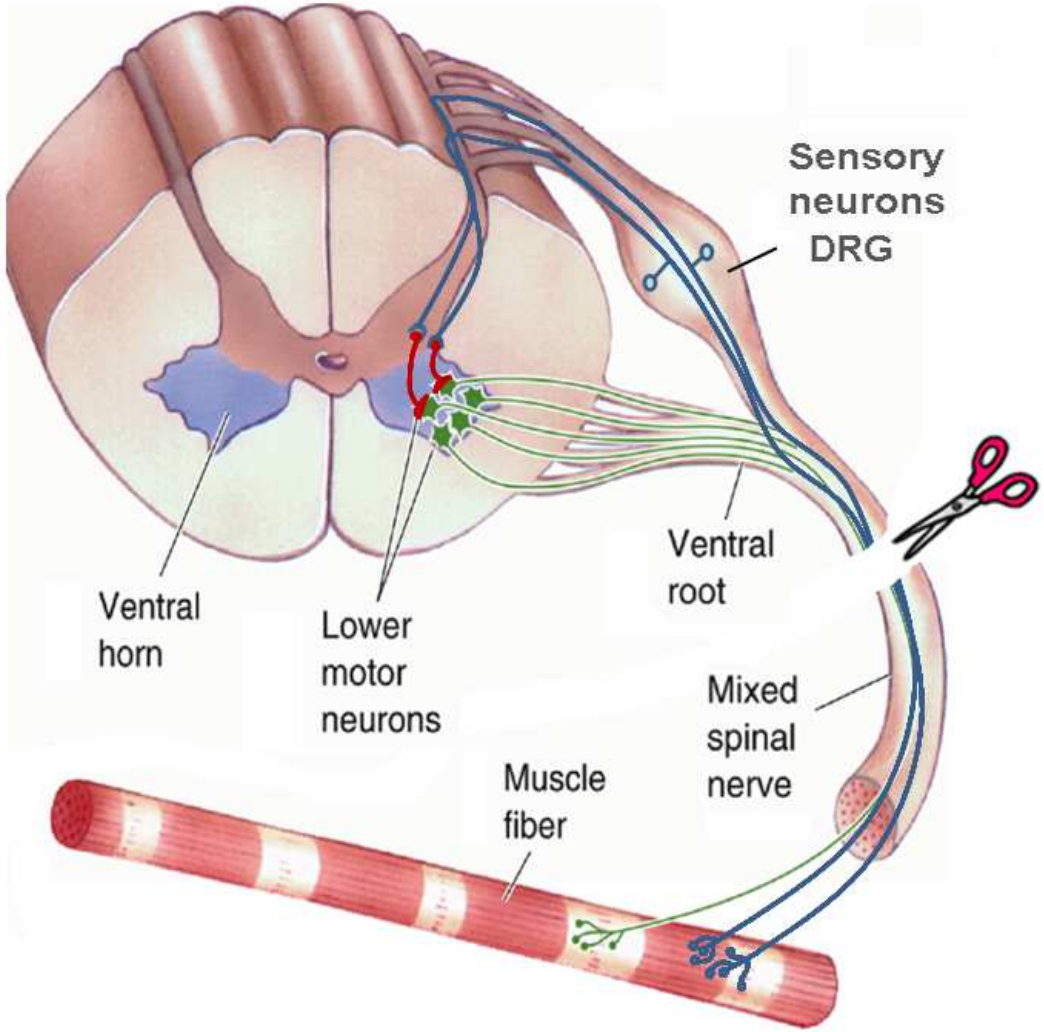
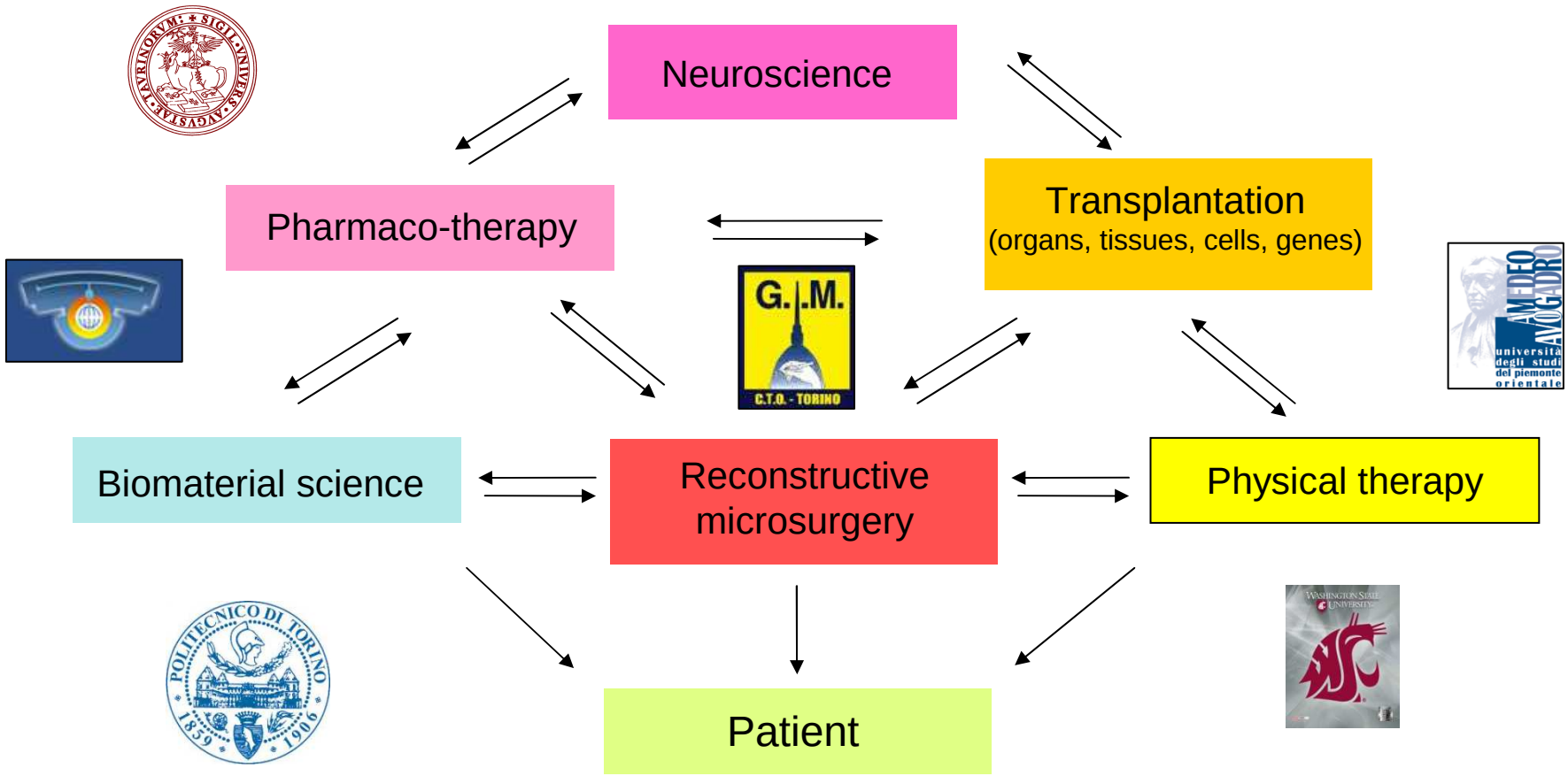
NERVE INJURY and PERIPHERAL NERVE REGENERATION



L’ingegneria tissutale per la riparazione dei nervi periferici richiede un approccio interdisciplinare



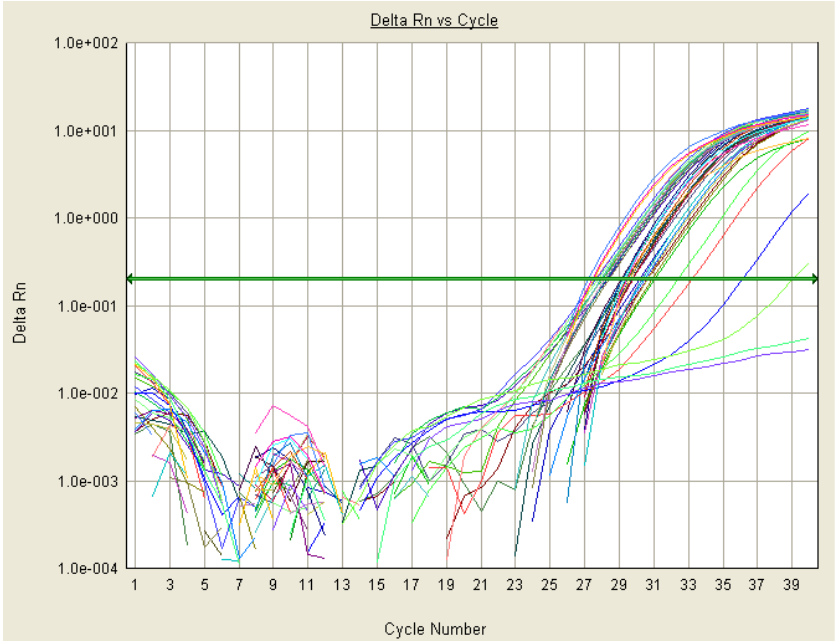
Quali geni sono regolati durante la rigenerazione del nervo periferico?

Quali proteine sono espresse durante la rigenerazione del nervo periferico?

Quali fattori possono favorire la rigenerazione nervosa?

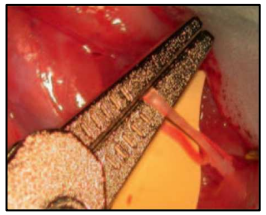
Quali biomateriali possono essere utilizzati per favorire la rigenerazione nervosa?

RNA expression analysis by quantitative real-time-PCR: do we have reliable housekeeping genes in the injured peripheral nerves?

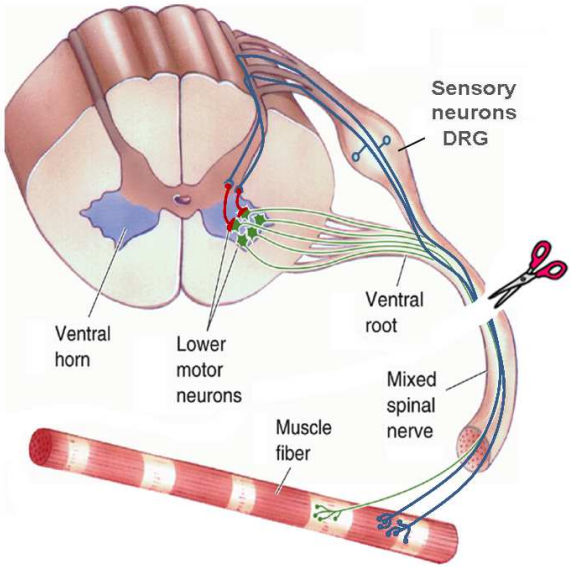


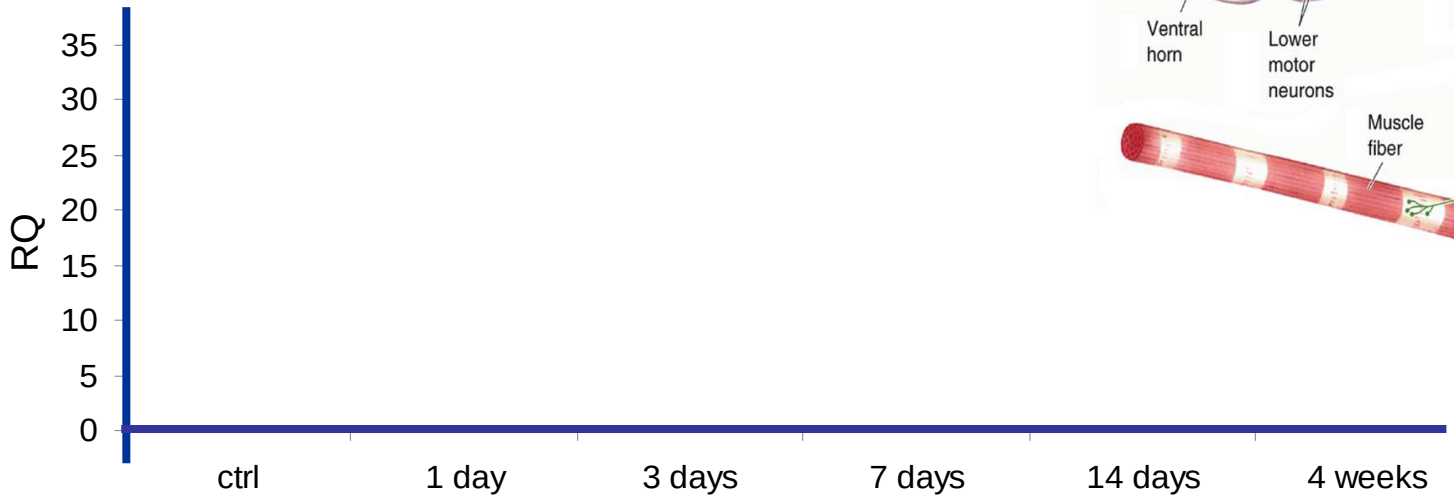
Housekeeping Genes

rat median nerve

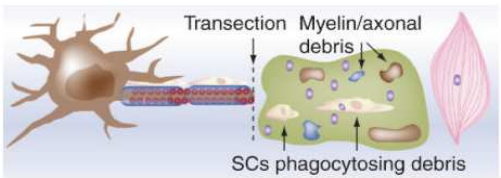


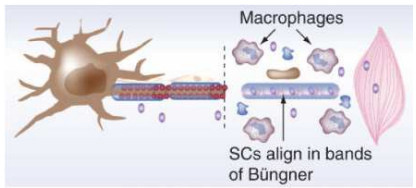
crush lesion
(*axonotmesis*)

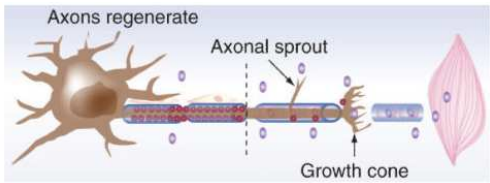


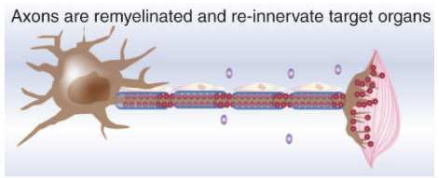


NERVE DEGENERATION
→ REGENERATION
→ REMYELINATION





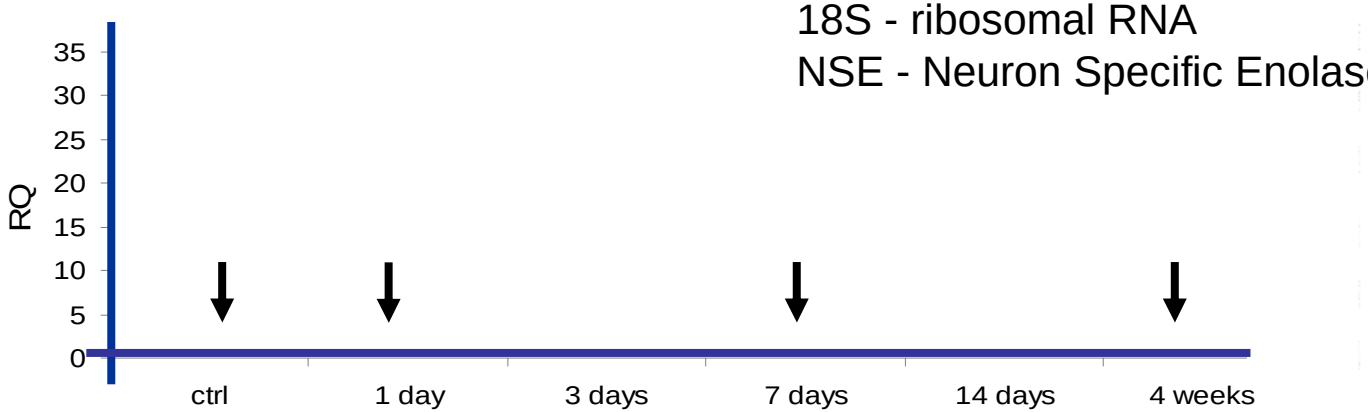




INJURY EXPERIMENTAL MODEL

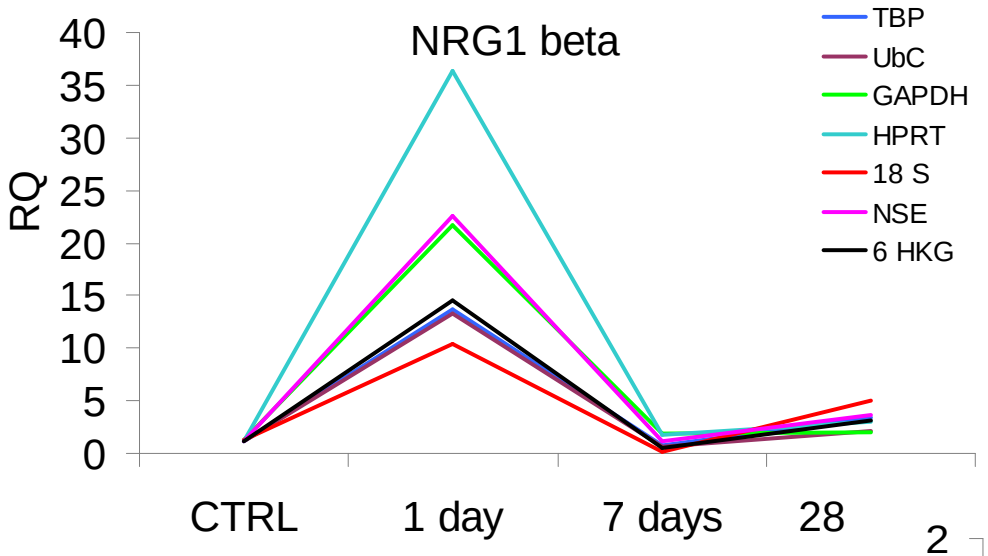
Total RNA extracted 1, 7, and 28 days after nerve crush lesion was analysed by qRT-PCR for the expression of 6 commonly used HKG:

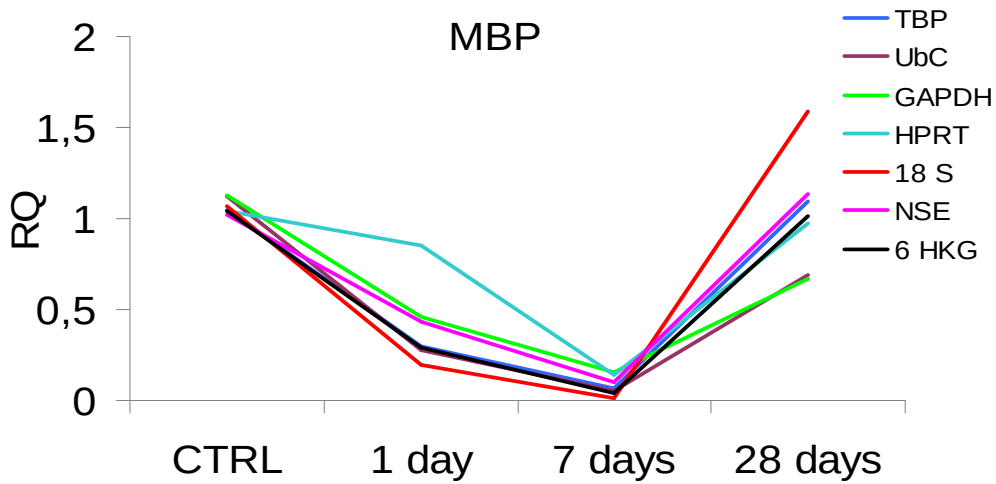
TBP - TATA box binding protein
UbC - Ubiquitin C
GAPDH - Glyceraldehyde-3-phosphate dehydrogenase
HPRT - Hypoxanthine phosphoribosyl-transferase
18S - ribosomal RNA
NSE - Neuron Specific Enolase



NERVE DEGENERATION
→ REGENERATION
→ REMYELINATION

NORMALIZED RELATIVE QUANTIFICATION

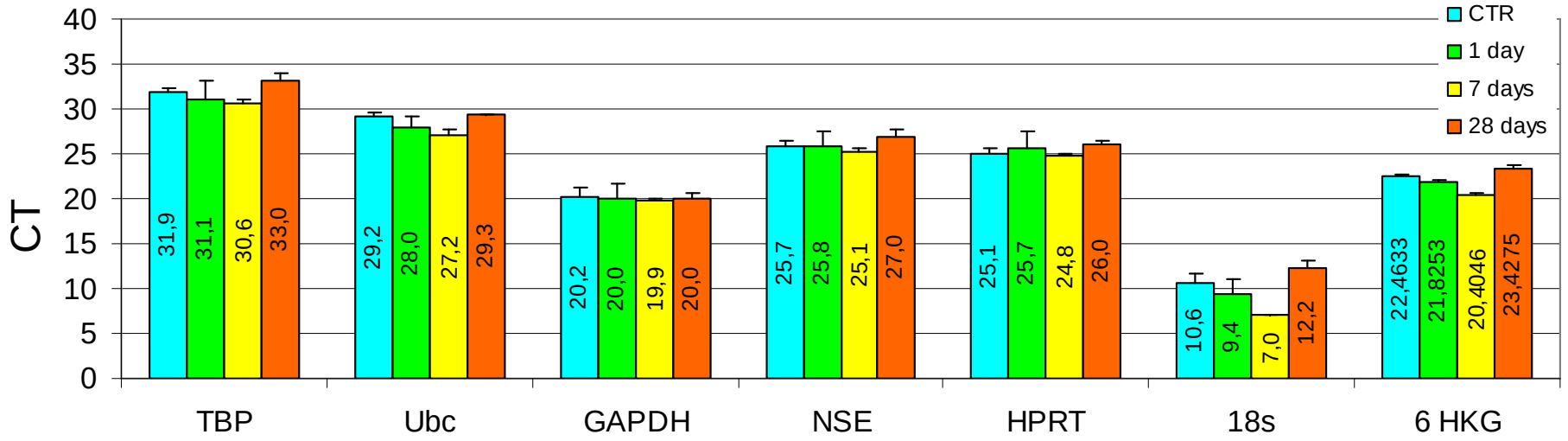


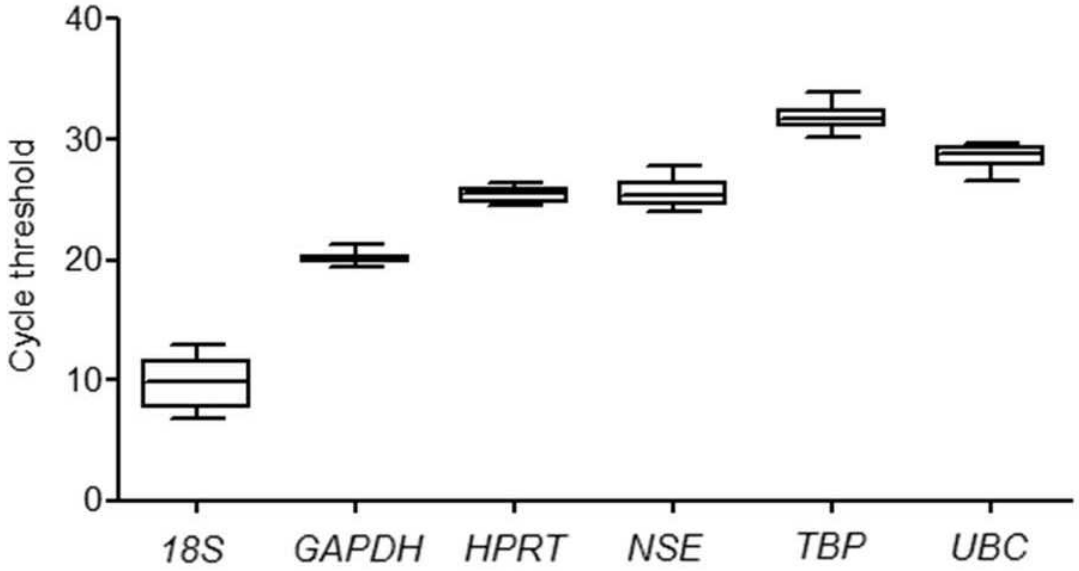


- the expression of genes regulated after lesion (up, NRG1 and down, MBP) is strongly influenced by the HKG used for normalization

- the strong variation of CT values, primarily in the first days after nerve injury, highlights the fact that all these genes are differently regulated after lesion

COMMONLY USED HKG ARE REGULATED FOLLOWING NERVE INJURY





NormFinder	geNorm
TBP	*TBP - UBC*
UBC	
HPRT	*HPRT*
NSE	*NSE*
GAPDH	*GAPDH*
18S	*18S*

Several commonly used HKG present pseudogenes or a single exon.

		PSEUDOGENES	PRIMERS on DIFFERENT EXONS
TBP	TATA box binding protein	NO	YES
UbC	Ubiquitin C	YES	YES
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	YES	YES
HPRT	Hypoxanthine phosphoribosyl-transferase	YES	YES
18S	ribosomal RNA	NO	NO
NSE	Neuron Specific Enolase	NO	YES

AIM

Identify new housekeeping genes (HKG)

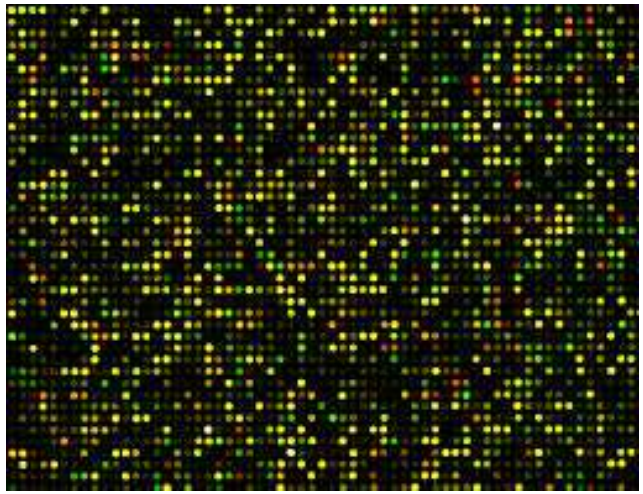
1 - which remain **stable** during **all steps** of nerve degeneration and regeneration

&

2 - that do not have pseudogenes
3 - that contain at least one intron, in order to localize forward and reverse primer on different exons

PUBLICLY AVAILABLE MICROARRAY DATA

TIME COURSE	NERVE	SPECIES	MICROARRAY PLATFORM	GEO, Series Accession	Reference
0 – 1 – 5 days after injury	sciatic	*Mus musculus*	Illumina MouseRef-8 v2.0 expression beadchip	# GSE33454	Kim *et al.,* 2012
0 – 7 days after injury	sciatic	*Mus musculus*	Affymetrix Mouse Genome 430 2.0 Array	# GSE38693	Arthur-Farraj *et al.,* 2012
0 – 1 – 3 – 7 -14 days after injury	sciatic	*Mus musculus*	Affymetrix Mouse Genome 430 2.0 Array	# GSE22291	Barrette et al., 2010



Housekeeping Genes

IDENTIFICATION OF GENES STABLE 1 AND 5 DAYS AFTER INJURY

Microarray analysis (1 and 5 days after injury) (Kim et al, 2012)	25697 gene probes
$0,99 \leq fc_{1/0} \leq 1,01$	1840 gene probes
$0,99 \leq fc_{5/0} \leq 1,01$	192 gene probes
$0,99 \leq fc_{5/1} \leq 1,01$	132 gene probes
Fluorescence ≥ 100	66 gene probes
≥ 2 probes	39 genes

Housekeeping Genes

IDENTIFICATION OF 3 GENES STABLE 1, 5 AND 7 DAYS AFTER INJURY

39 genes

Microarray analysis
(7 days after injury)
(Arthur-Farraj et al., 2012)

$0{,}99 \leq fc_{1/0}; fc_{5/0}; fc_{5/1}; fc_{7/0}$ average $\leq 1{,}01$
standard deviation $\leq 0{,}05$

4 genes

- Rictor (RPTOR Independent Companion Of MTOR, Complex 2)
- Ankrd27 (ankyrin repeat domain 27)
- Mrpl10 (mitochondrial ribosomal protein L10)
- UbXN11 (UbX domain protein N11)

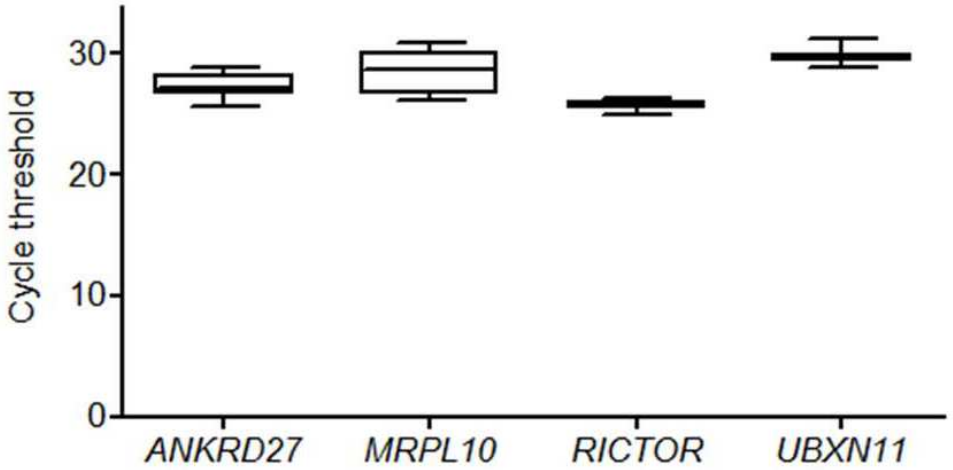
Microarray analysis
(1, 3, 7, 14 days after injury)
(Barrette et al., 2010)

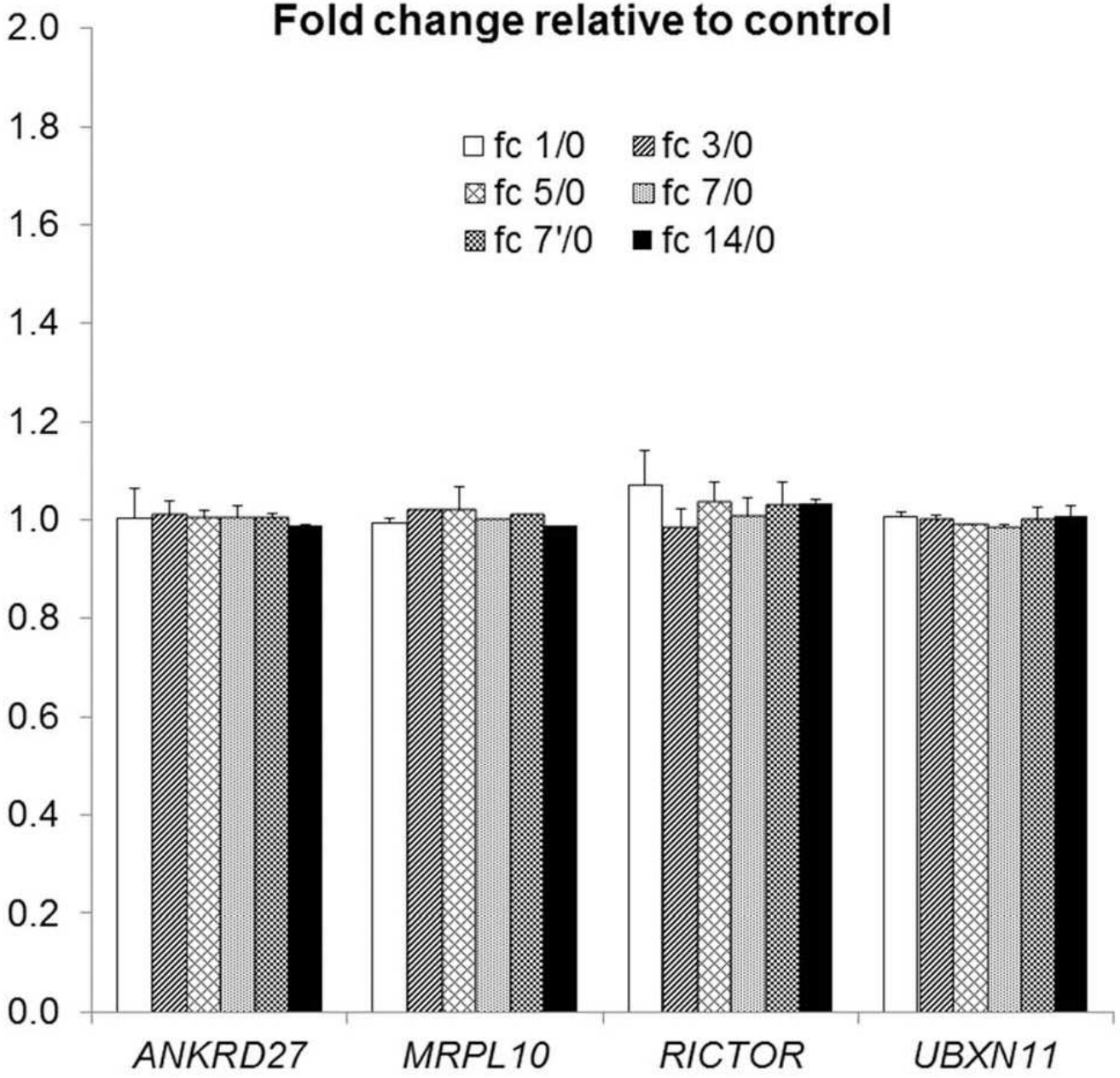
Stability confirmed

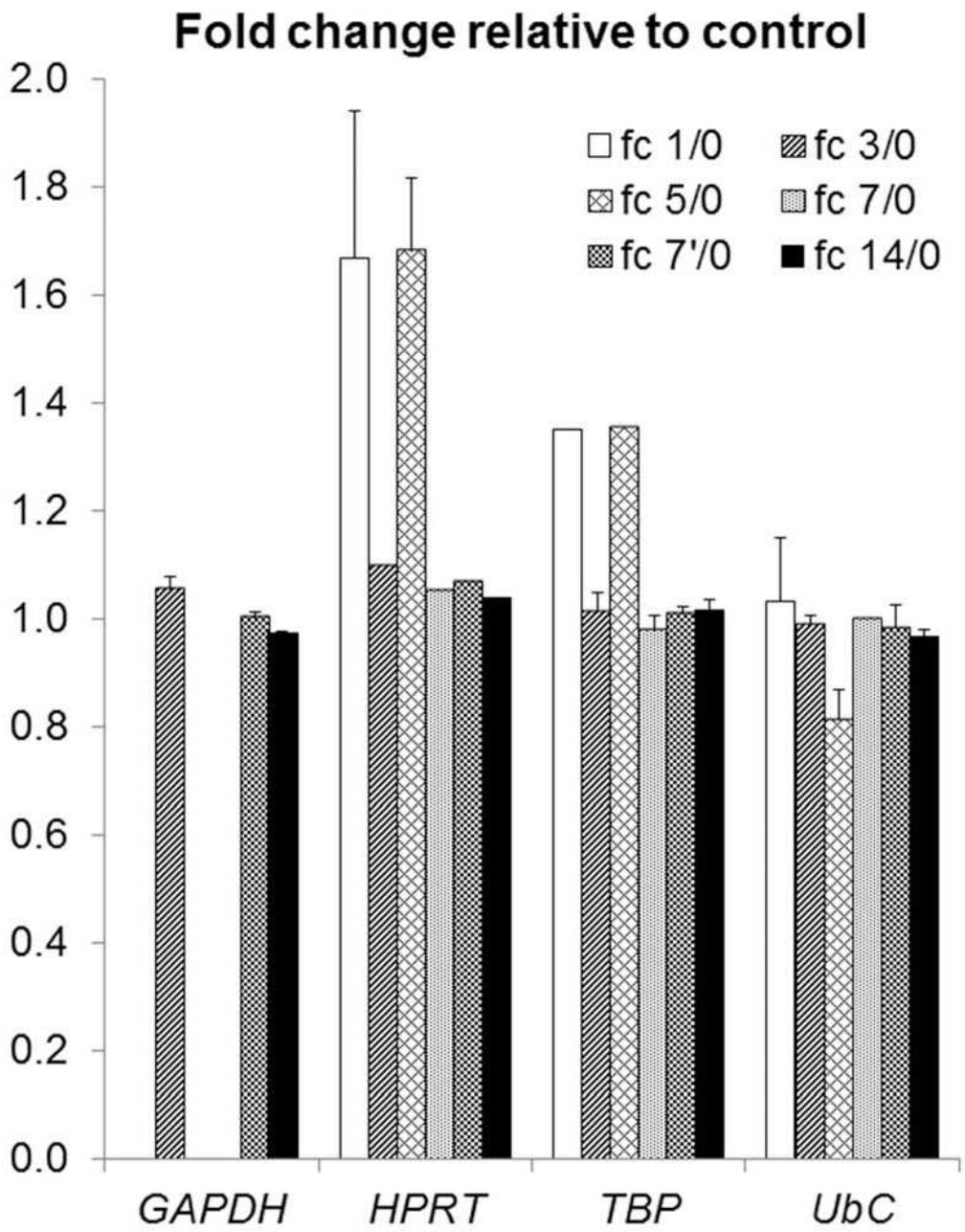
IDENTIFICATION OF HIGHLY STABLE GENES

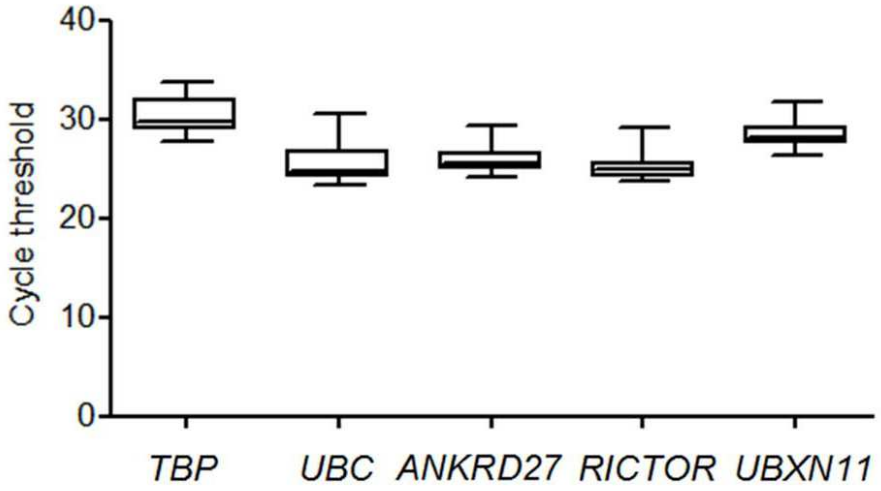
Symbol	Gene name	Accession no.	Function	Pseudogenes	Introns
ANKRD27	ankyrin repeat domain 27 (VPS9 domain)	NM_001271264.1	guanyl-nucleotide exchange factor activity	No	Yes
MRPL10	mitochondrial ribosomal protein L10	NM_001109620.1	structural constituent of ribosome	No	Yes
RICTOR	RPTOR independent companion of MTOR, complex 2	XM_001055633.3	regulation of Rac GTPase activity, positive regulation of TOR signaling	No	Yes
UBXN11	UBX domain protein 11	NM_138853.2	positive regulation of Rho GTPase activity	No	Yes

NormFinder	geNorm
ANKRD27	*RICTOR - UBXN11*
RICTOR	
UBXN11	*ANKRD27*
MRPL10	*MRPL10*

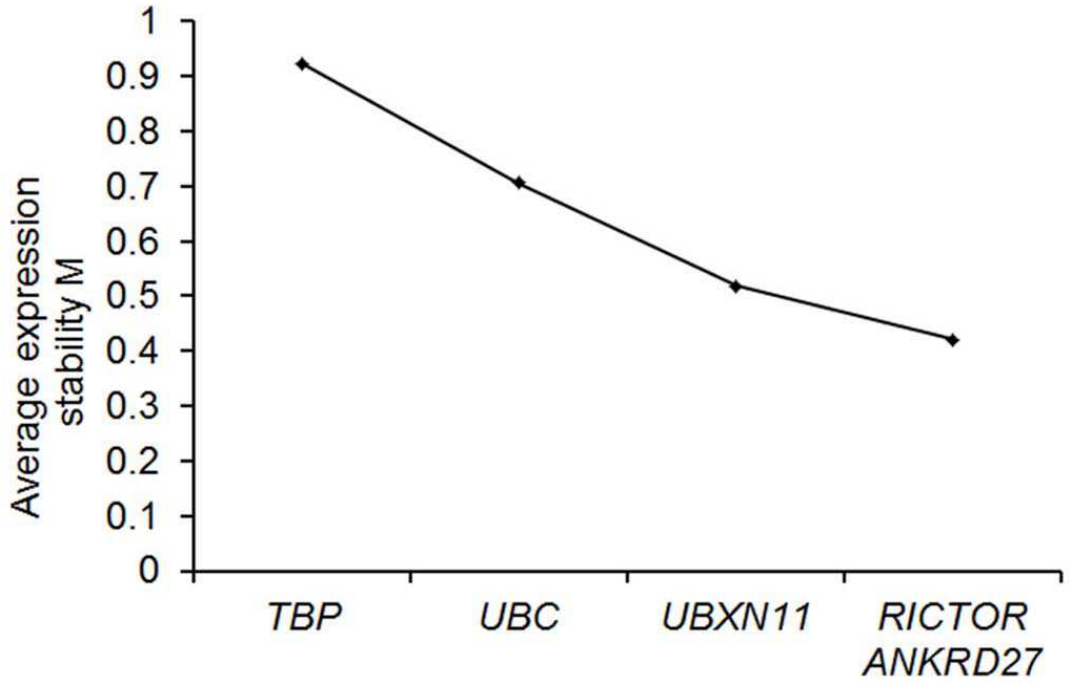


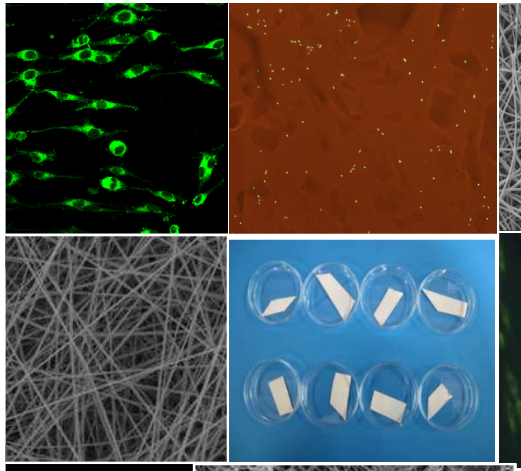




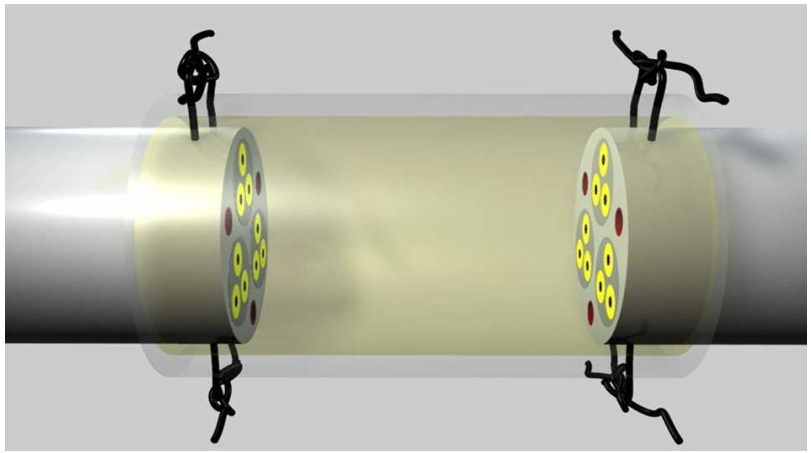


NormFinder	geNorm
ANKRD27	*RICTOR - ANKRD27*
RICTOR	
UBXN11	*UBXN11*
UBC	*UBC*
TBP	*TBP*





Develoment of artificial nerve guidance channels as alternatives to autologous nerve grafts



Ideal scaffold requirements

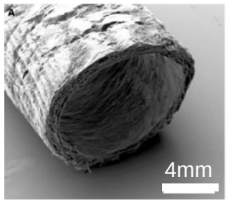
- Biocompatibility
- Biodegradability
- Permeability
- Mechanical strength

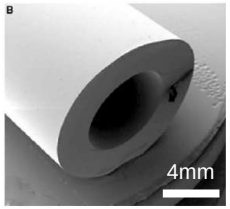
Clinically approved materials for bridging peripheral nerve injuries

Salubridge from Salumedica

Neuromatrix or Neuroflex from Collagen Matrix Inc
type-I bovine collagen,
2-6 mm X 2.5 cm

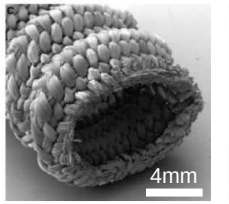
Neurolac from Polyganics BV
Poly(DL-lactide-e-caprolactone
1.5-10 mm X 3 cm





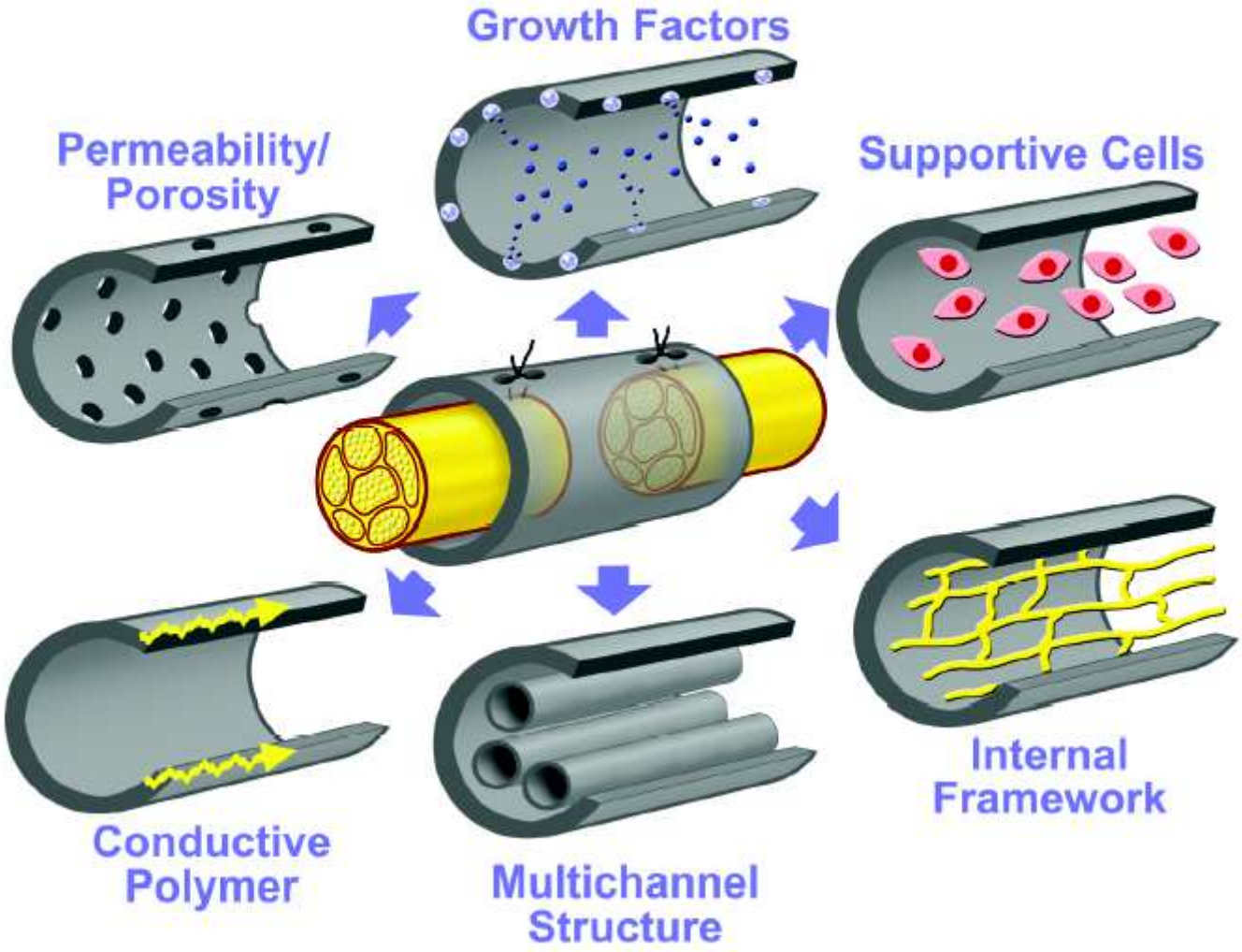
Neuragen from Integra Neuroscience
Semi-permeable type-I bovine collagen,
1.5-7 mm X 3 cm

Neurotube from Synovis Micro Companies Alliance
Poly-glycolic acid
2.3-4-8 mm X 4 cm

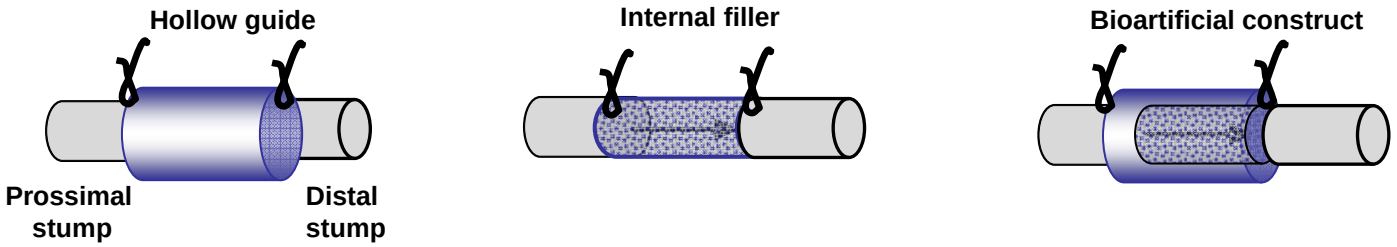


Deumens R., et al, 2010

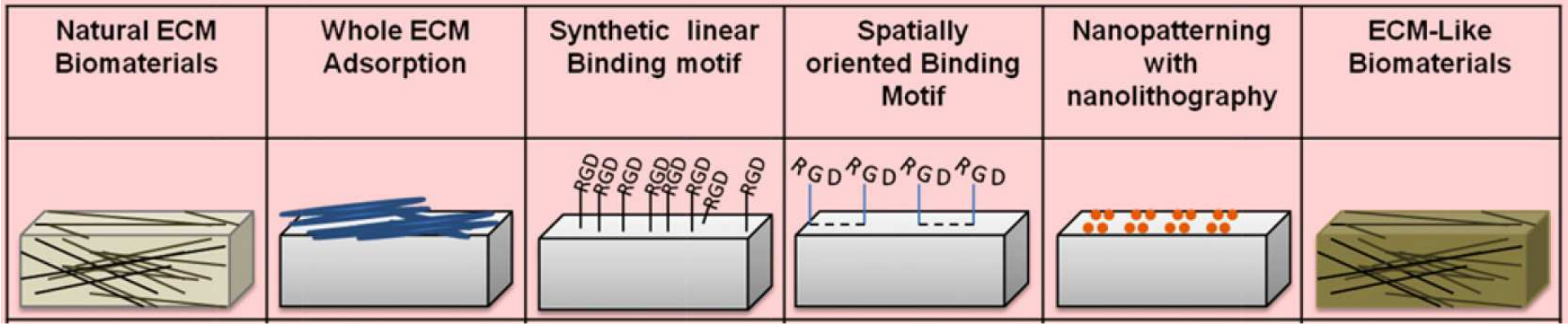
Modifications to the single lumen nerve tubes



De Ruiter G. C. W., et al.,2009



Modifiche dei biomateriali mediante l'uso di molecole della matrice extracellulare

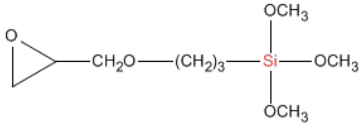


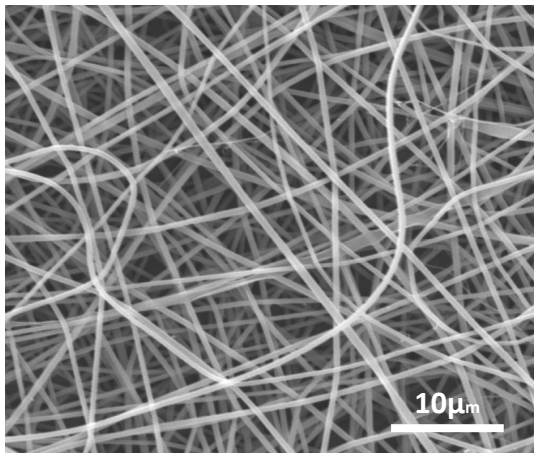
Rahmany and Van Dyke 2013

Scaffold made of electrospun gelatin nanofibres

Crosslinking: 3-Glycidoxypropyl-trimethoxysilane, GTPMS

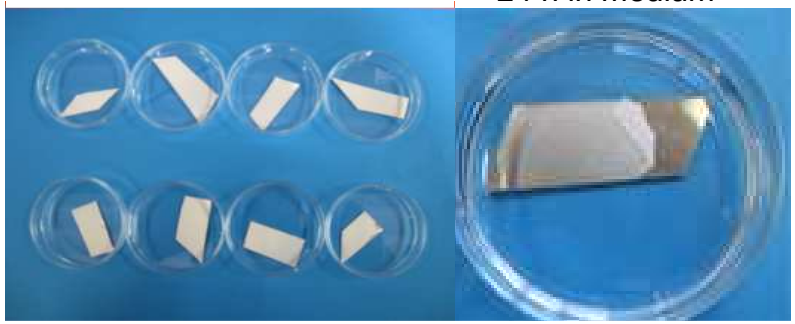
GL/GPTMS-NF: produced by electrospinning.





Fiber diameter
356 ± 59 nm

After immersion of
24 h in medium



Injectable hydrogels for growth factors release

Hydrogels are crosslinked hydrophilic polymers

- high water content (up to 98 %)
- porous structure
- thixotropic and injectable nature

Internal fillers for hollow guide:
electrospun nanofibers