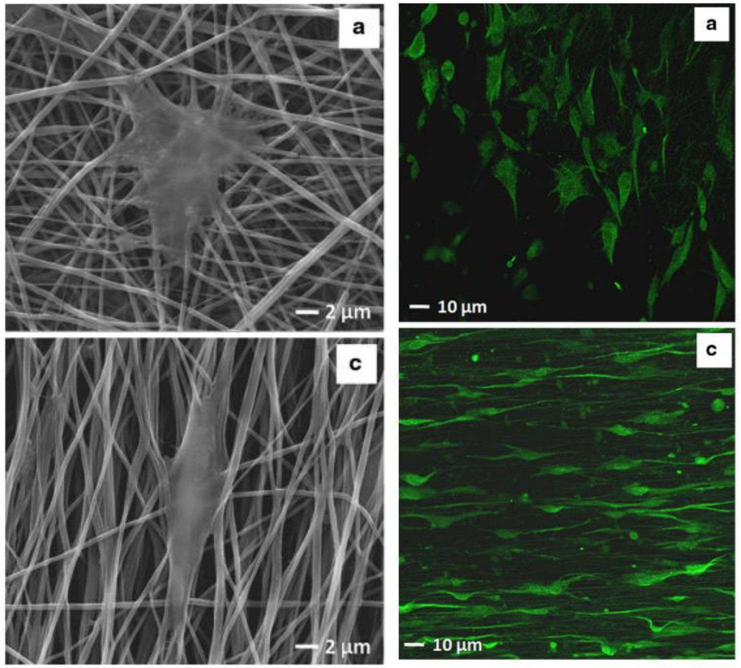


S. G. Higgins et al., Adv. Mater. 2020, 32, 1903862



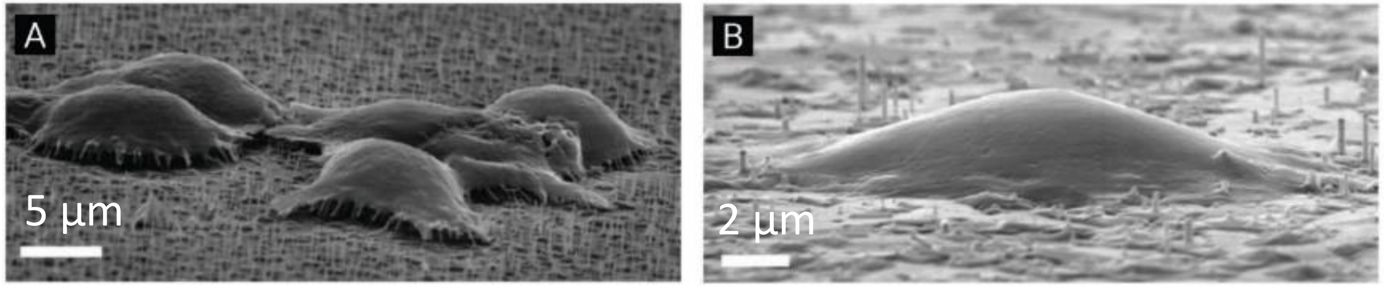
D. Scaini and L. Ballerini, Current Opinion in Neurobiology 2018, 50,50–55

Nanomaterials – cell interaction



hMSC after 7 days

V. Cirillo et al., J Mater Sci: Mater Med 2014, 25, 2323–2332



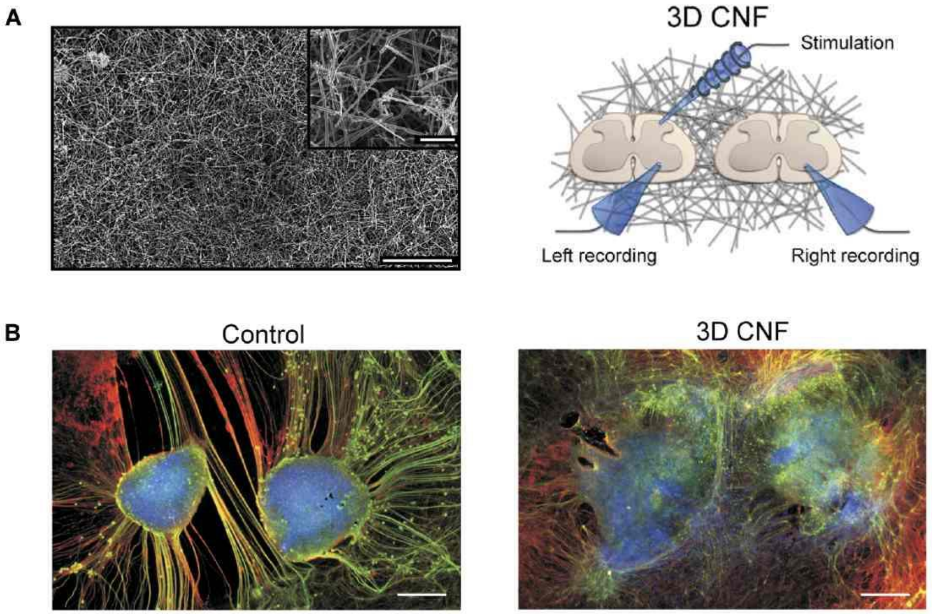
Cell settling depends on:

- dimensions of the individual nanowire
- density of the nanowire network

N. Buch-Månson et al., Adv. Funct. Mater. 2015, 25, 3246–3255

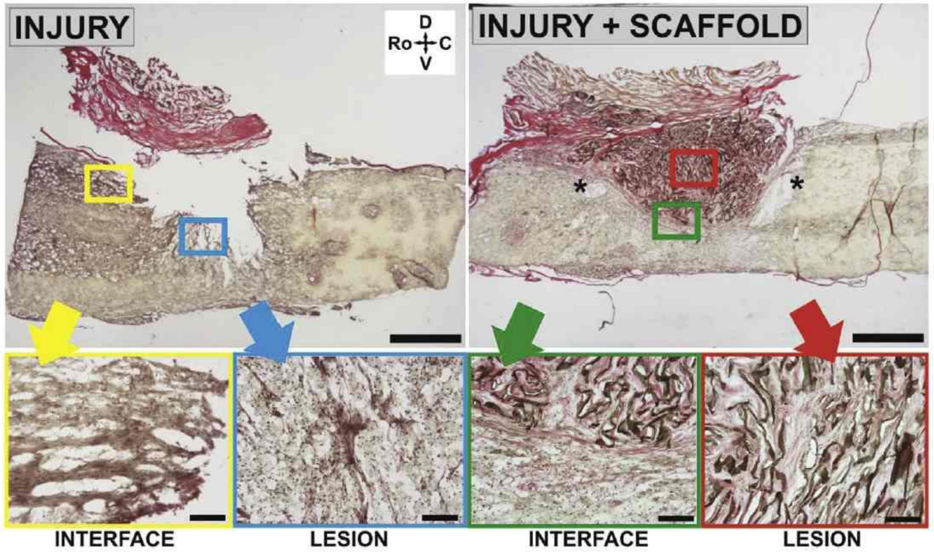
Nanotechnology for regeneration

Carbon-based nanomaterials: Carbon Nanotubes and graphene



A 3D carbon-nanotube mesh promotes the spontaneous regrowth of neurite bundles

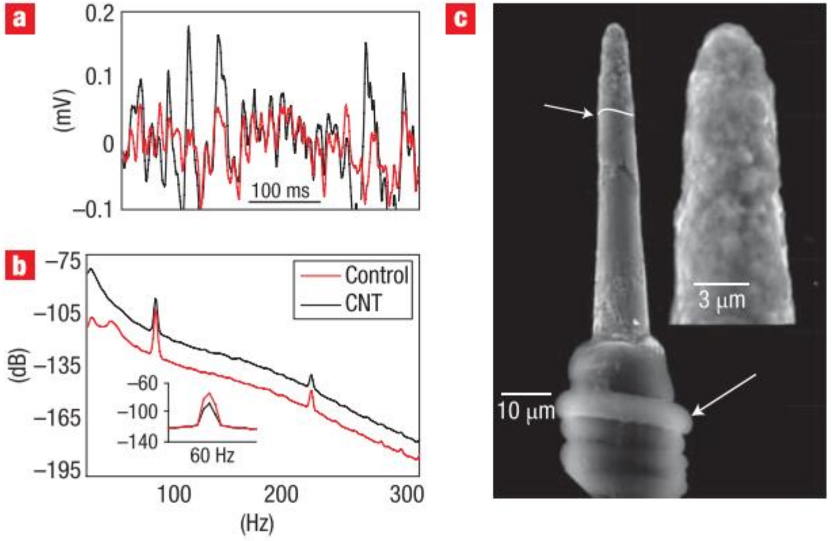
S. Usmani, L. Ballerini et al. Sci. Adv. 2016, 2, e1600087



A reduced graphene oxide scaffold promotes the growth of new blood vessels and neural axons

E. López-Dolado, M. C. Serrano et al. Biomaterials 2016, 99, 72-81

Nanostructured electrodes



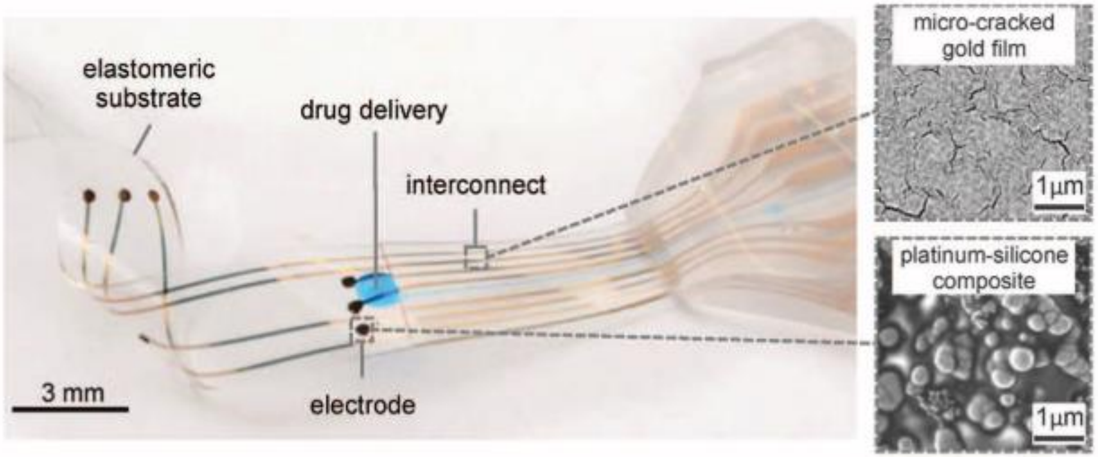
Carbon nanotube coated electrode

Improved neural recordings from the monkey visual cortex

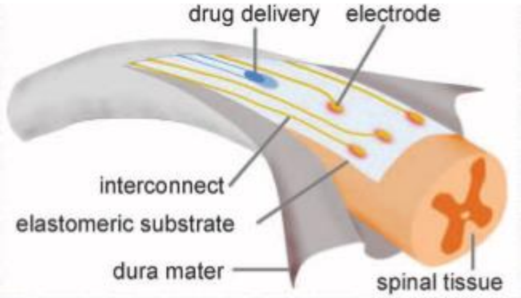
larger amplitude responses

lower 60-Hz line-noise peak

E. W. Keefer et al., Nature Nanotech. 2008, 3, 434–439



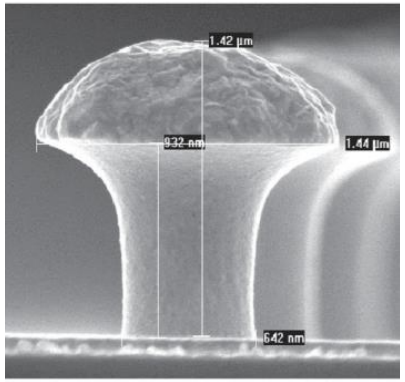
Platinum-silicone composite electrode

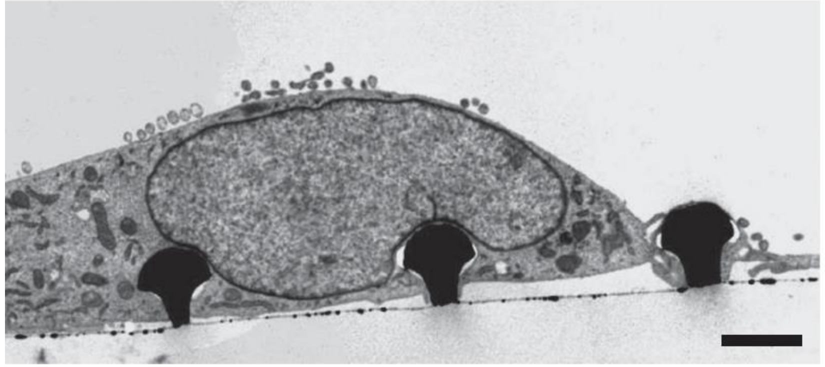


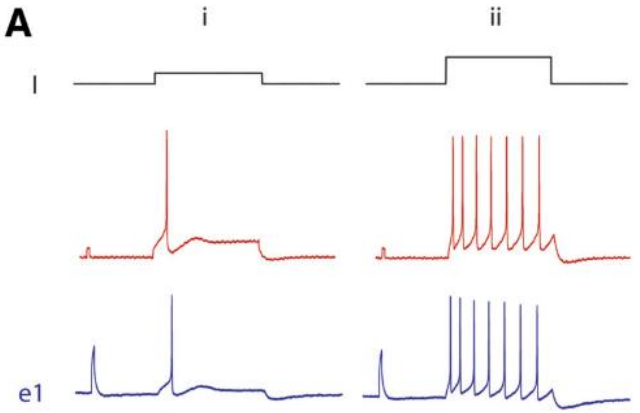
I. R. Minev et al., Science 2015, 347, 6218, 159-163

Nanostructured electrodes

Metallic electrodes for sensing and stimulation at a single cell level





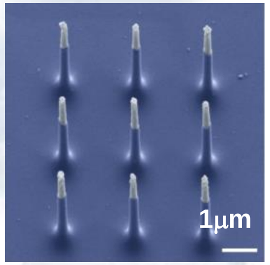


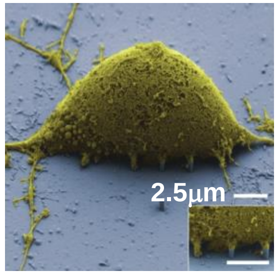
stimulus

pipette

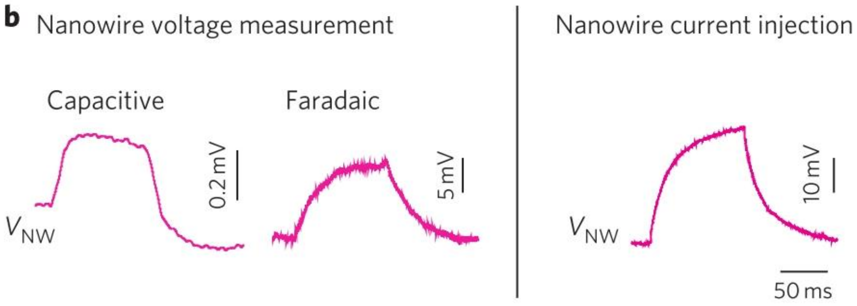
microelect.

A. Hai et al., J Neurophysiol. 2010, 104, 559–568
M. E. Spira and A. Hai, Nature Nanotechnology 2013, 8, 119–124





Many steps - expensive



J. T. Robinson, H. Park et al. Nature Nanotechnology, 7, 180–184

Nanomaterials at IMDEA Nanociencia

FET-OPEN proyect ByAxon

Prof. M. Teresa González

Prof. Laura Ballerini

Dr. Laurence Méchin

Sensing and stimulating neural interfaces based in nanotechnology

Budget: 3.7 M€
Duration: January 2017- December 2020

Dr. Elisa López-Dolado

Dr. M. Concepción Serrano

Dr. Bernd Lecher

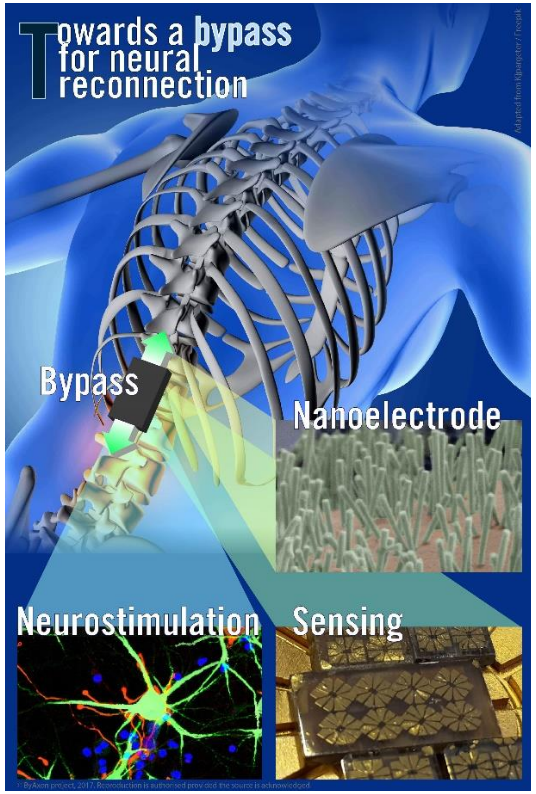
Nanomaterials at IMDEA Nanociencia

FET-OPEN proyect ByAxon

Aim: use NANOTECHNOLOGY to improve NEURAL INTERFACES
- Minimize the risk of **infections** and **foreign-body rejection**
- Enhance **efficiency** and **resolution**

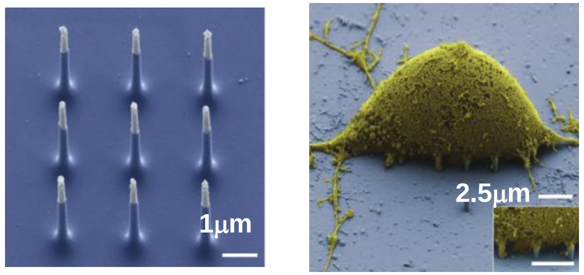
To be able to address directly the SPINAL CORD

We tackle both directions of interfacing
- **Stimulating:** flexible nanostructured ELECTRODES
- **Sensing**: room-temperature ultrasensitive MAGNETIC SENSORS

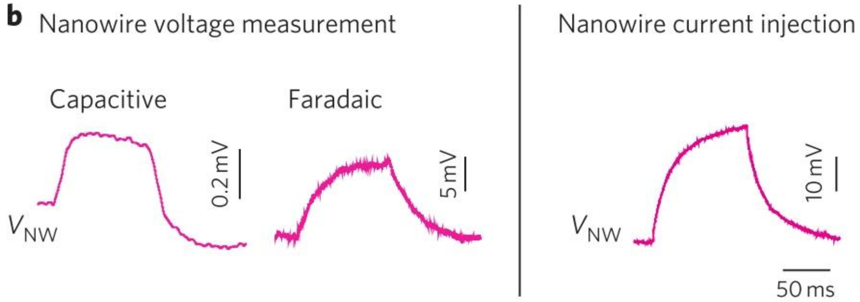


Stimulating electrodes

We take inspiration from previous works:



Many steps - expensive

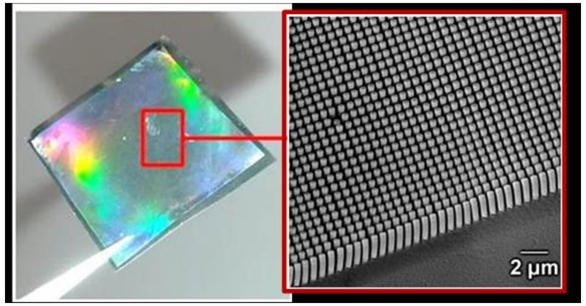


J. T. Robinson, H. Park et al. Nature Nanotechnology, 7, 180–184

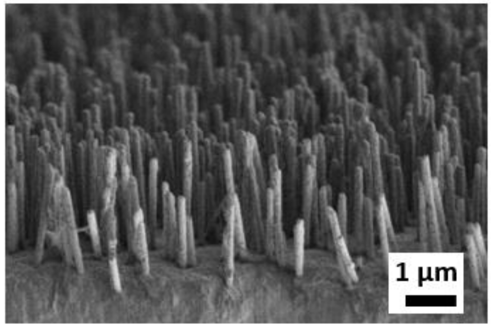
We want electrodes:

- High efficiency
- Low tissue disturbance: flexible electrodes
- Fast mass production

1. Polymeric nanopillars fabricated by nanoimprinting



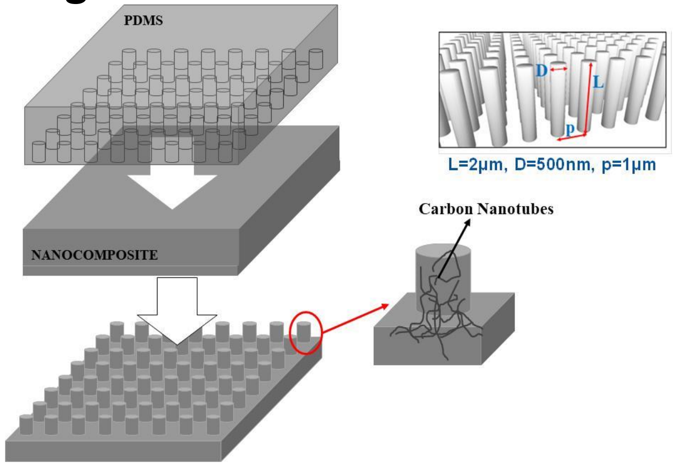
2. Metallic nanowires grown by template-assisted electrochemical deposition

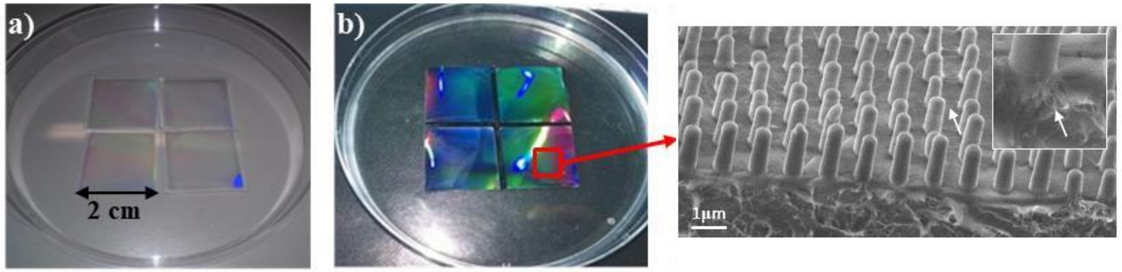


Stimulating electrodes

1. *Polymeric* (PS, PDMS) electrodes with nanopillars

Nanoimprinting



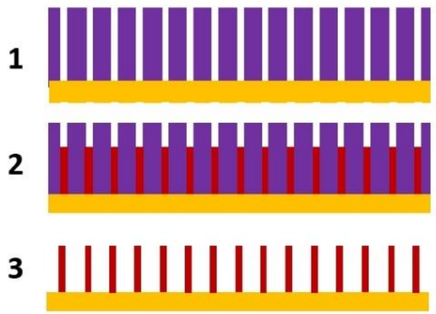


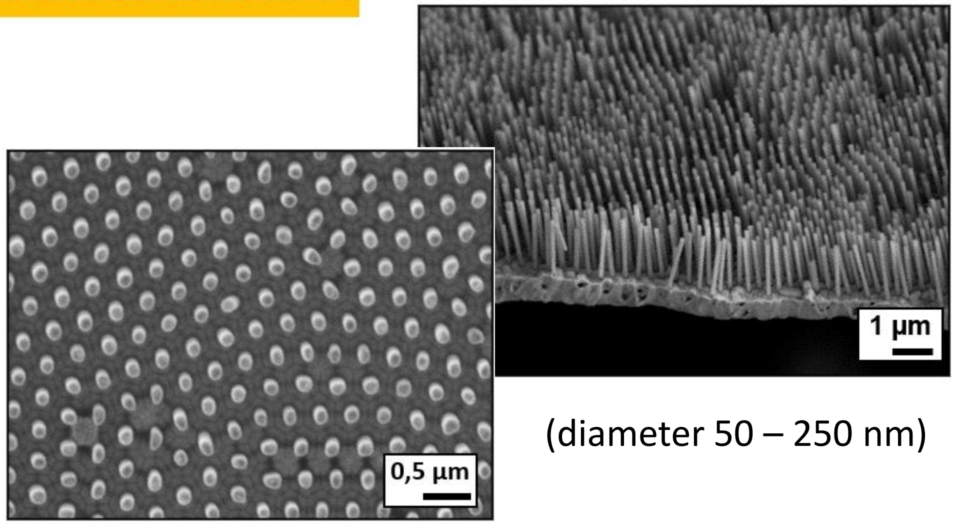
PS **PS-SWCNT** (diameter 500 nm)

Isabel Rodríguez team

2. *Metallic* (Au, Ni, Pt) electrodes nanostructured by nanowires

Template-assisted electrochemical deposition





(diameter 50 – 250 nm)

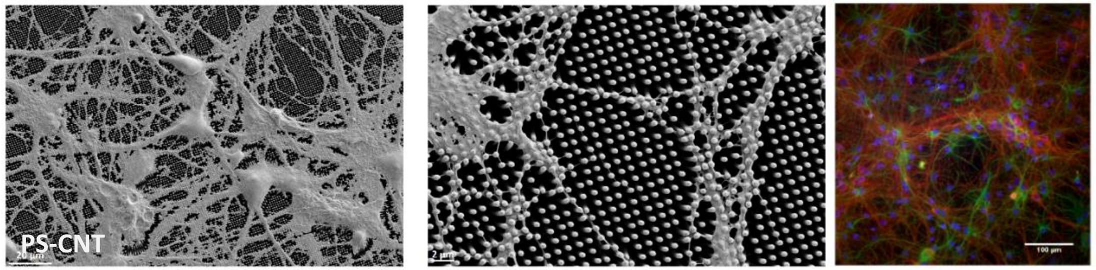
Polymeric nanostructured surfaces

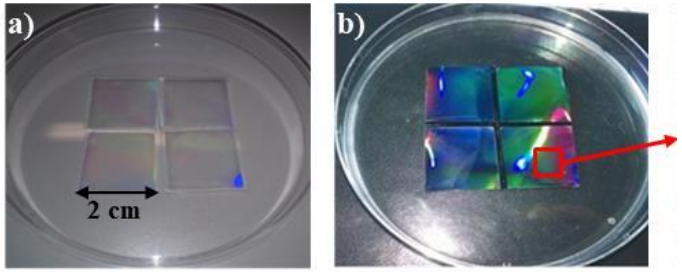
Polymeric nanopillars fabricated by nanoimprinting

with Laura Ballerini team

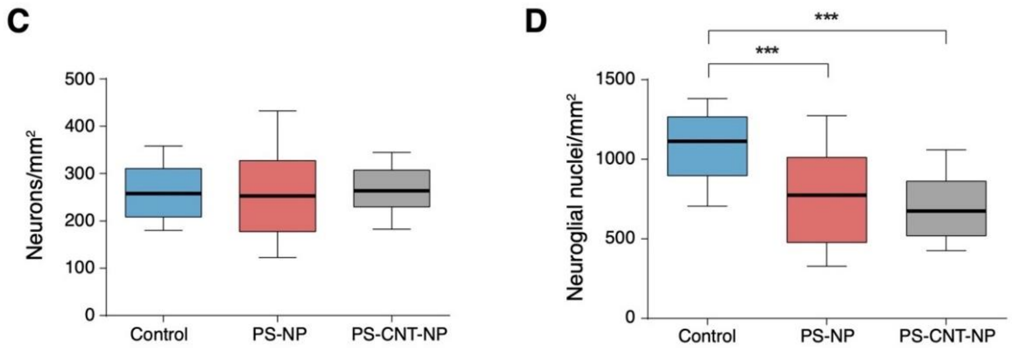
Biocompatibility study:

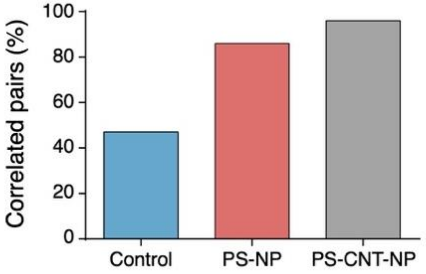
Viability/proliferation/adhesion





postnatal rat hippocampus neurons





Two patents:
PCT/EP2020/078301
EP20382637.5

I. Calaresu et al., Adv. Mater. Interfaces **2021**, 8, 2002121

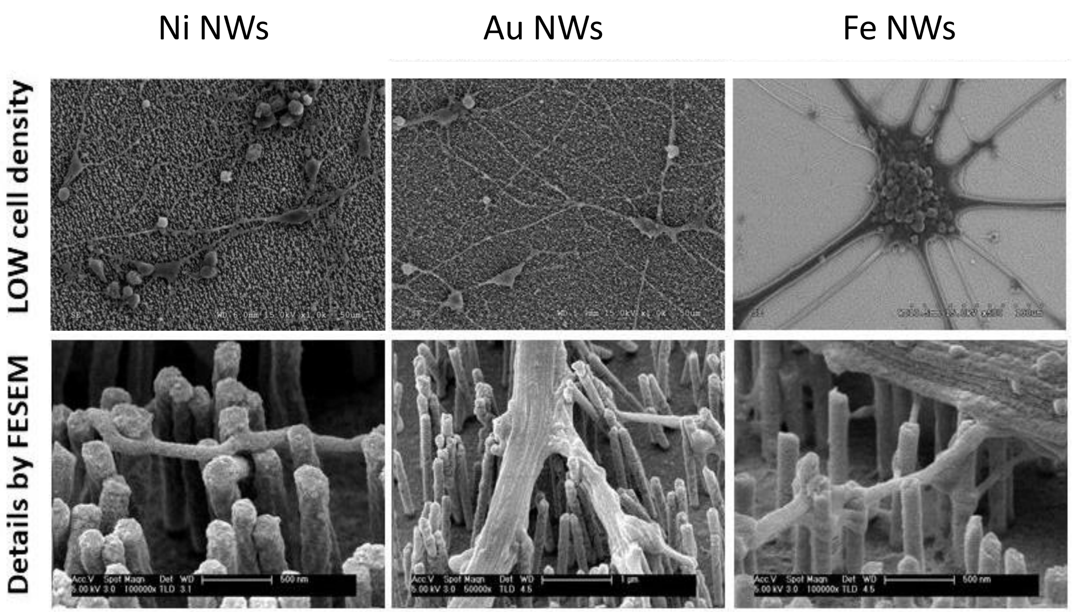
Metallic nanostructured electrodes

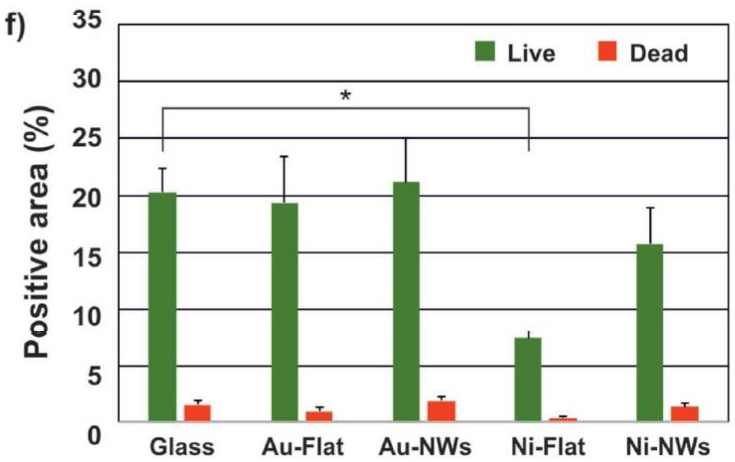
with M. C. Serrano team

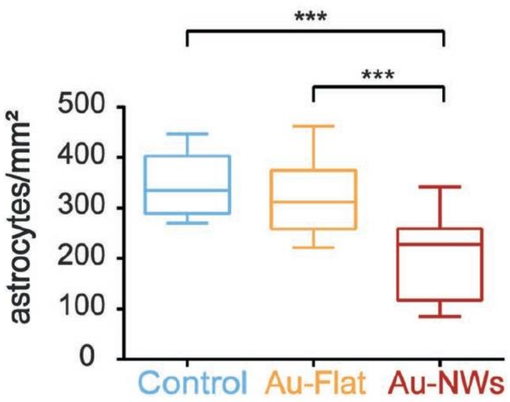
Metallic nanowires grown by template-assisted electrochemical deposition

Biocompatibility study:

- Cells properly adhered in all cases (Cell clumpling for Fe)
- Intimate contact of the cell somas and neurites with the NWs
- Satisfactory viability/proliferation results
- ENPCs differentiation: neuron networks with negligible glial cells presence







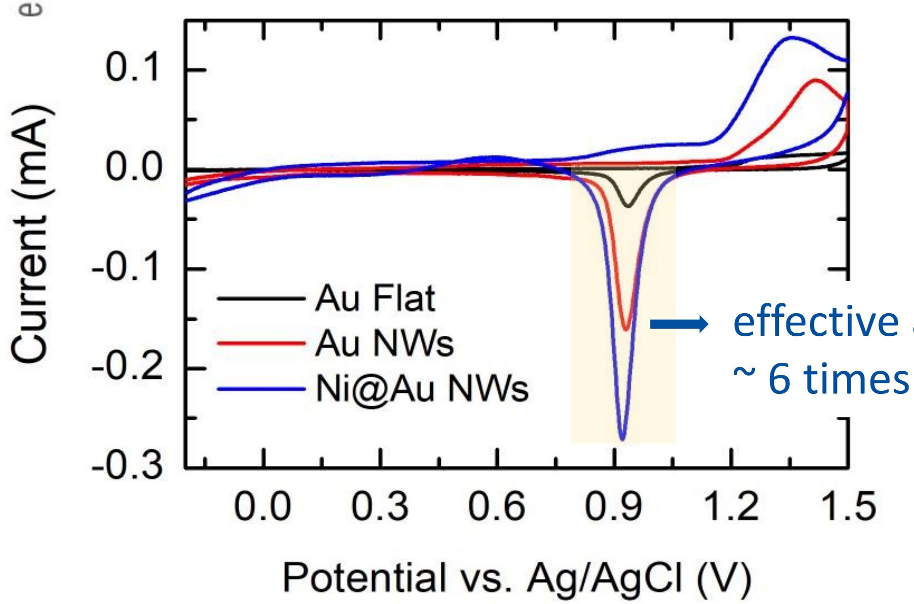
ENPC: embryonic neural progenitor cells

A. Domínguez-Bajo *et al. Adv. Biosys.* **2020**, 2000117

On-bench electrode performance

Nanostructured electrodes (NWs) vs. **Flat** electrodes

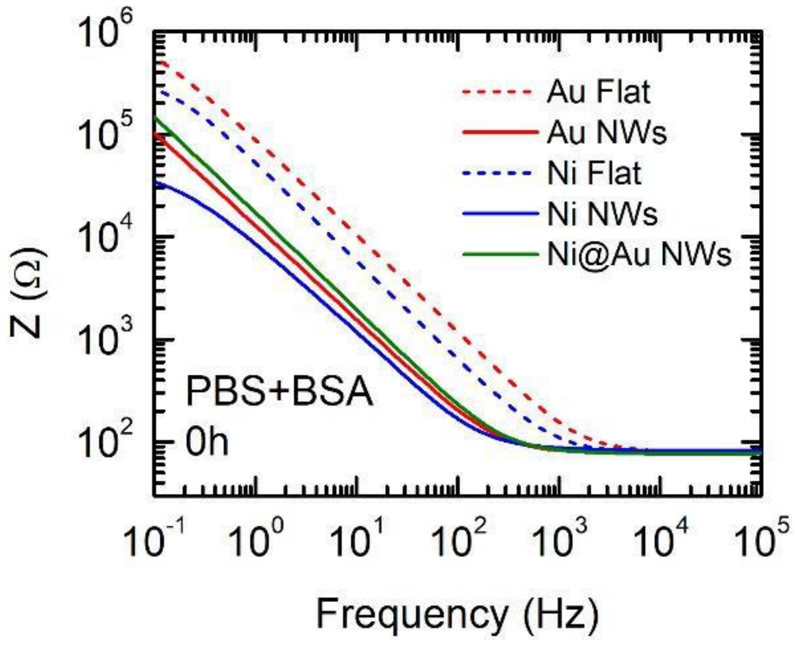
Electrochemical effective area:



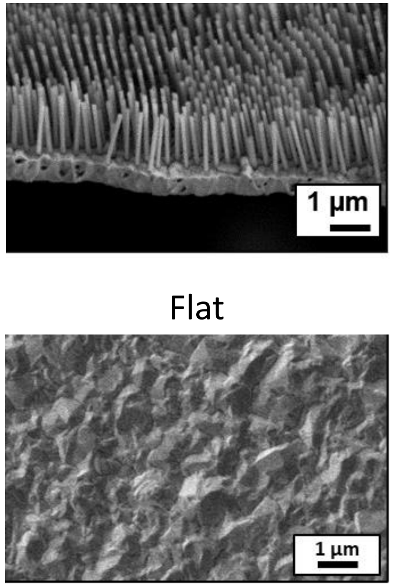
effective area
~ 6 times larger

Nanostructure increases the effective area

Electrical Impedance Spectroscopy :

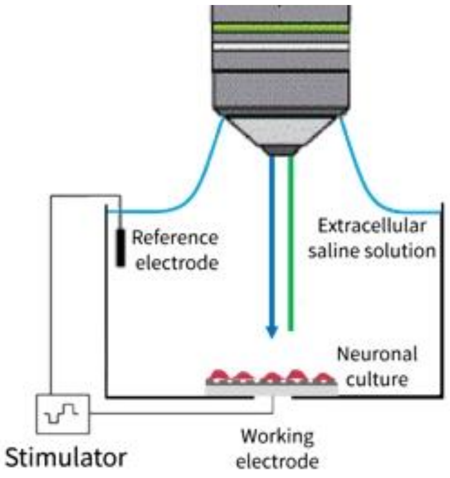


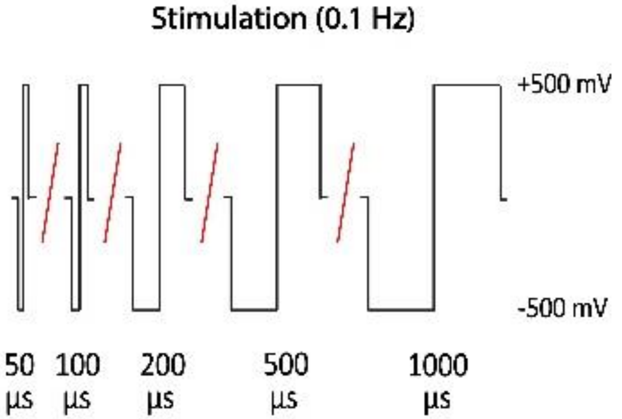
Nanostructured (NWs)



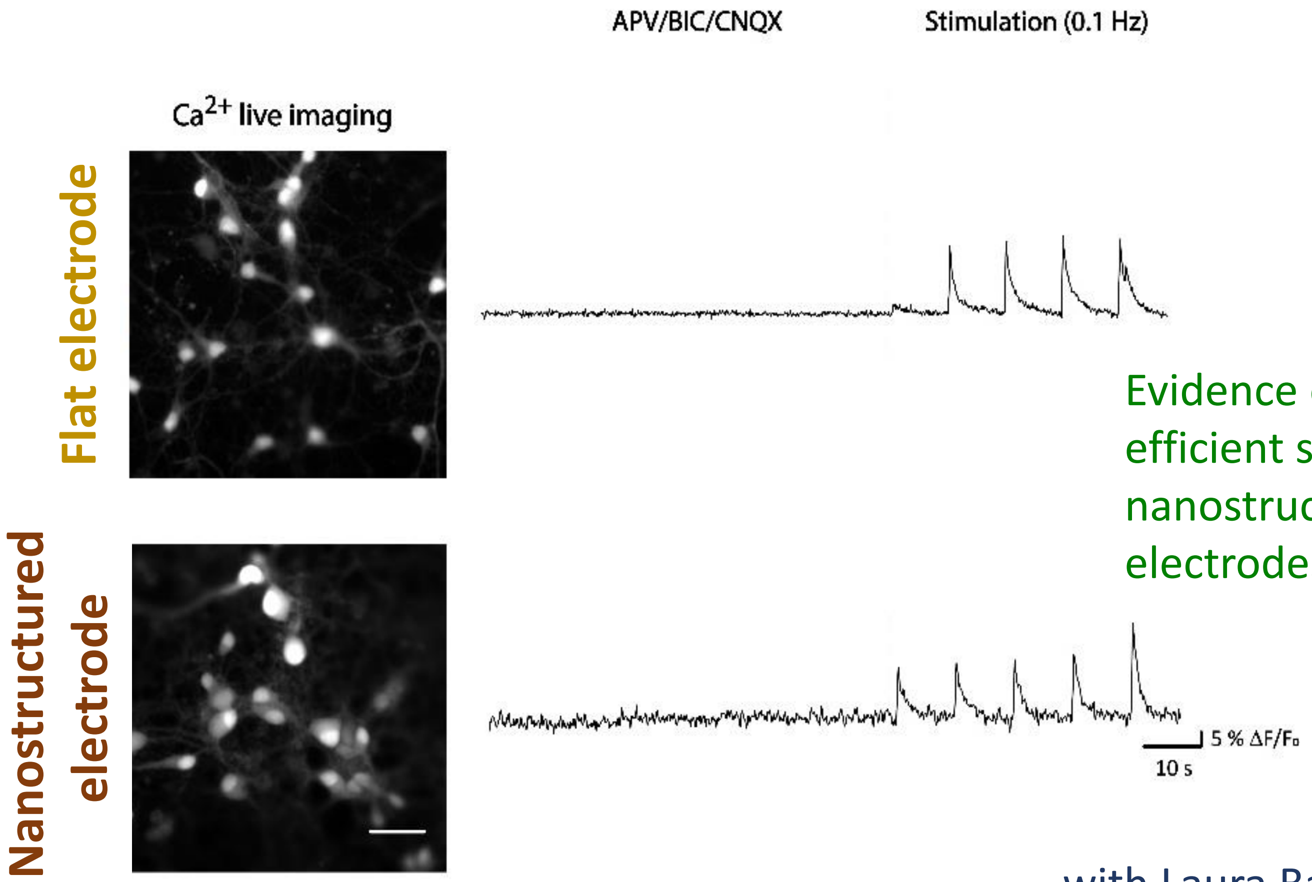
Nanostructure reduces the impedance and increases the electrode stability

Neural stimulation demonstration





Stimulation tests on primary neural cultures by graded waveforms of increasing duration



Evidence of enhanced efficient stimulation for nanostructured electrodes

with Laura Ballerini team

Nanomaterials at IMDEA Nanociencia

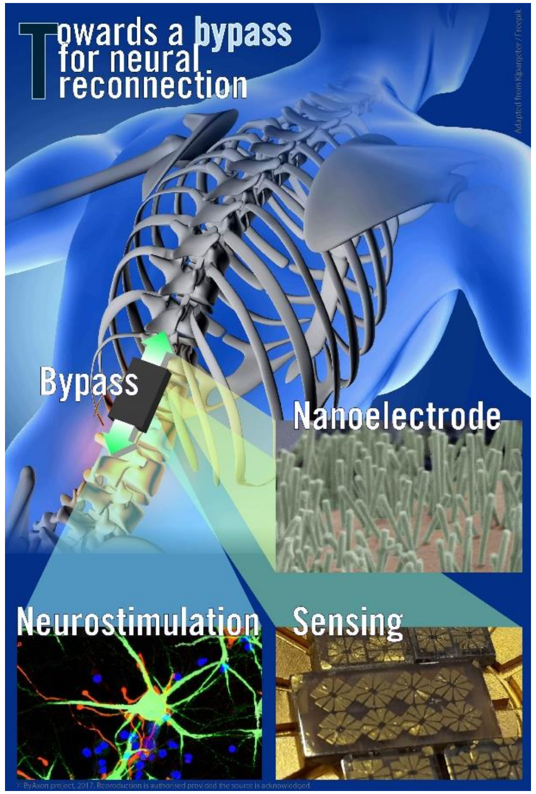
FET-OPEN proyect ByAxon

Aim: use NANOTECHNOLOGY to improve NEURAL INTERFACES
- Minimize the risk of **infections** and **foreign-body rejection**
- Enhance **efficiency** and **resolution**

To be able to address directly the SPINAL CORD

We tackle both directions of interfacing
- **Stimulating:** flexible nanostructured ELECTRODES
- **Sensing**: room-temperature ultrasensitive MAGNETIC SENSORS

neuronal activity (100 pT – 1 nT)



Magnetic sensors

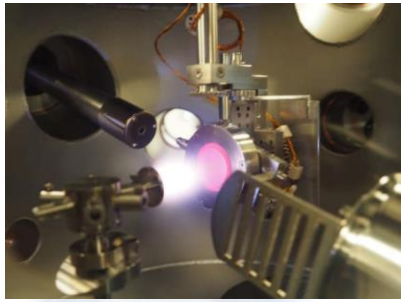
Magnetoencephalography
Commercial system

SQUIDs
(fTesla resolution)

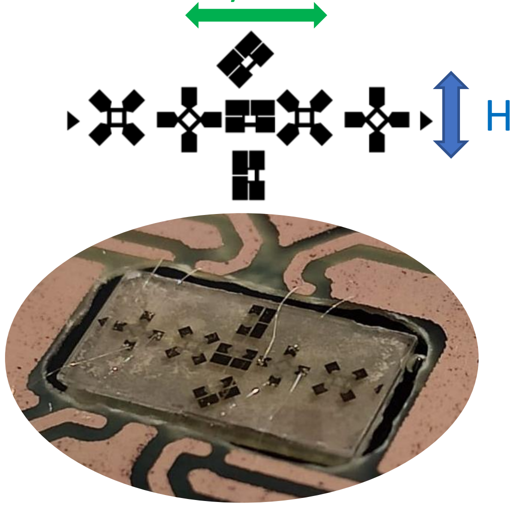
- need for cryogenics
- bulky devices
- need for screening

Sensors based in Anisotropic MagnetoResistance (spintronic)

- Non-contact: sense the magnetic field produced by neuronal activity (100 pT – 1 nT)
- Scalable: arrays of hundreds of detectors
- Portable: work at room temperature / body temperature
- Remove external contributions: Wheatstone config./differential meas.

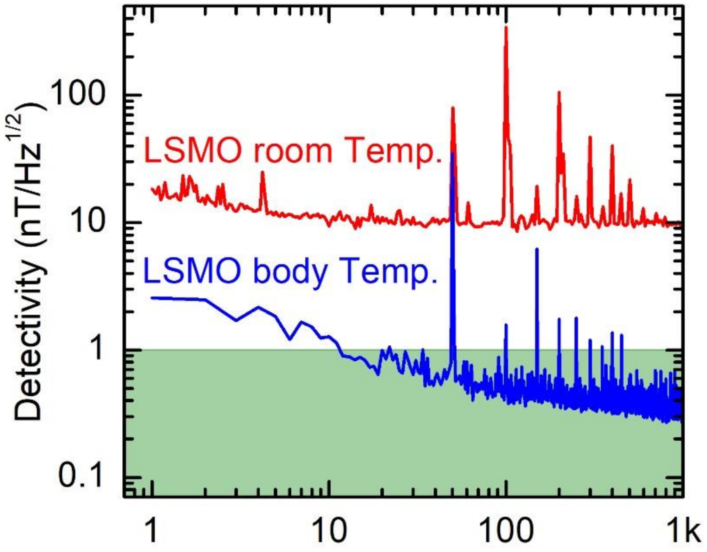


- $La_{0.7}Sr_{0.3}MnO_3$ (LSMO) thin films
- Pulsed Laser Deposition
- On substrates with nanometric terraces



On-bench characterization

with Laurence Mechin team



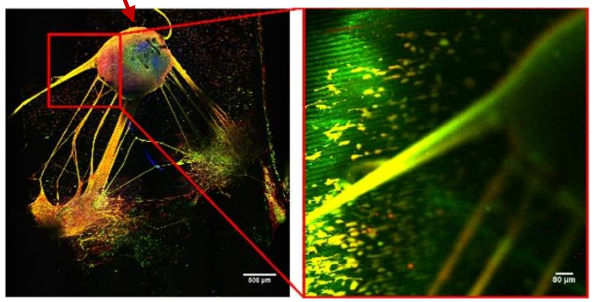
Sub 1 nT resolution achieved!

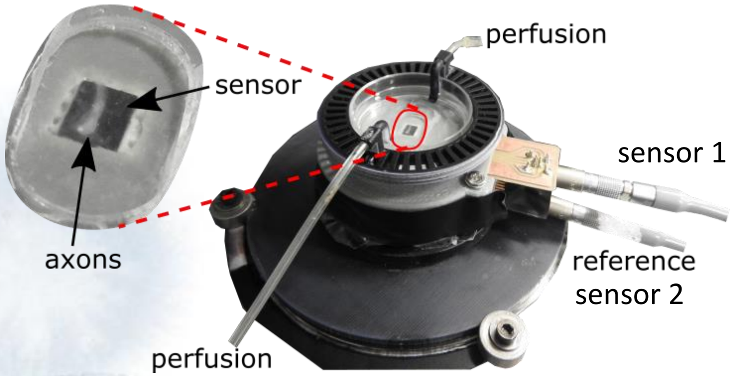
Detectivity: noise floor, error bar in the measured H

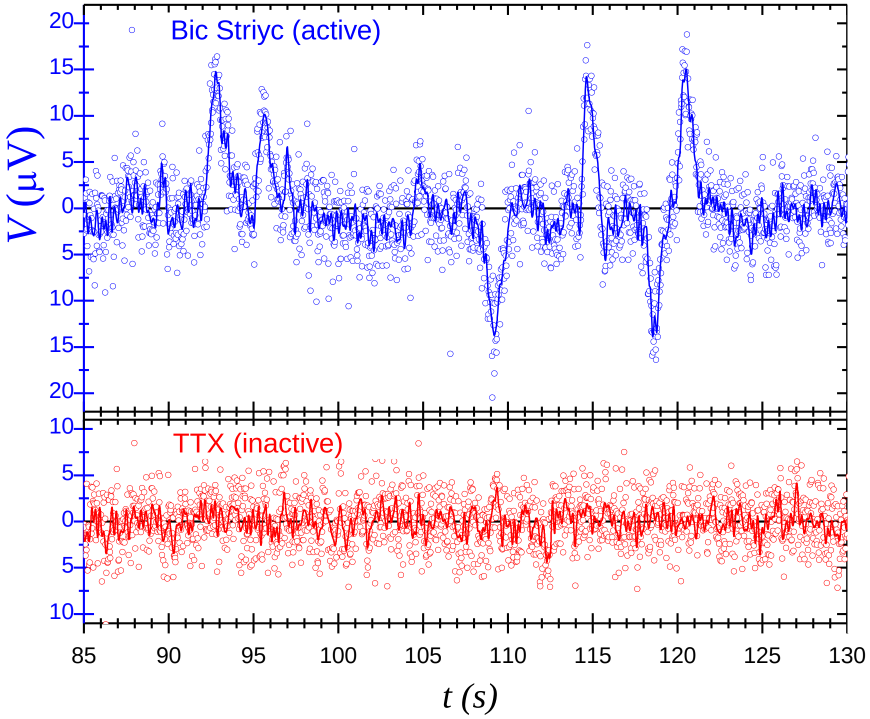
In-vitro detection demonstration

In-vitro detection test with commercial sensors

Mouse-embryo spinal cord slice







ON

Activity in axon bundles pharmacologically induced

OFF

Activity pharmacologically blocked

Neural activity detected
- In **real-time** measurements!
(No average performed)
- **Without** magnetic **shields**

with Laura Ballerini team

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- **Grade A:** The impairment is complete. There is no motor or sensory function left below the level of injury.
  Grade B: The impairment is incomplete. Sensory function, but not motor function, is preserved below the neurologic level (the first normal level above the level of injury) and some sensation is preserved in the sacral segments S4 and S5.
  Grade C: The impairment is incomplete. Motor function is preserved below the neurologic level, but more than half of the key muscles below the neurologic level have a muscle grade less than 3 (i.e., they are not strong enough to move against gravity).
  Grade D: The impairment is incomplete. Motor function is preserved below the neurologic level, and at least half of the key muscles below the neurologic level have a muscle grade of 3 or more (i.e., the joints can be moved against gravity).
  Grade E: The patient's functions are normal. All motor and sensory functions are unhindered.