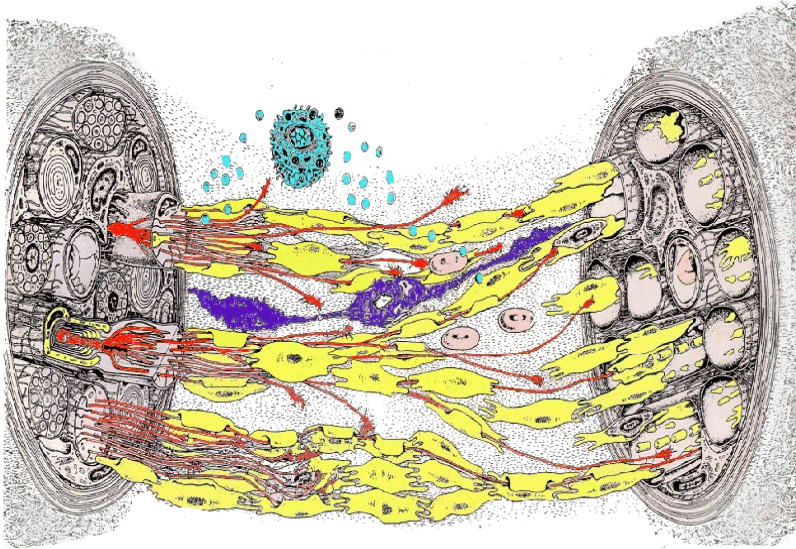


# Rigenerazione dei nervi periferici

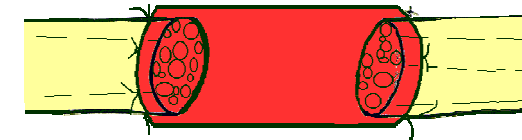
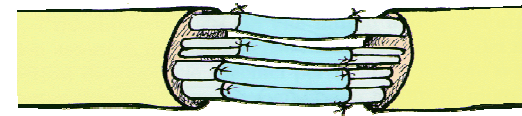
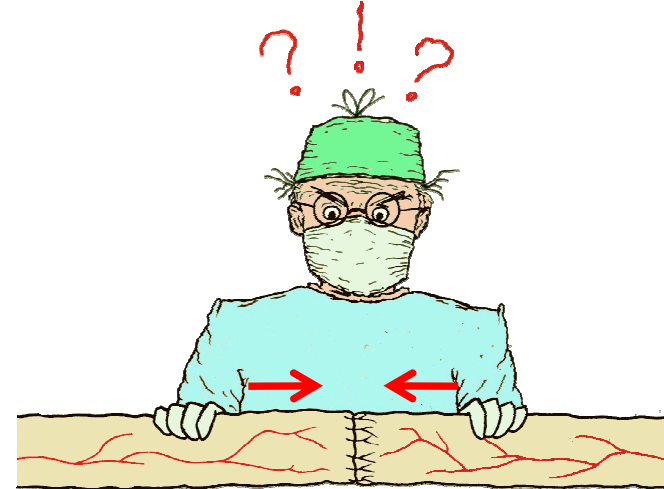


## Ricerca di base

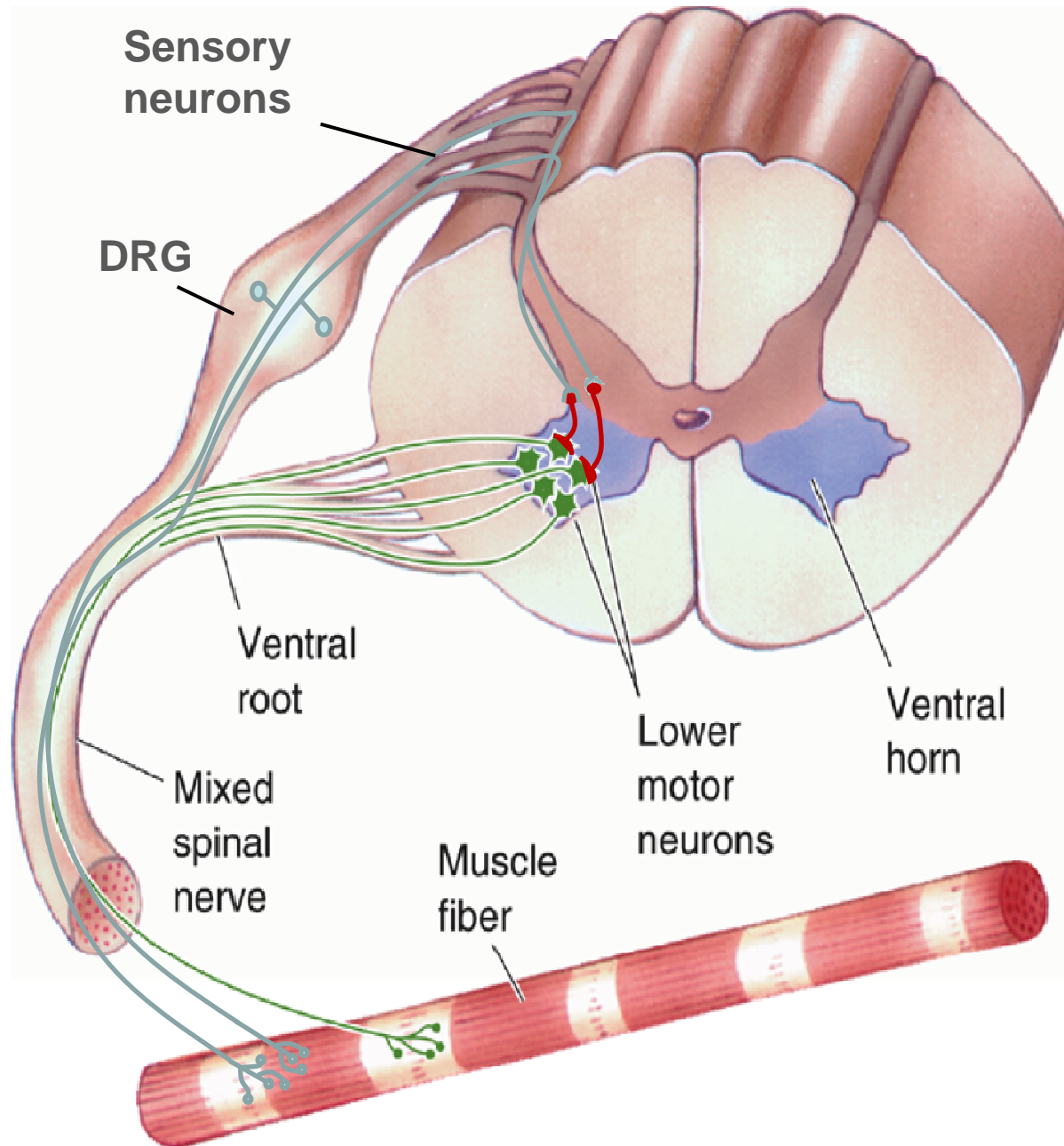


Studio dei processi di  
rigenerazione

## Ricerca applicata

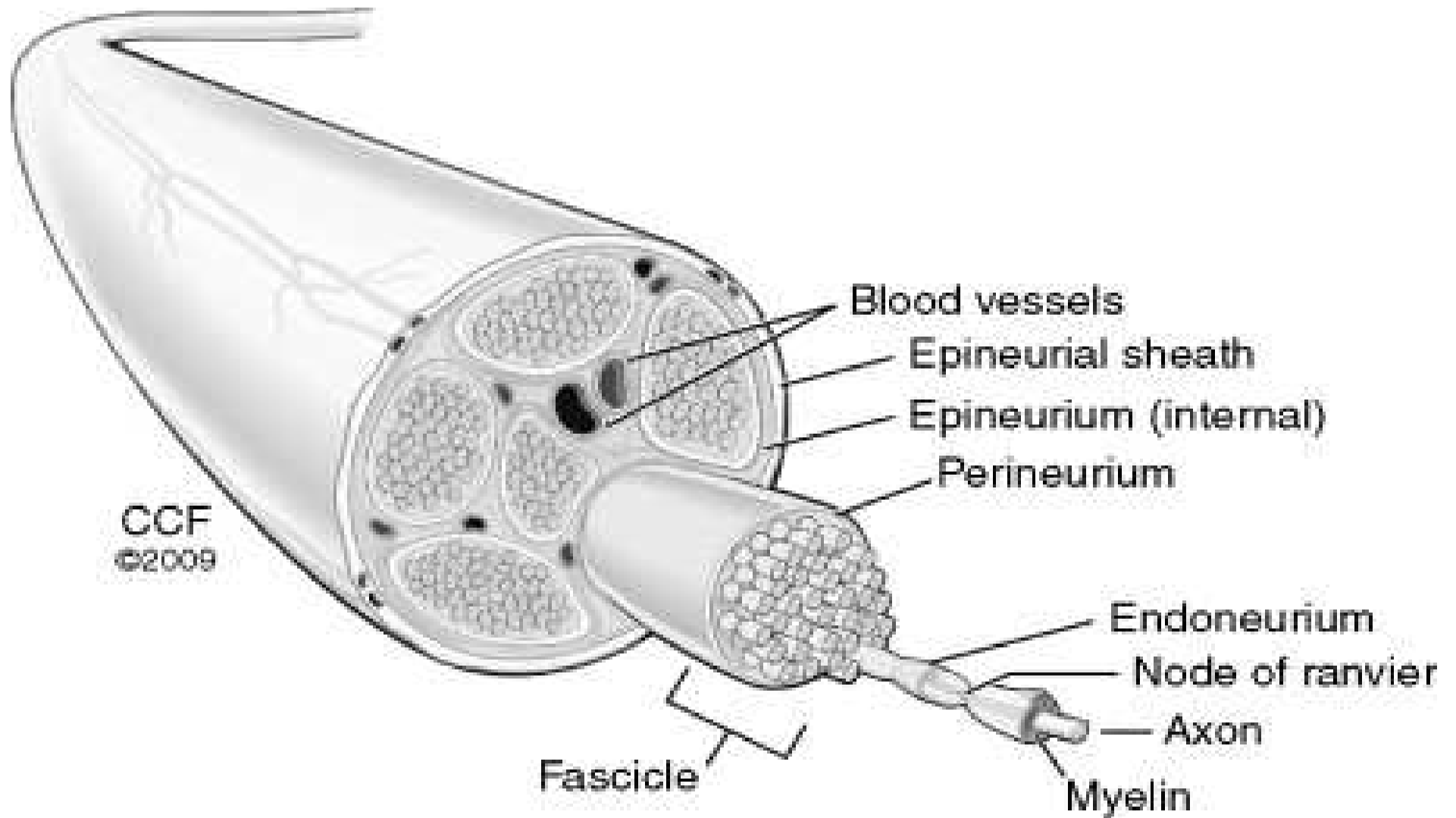


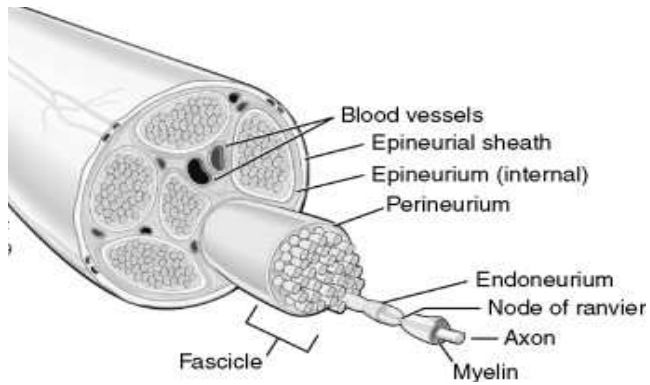
Studio delle tecniche di  
riparazione dei nervi periferici



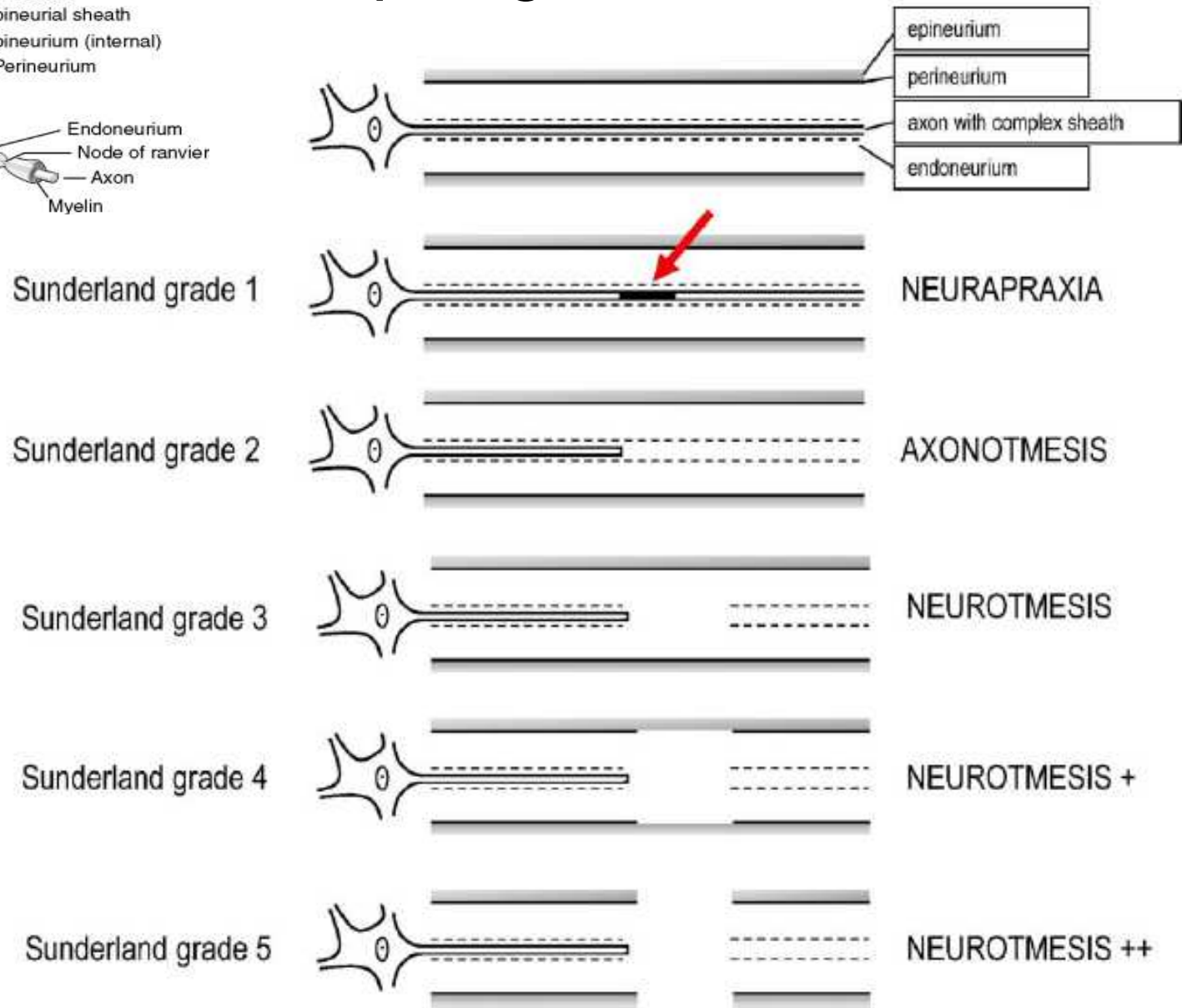
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Solo per uso didattico - vietata la riproduzione o la vendita

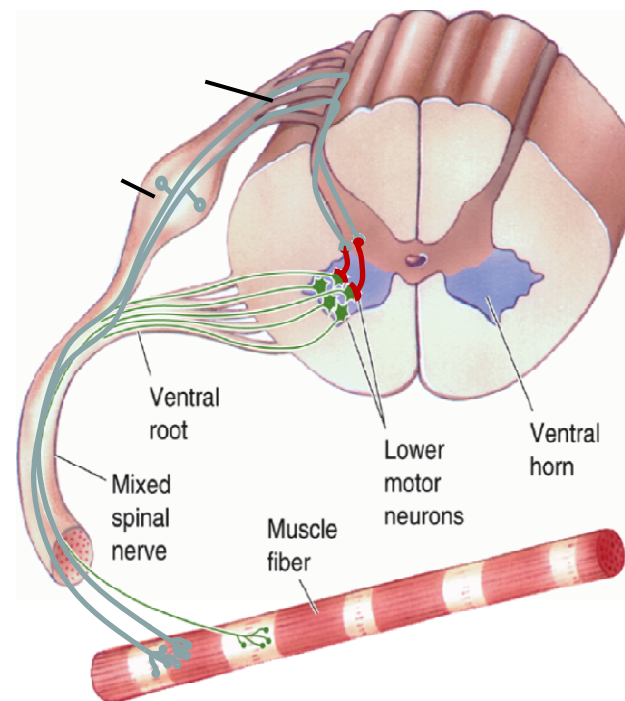
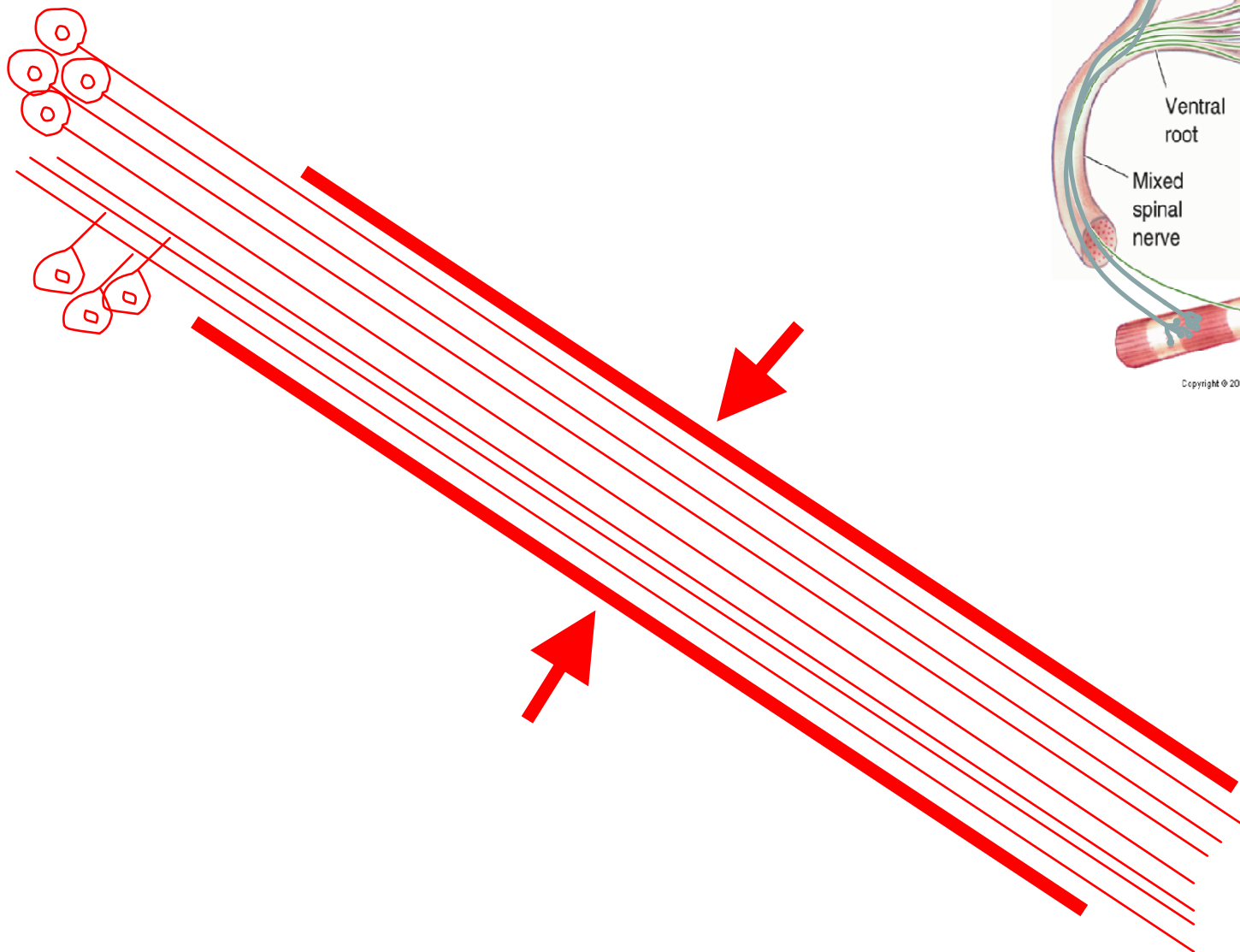




# Tipologie di lesione

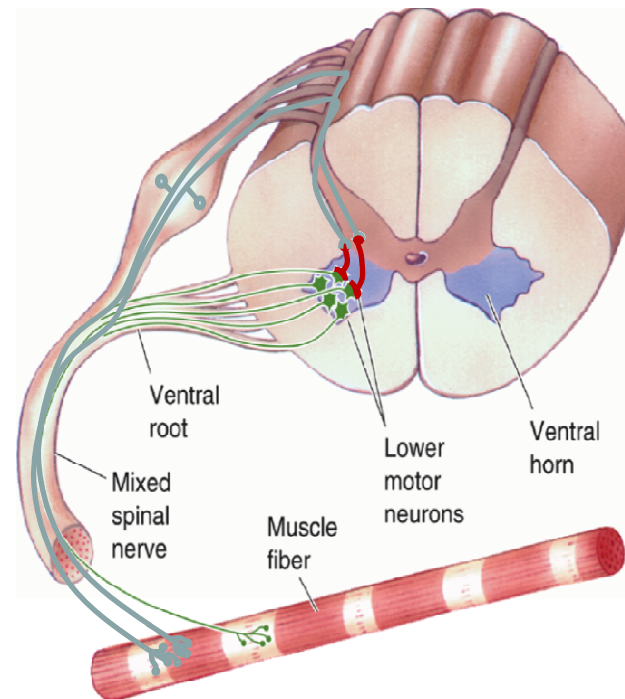


# Axonotmesi (mild nerve lesion)

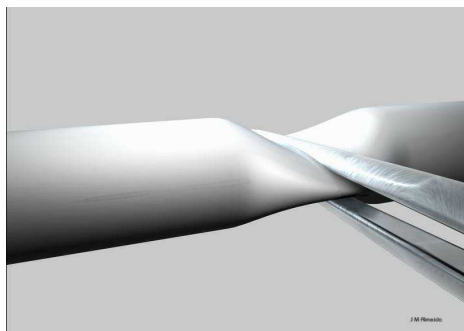
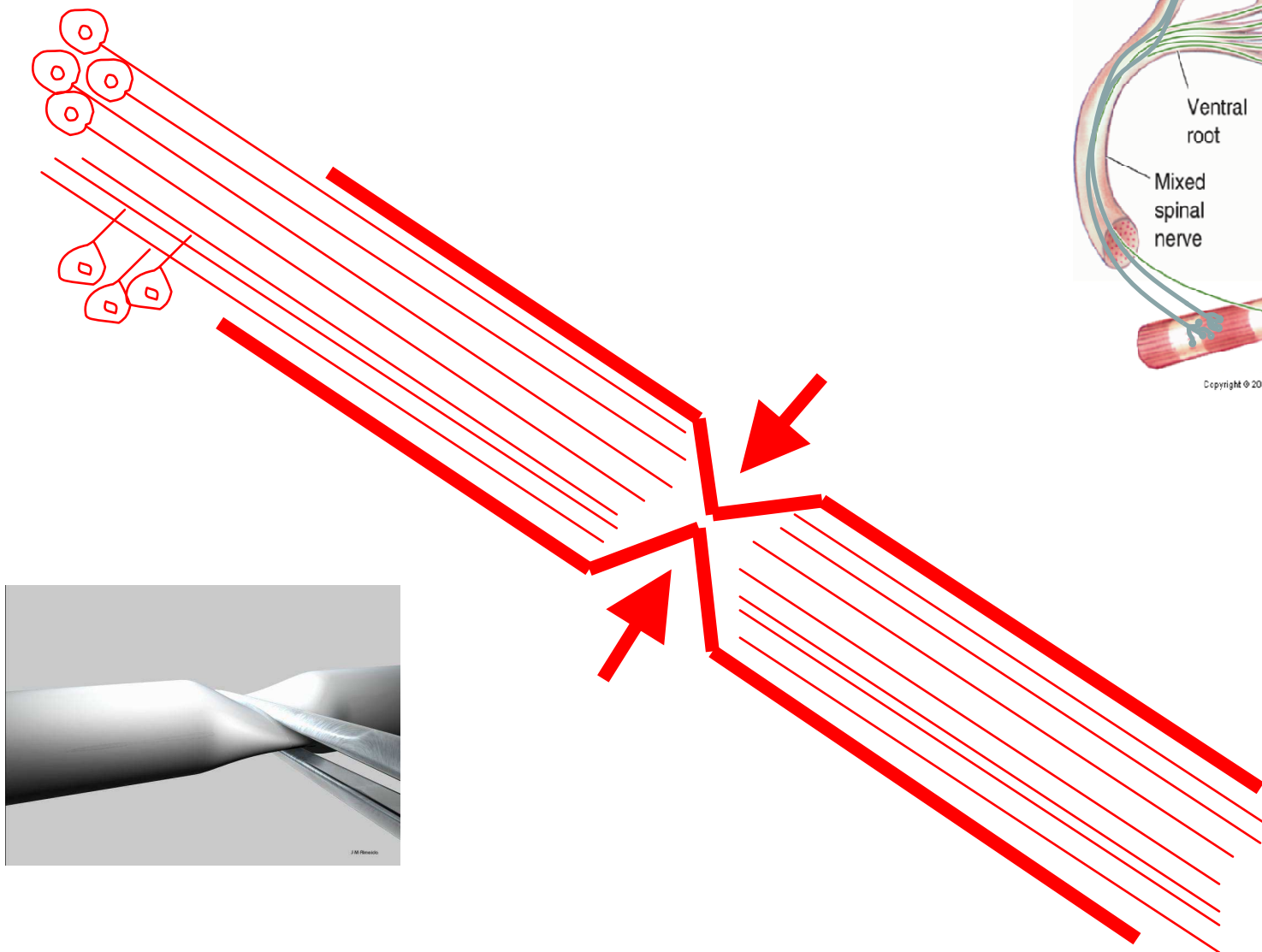


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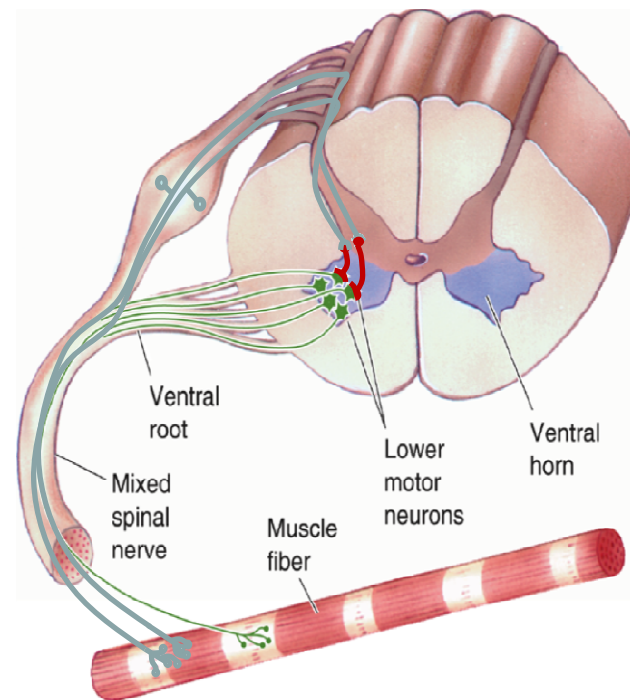
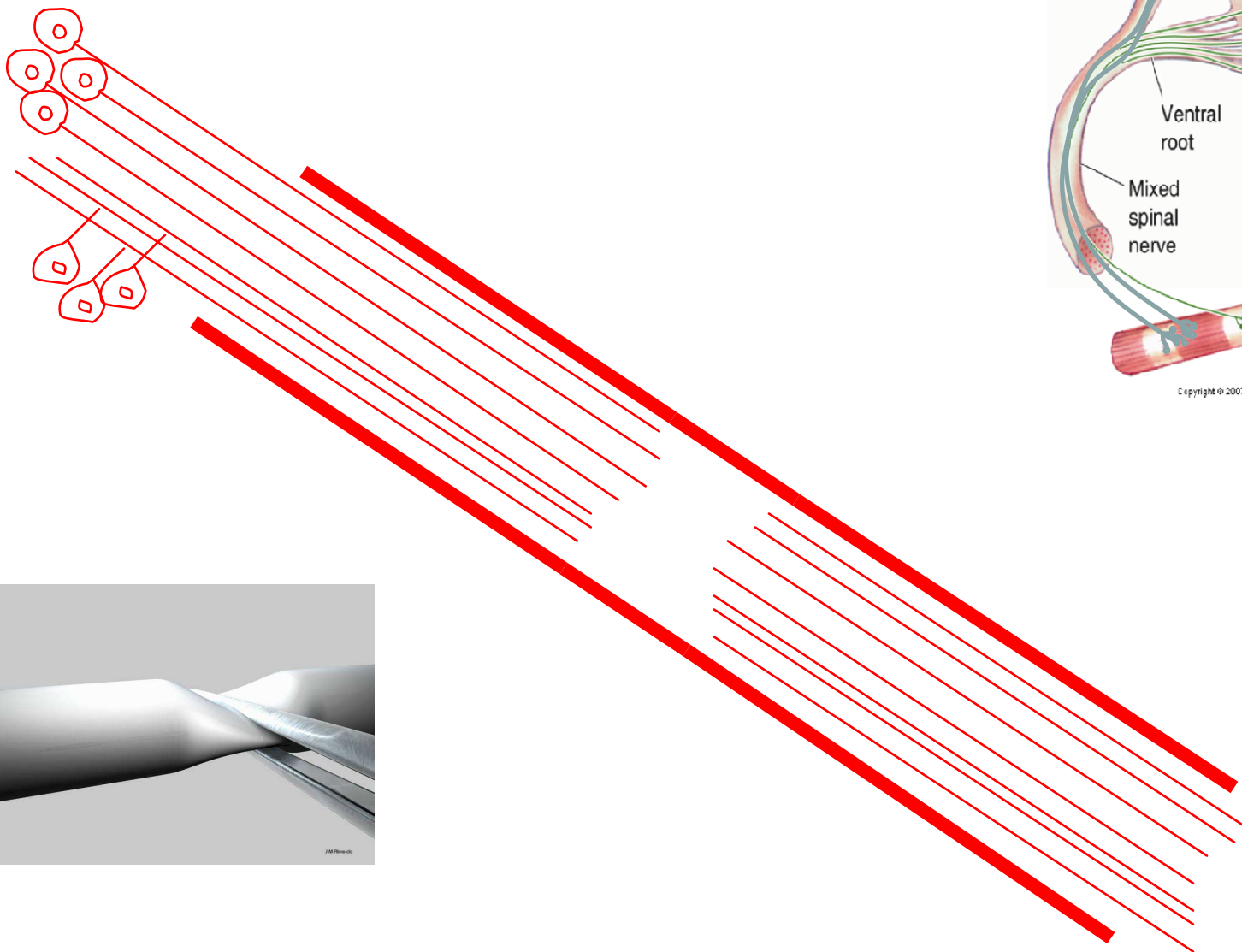
# Axonotmesis (crush)



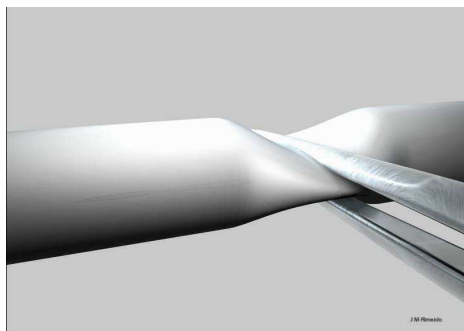
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# Axonotmesis (crush)

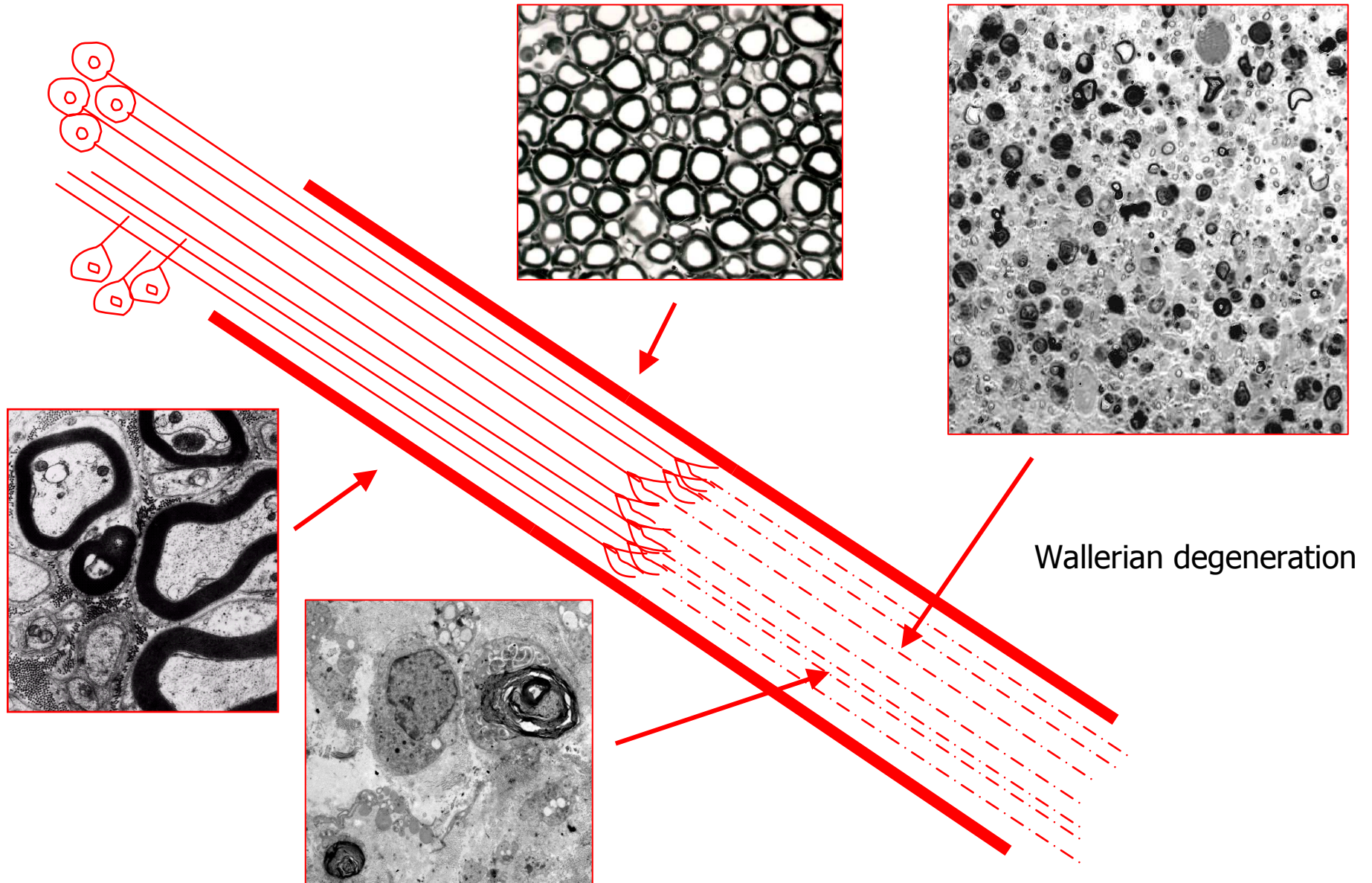


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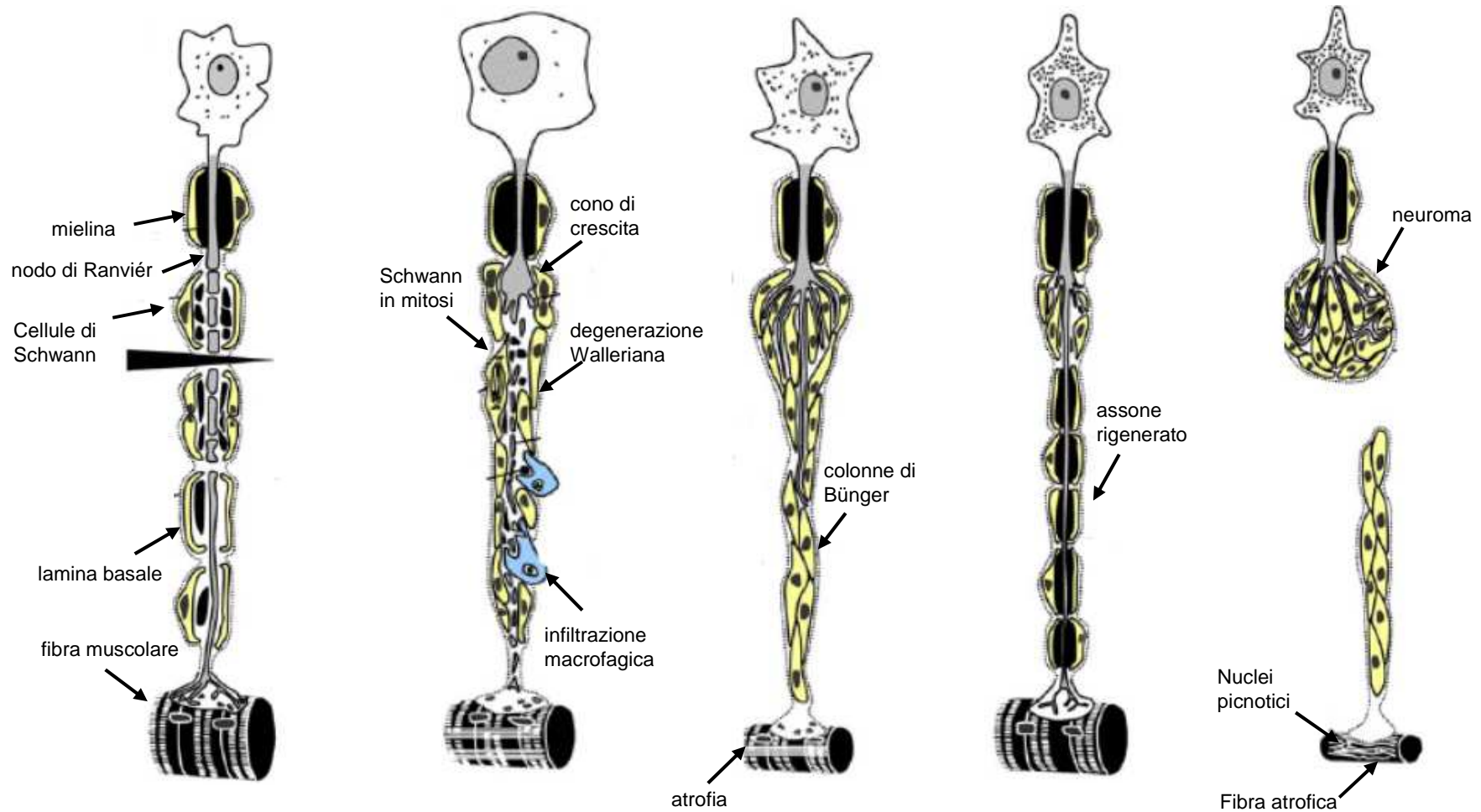




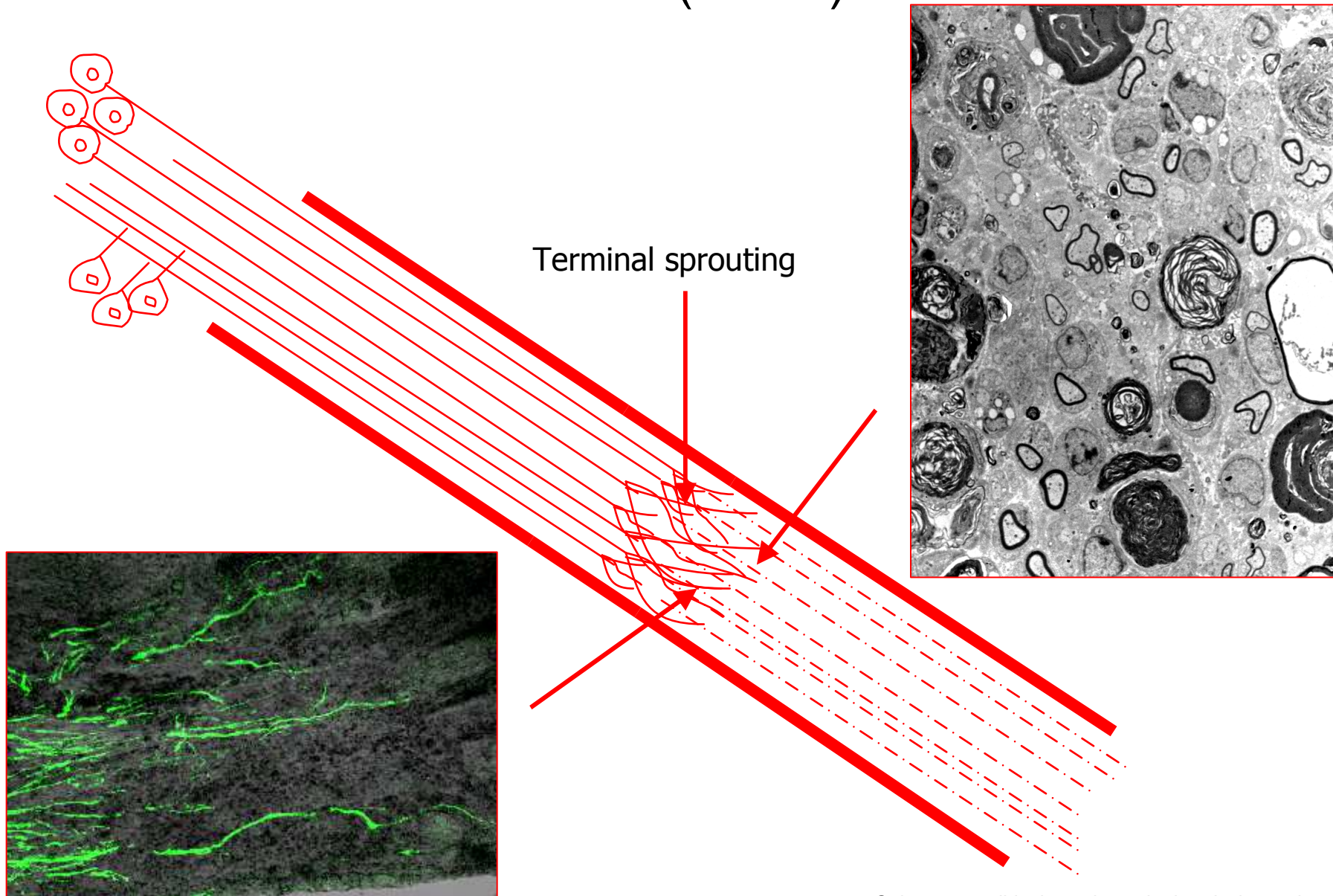
# Axonotmesis (crush)



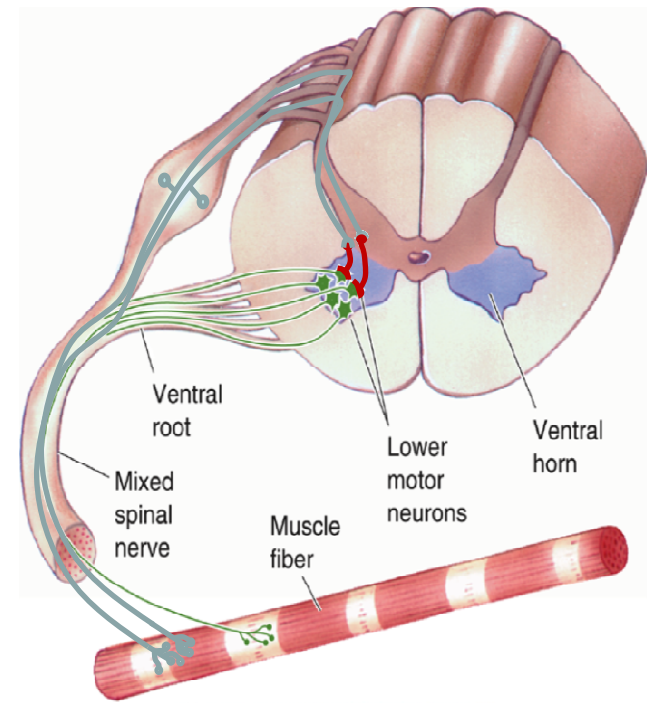
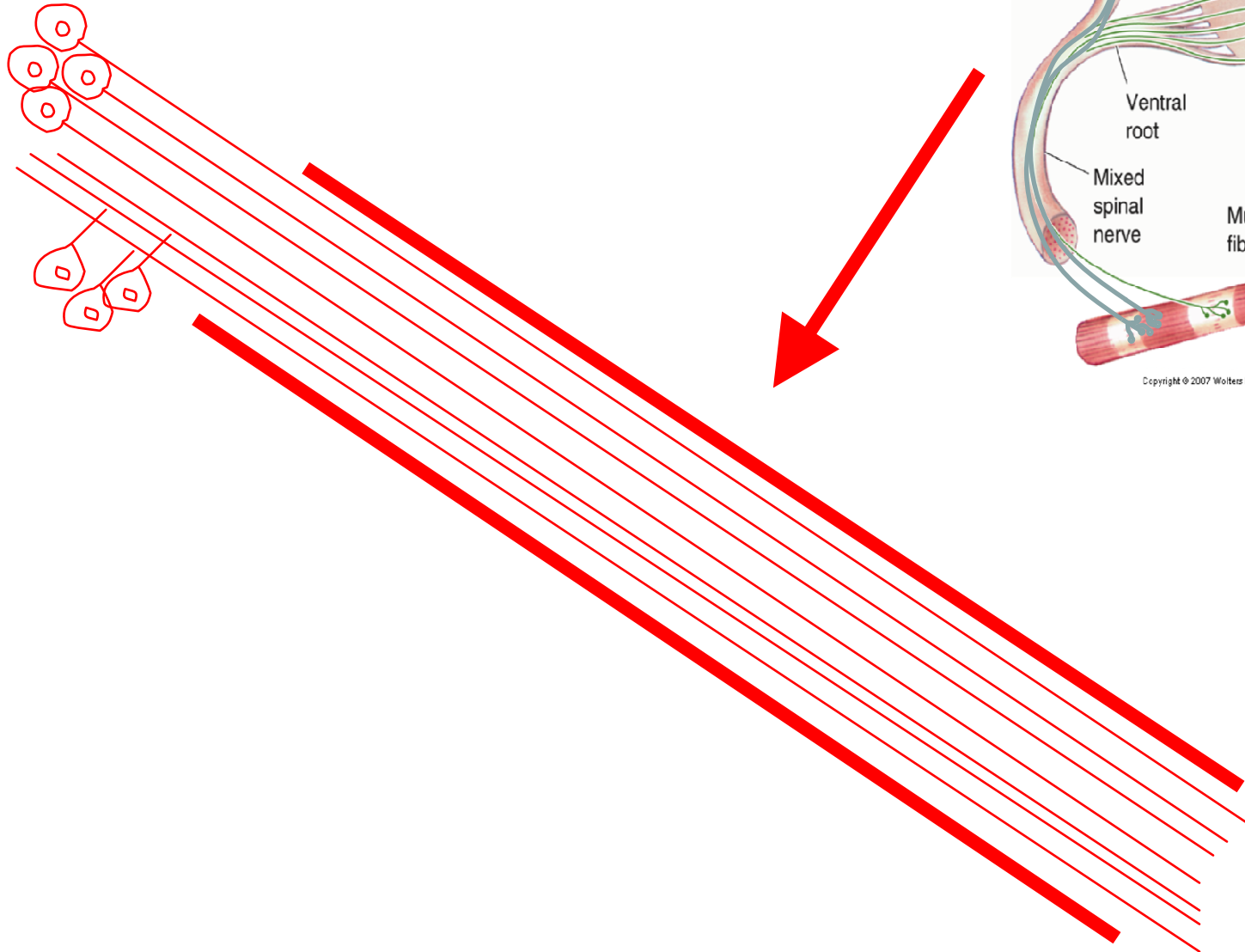
# Eventi degenerativi e rigenerativi associati alla lesione al nervo periferico



# Axonotmesis (crush)

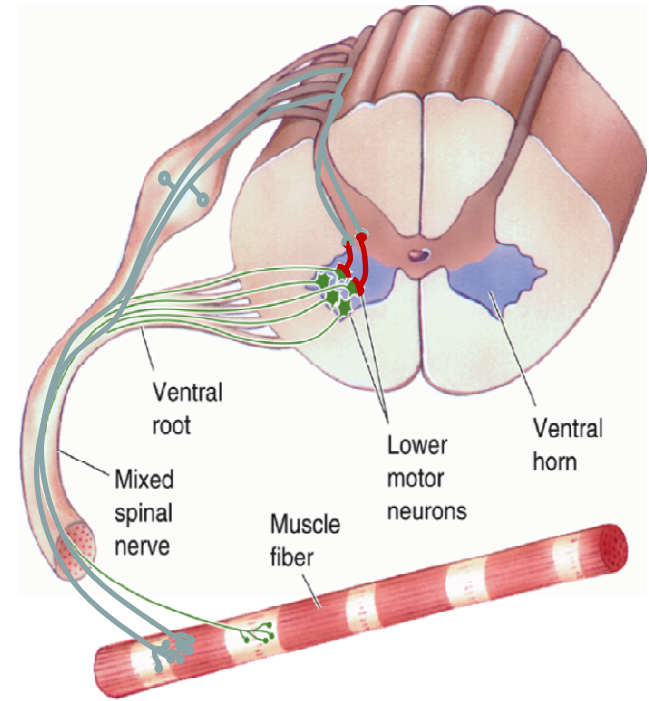
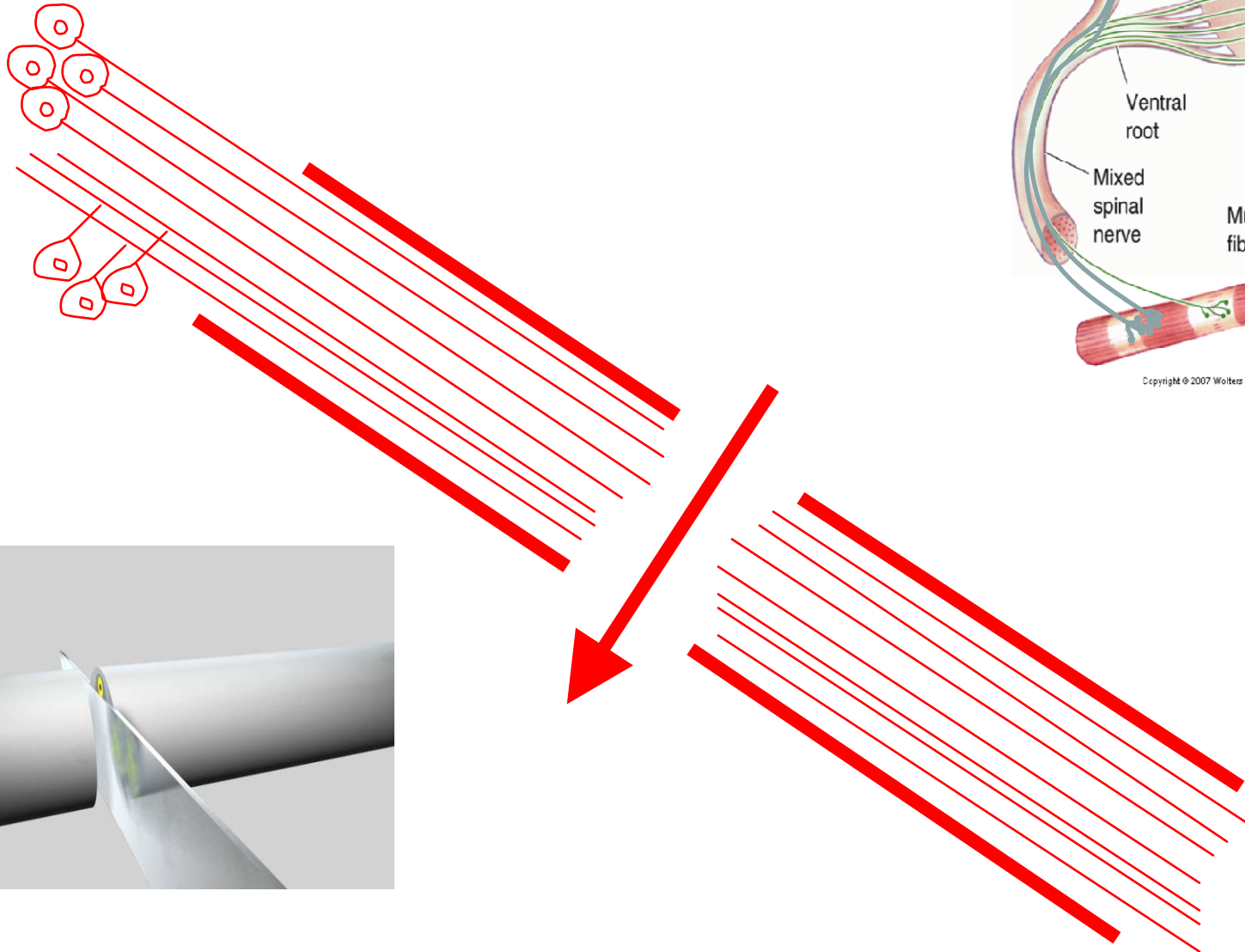


# Neurotmesis (SEVERE nerve lesion)

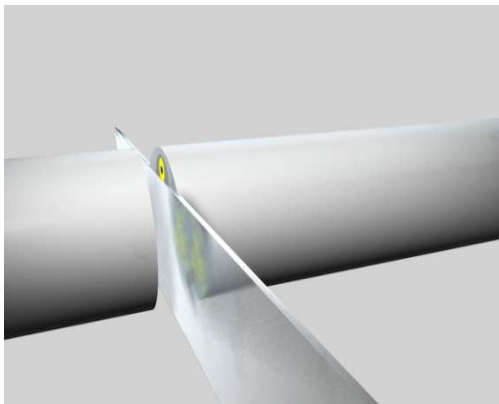


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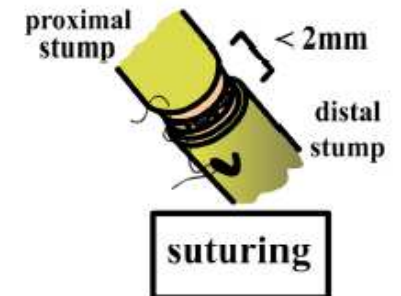
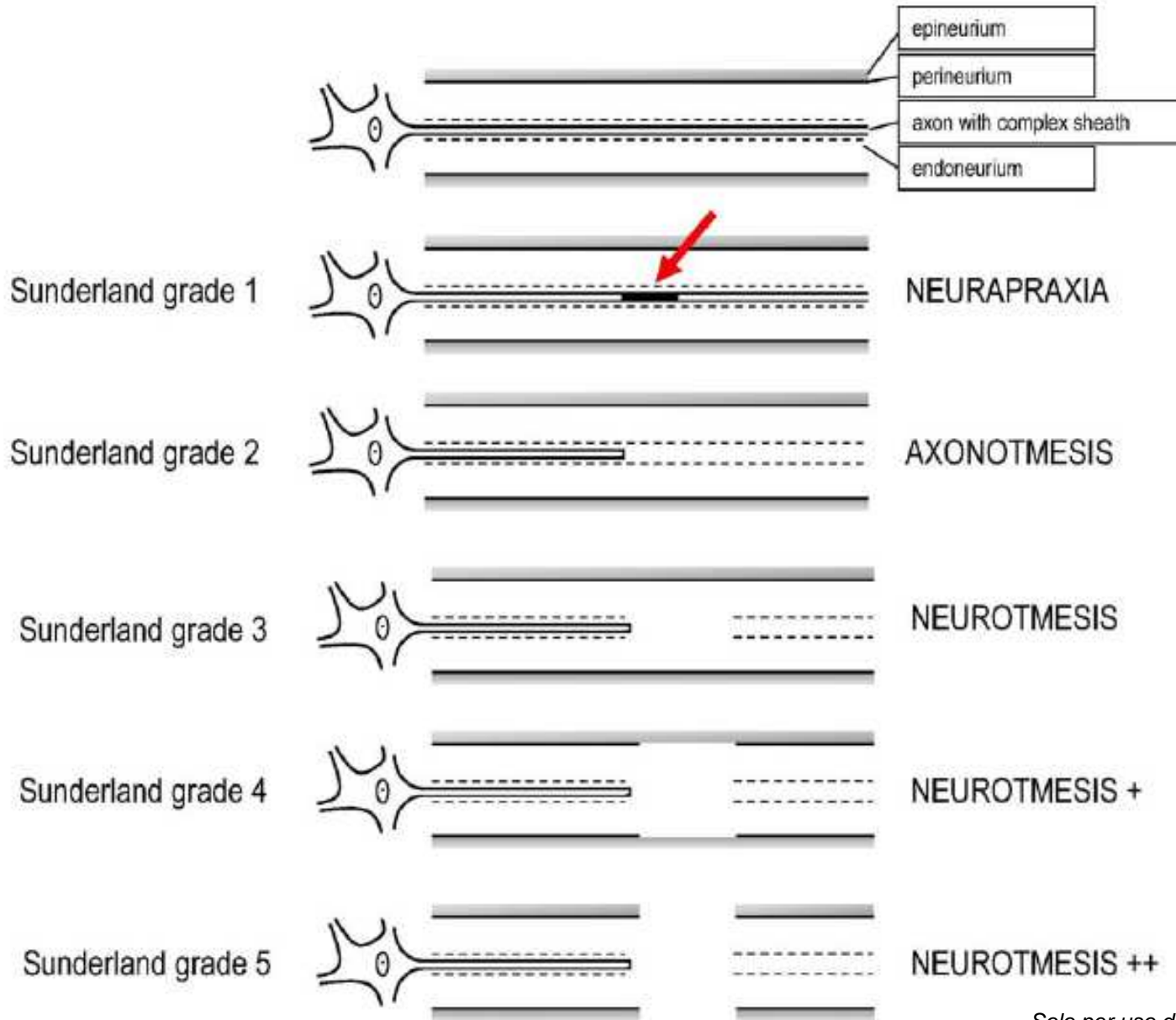
# Neurotmesis (SEVERE nerve lesion)



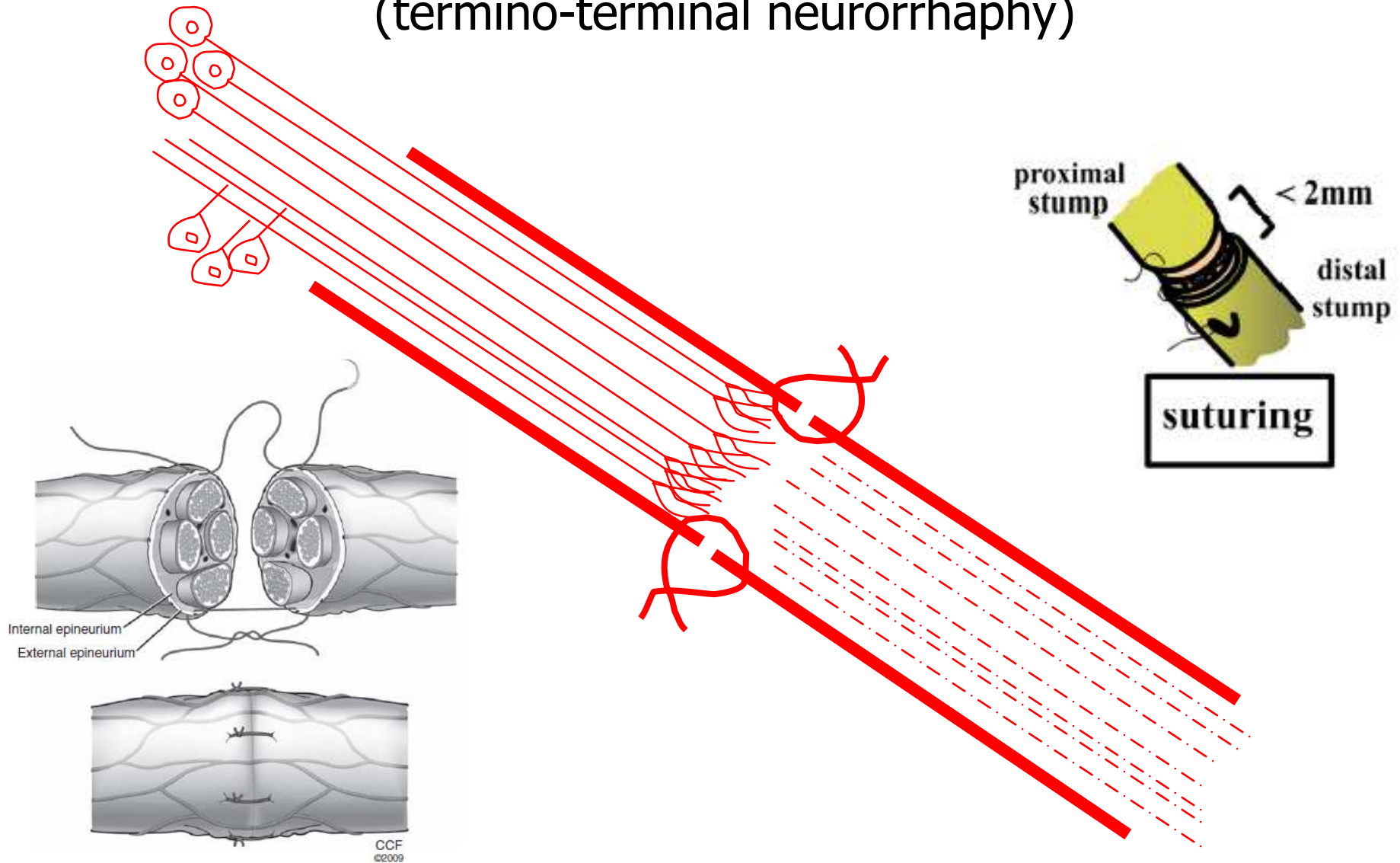
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# Tipologie di lesione



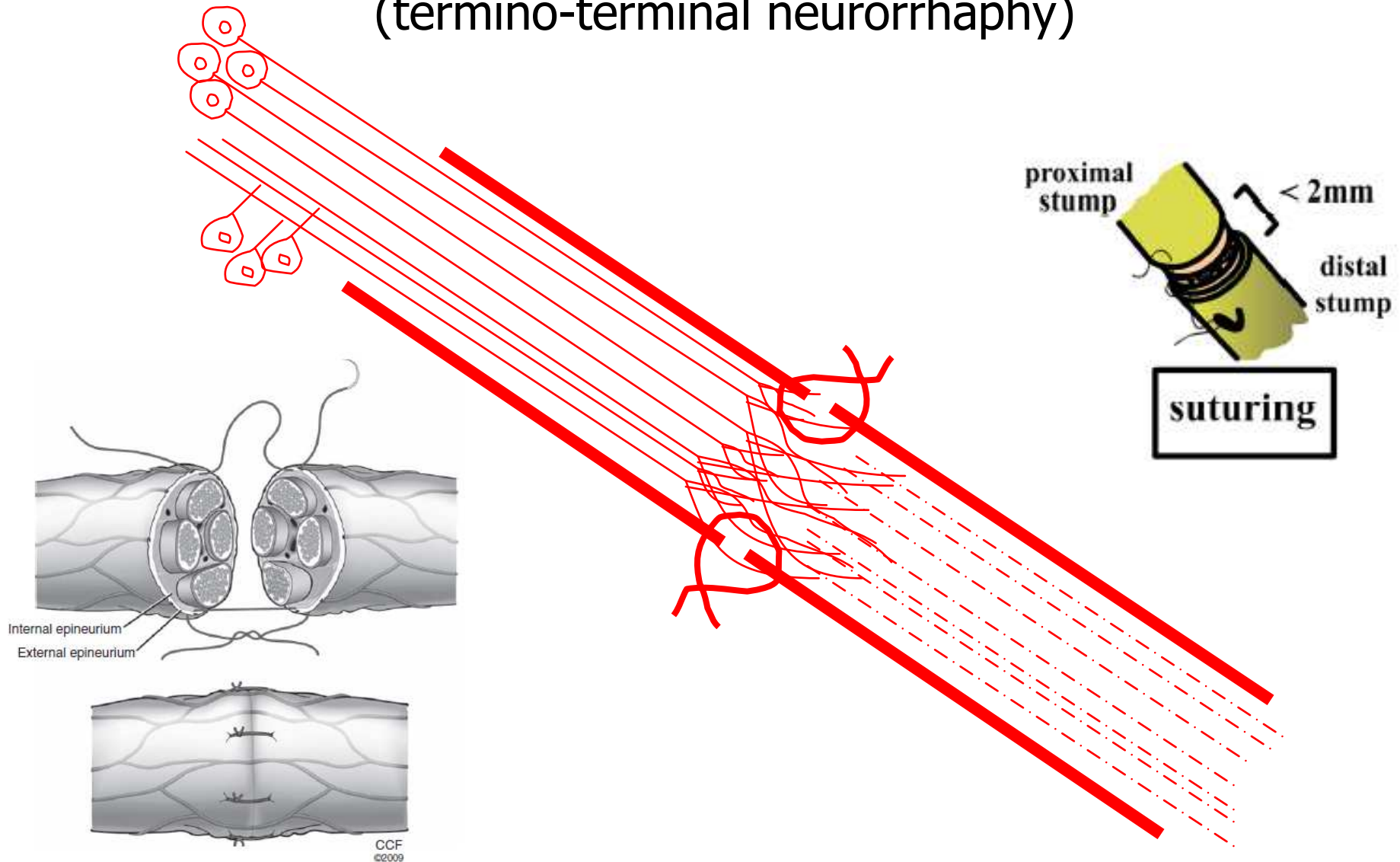
# Neurotmesis without substance loss Direct epineurial/perineurial suture (termino-terminal neurorrhaphy)



(Siemionow and Brzezicki 2009)

Solo per uso didattico - vietata la riproduzione o la vendita

# Neurotmesis without substance loss Direct epineurial/perineurial suture (termino-terminal neurorrhaphy)

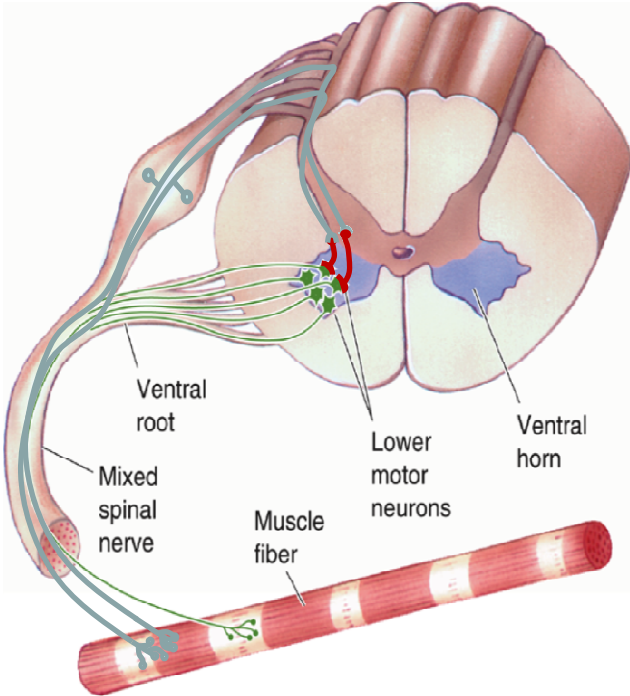
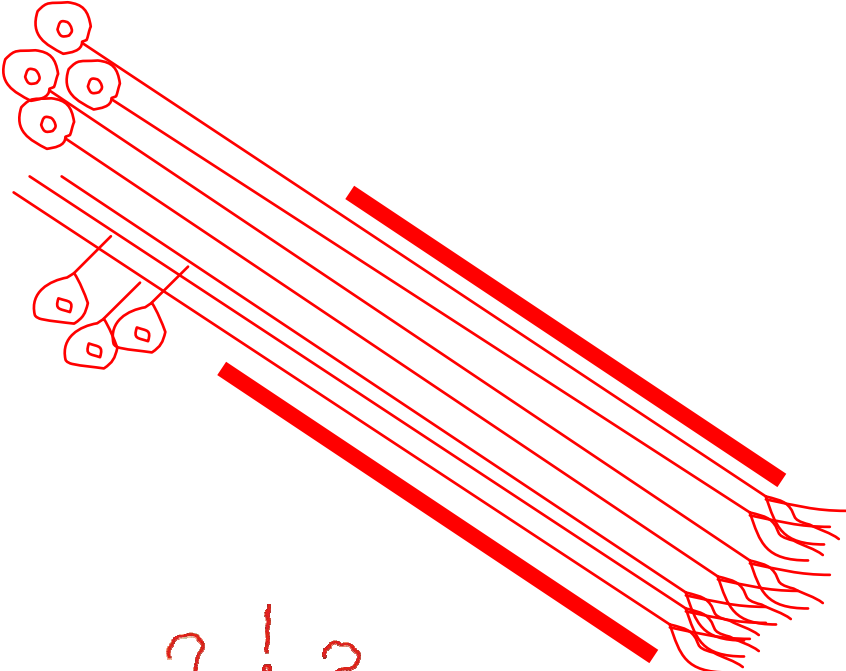


(Siemionow and Brzezicki 2009)

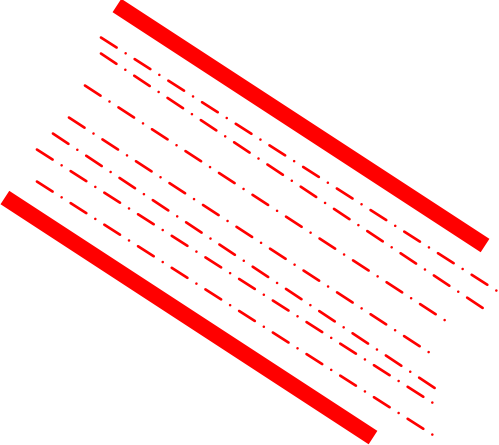
Solo per uso didattico - vietata la riproduzione o la vendita



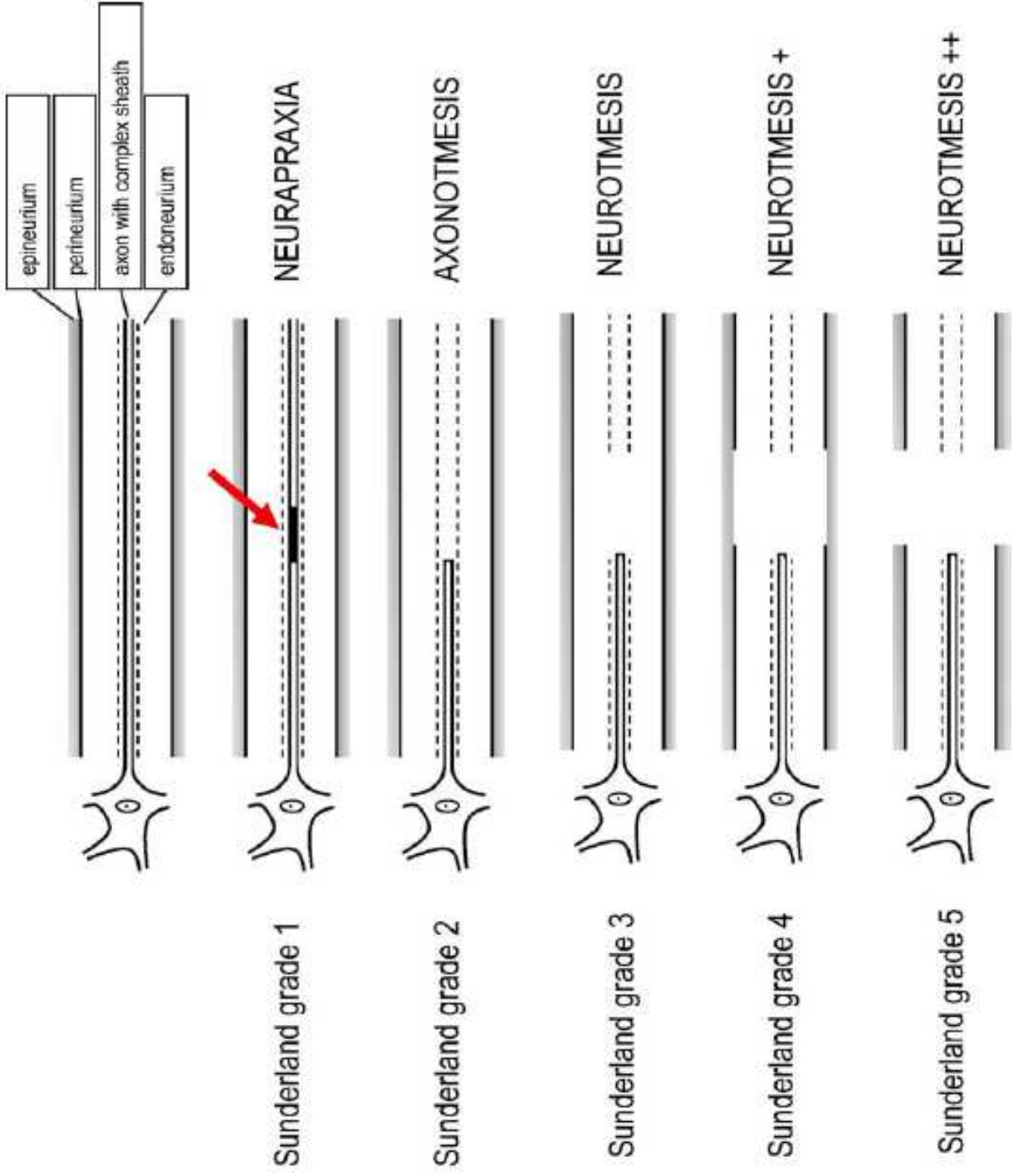
# Neurotmesis WITH substance loss



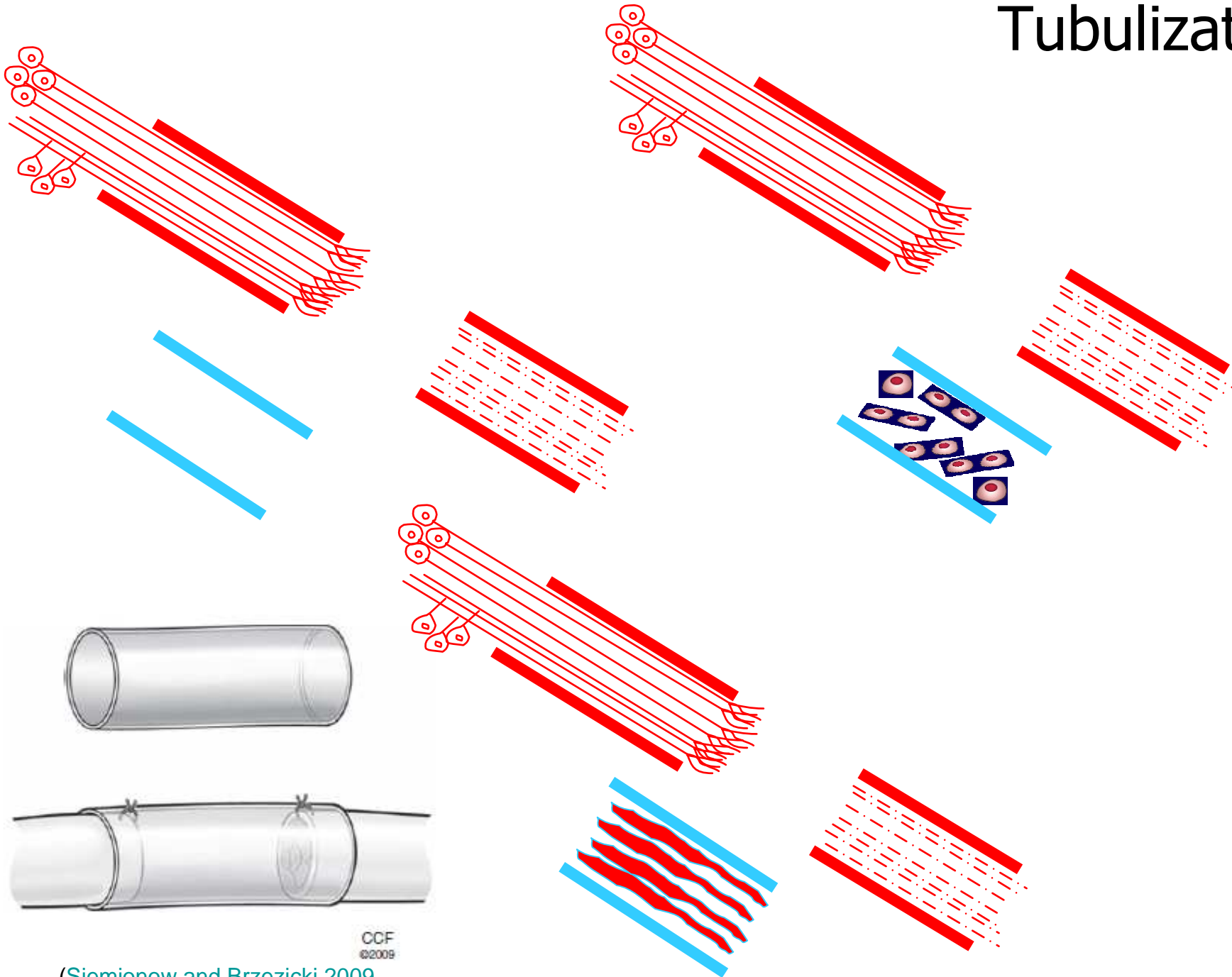
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# Tipologie di lesione



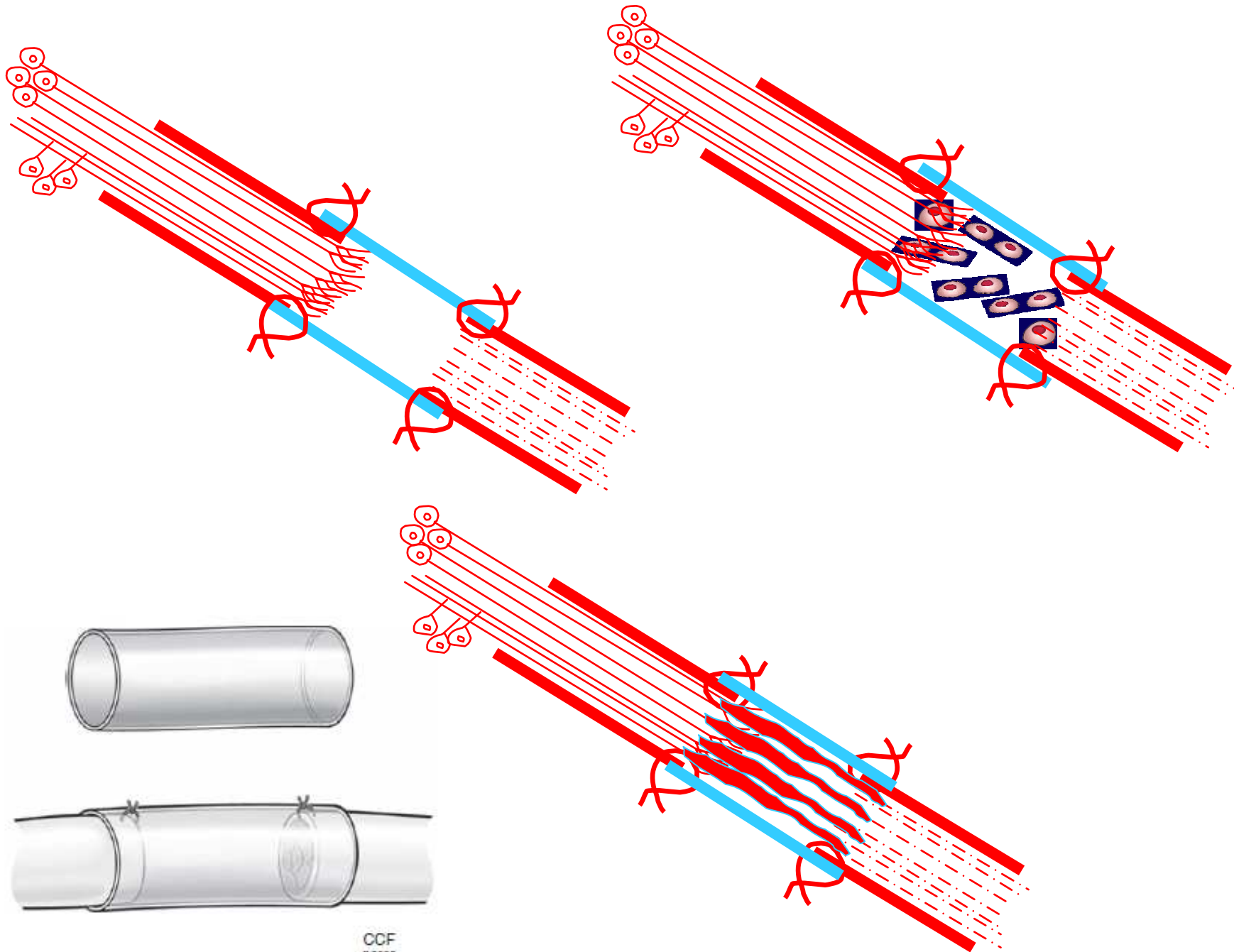
# Tubulization



(Siemionow and Brzezicki 2009)

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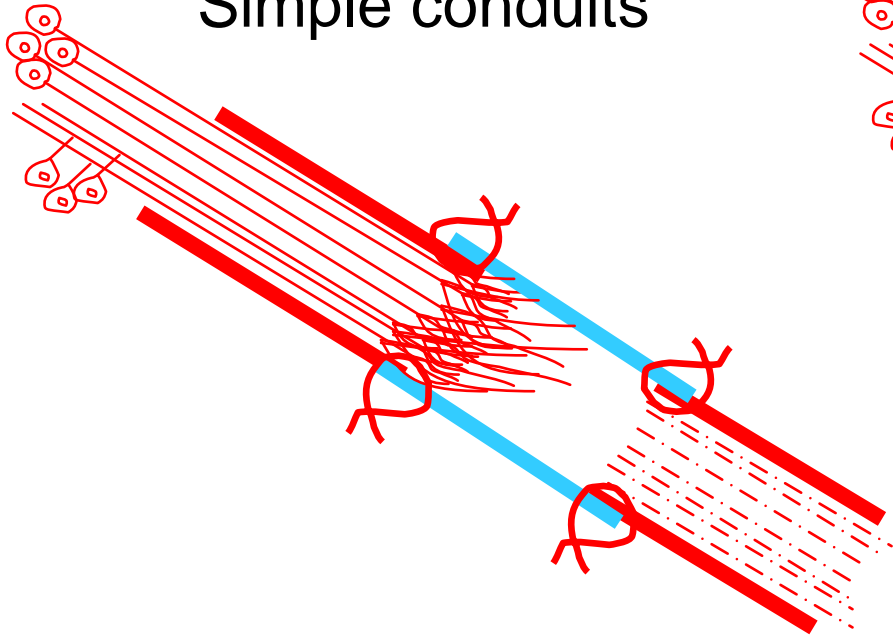


(Siemionow and Brzezicki 2009)

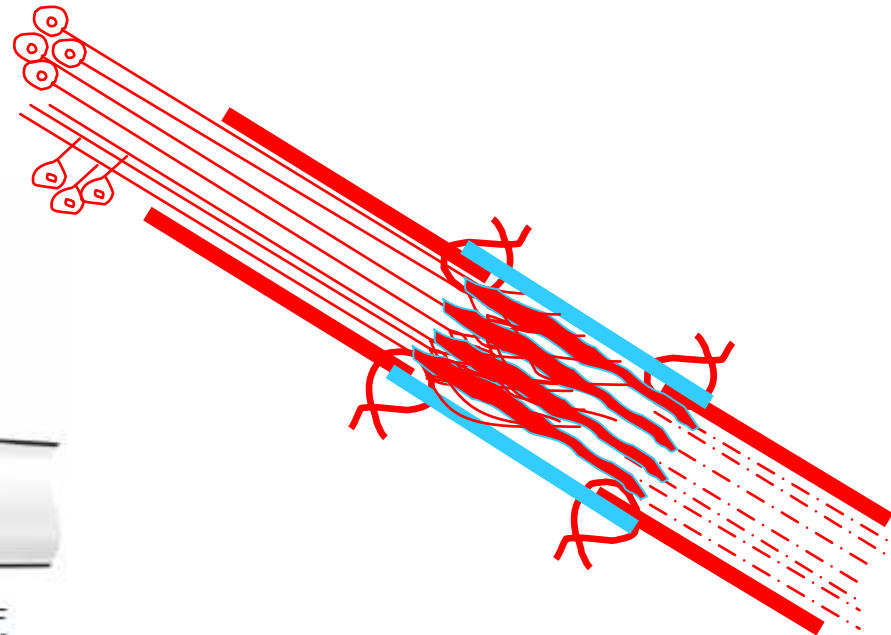
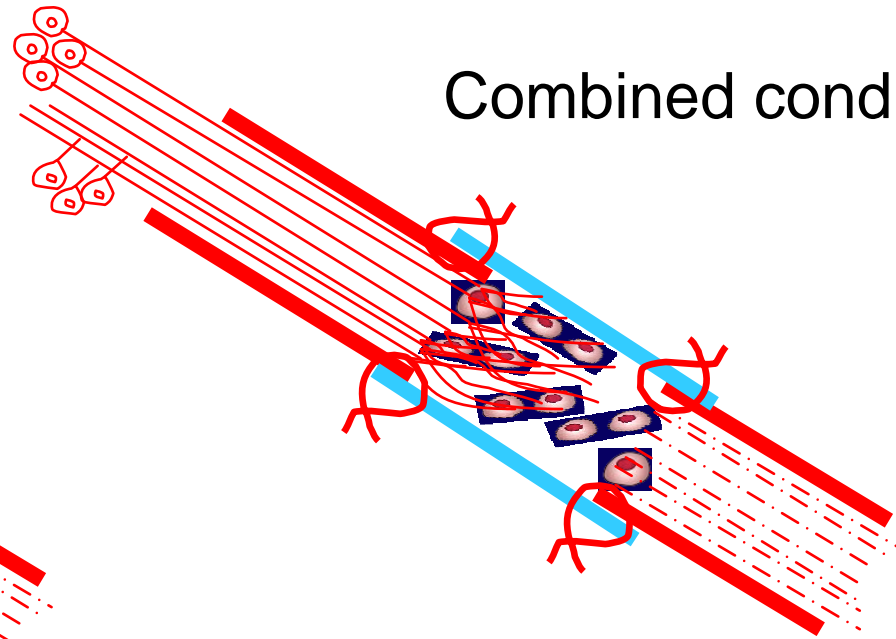
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## Simple conduits



## Combined conduits

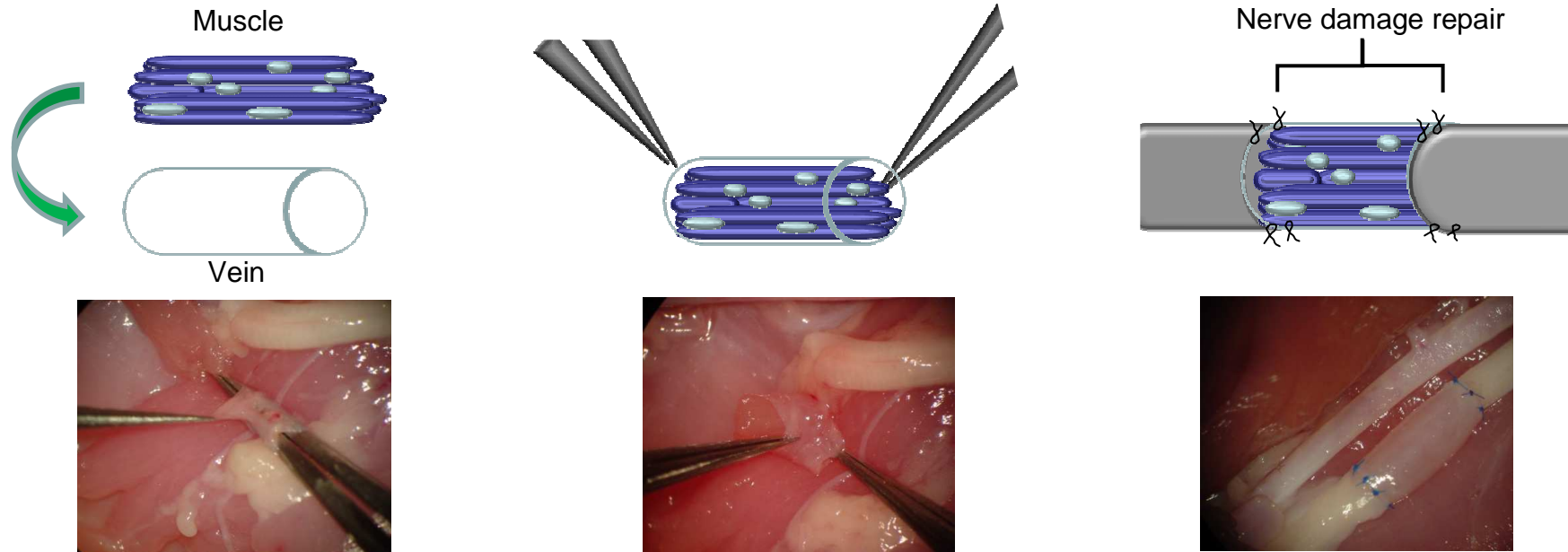


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([Siemionow and Brzezicki 2009](#))

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# The **M**uscle **I**n **V**ein combined technique

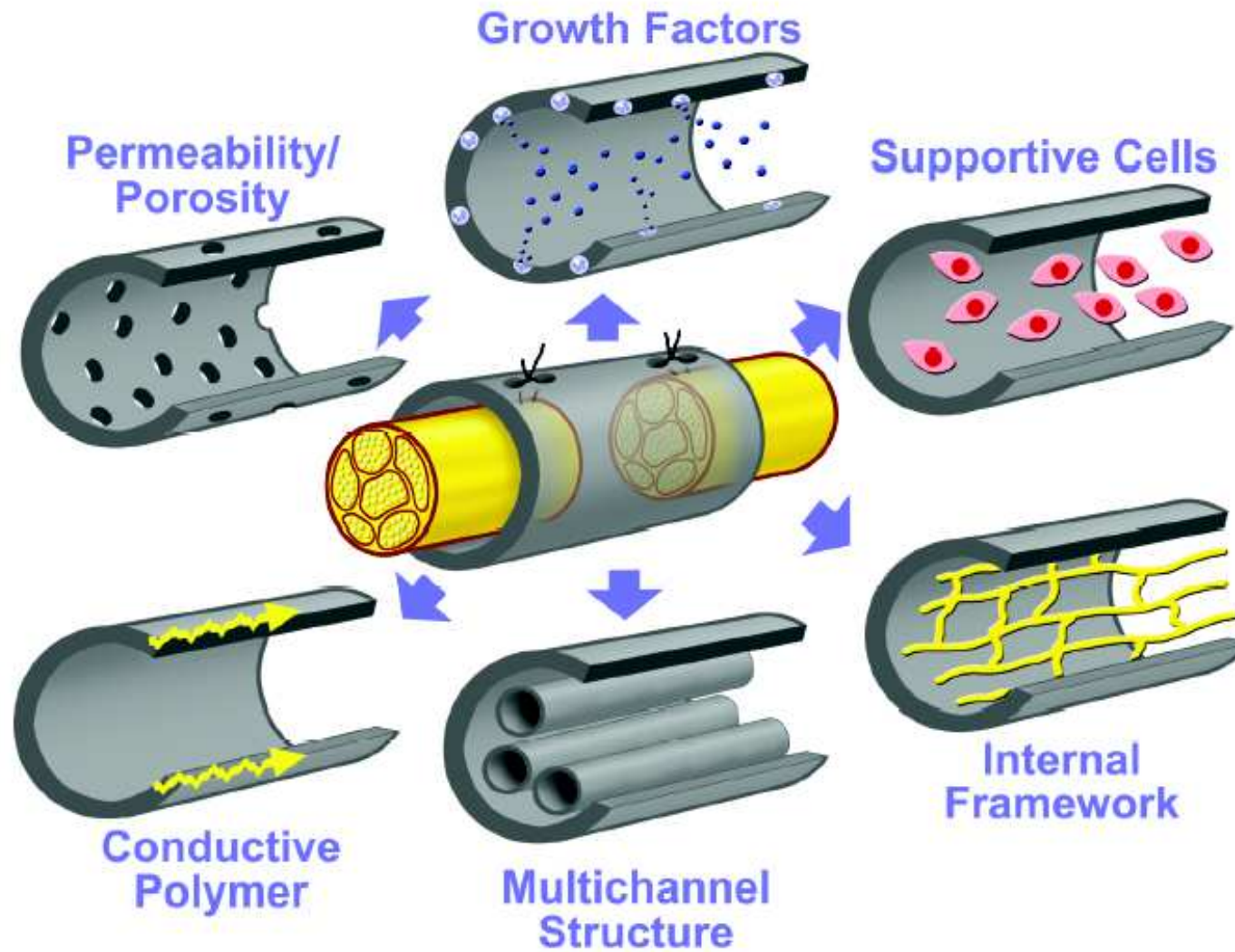


**MIV** avoids withdrawal of autologous nerve

The vein prevents axons dispersion

The muscle avoids vein collapse and guide axon regrowth and Schwann cells migration

This technique was applied in repair of 40 cases of sensory and mixed nerve defects (0.5-6 cm) with good results achieved in 85% of patients” (Battiston et al.,2000).



## Modelli animali

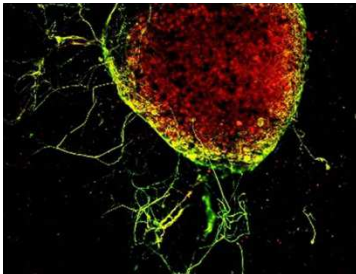
RATTO



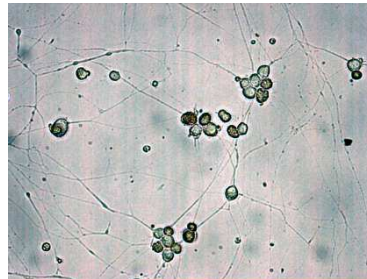
TOPO



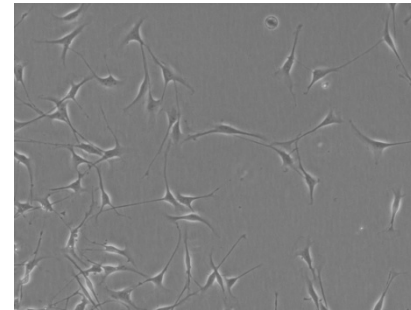
## Modelli *in vitro*



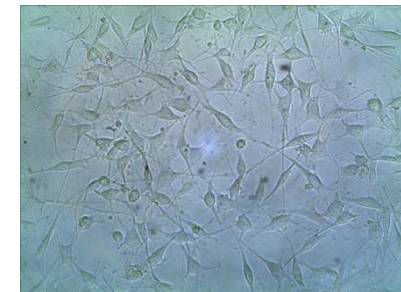
ESPIANTI DI DRG



COLTURE DI  
NEURONI SENSITIVI  
DISSOCIATI DA DRG



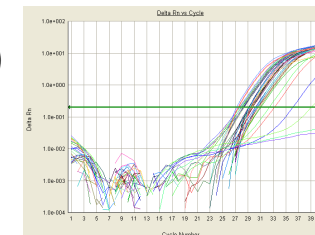
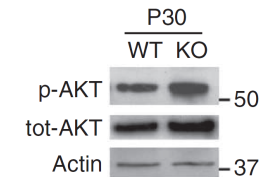
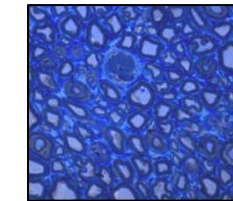
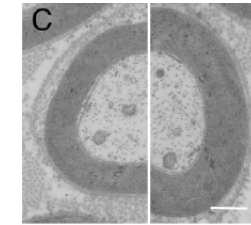
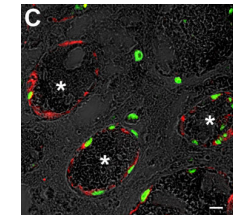
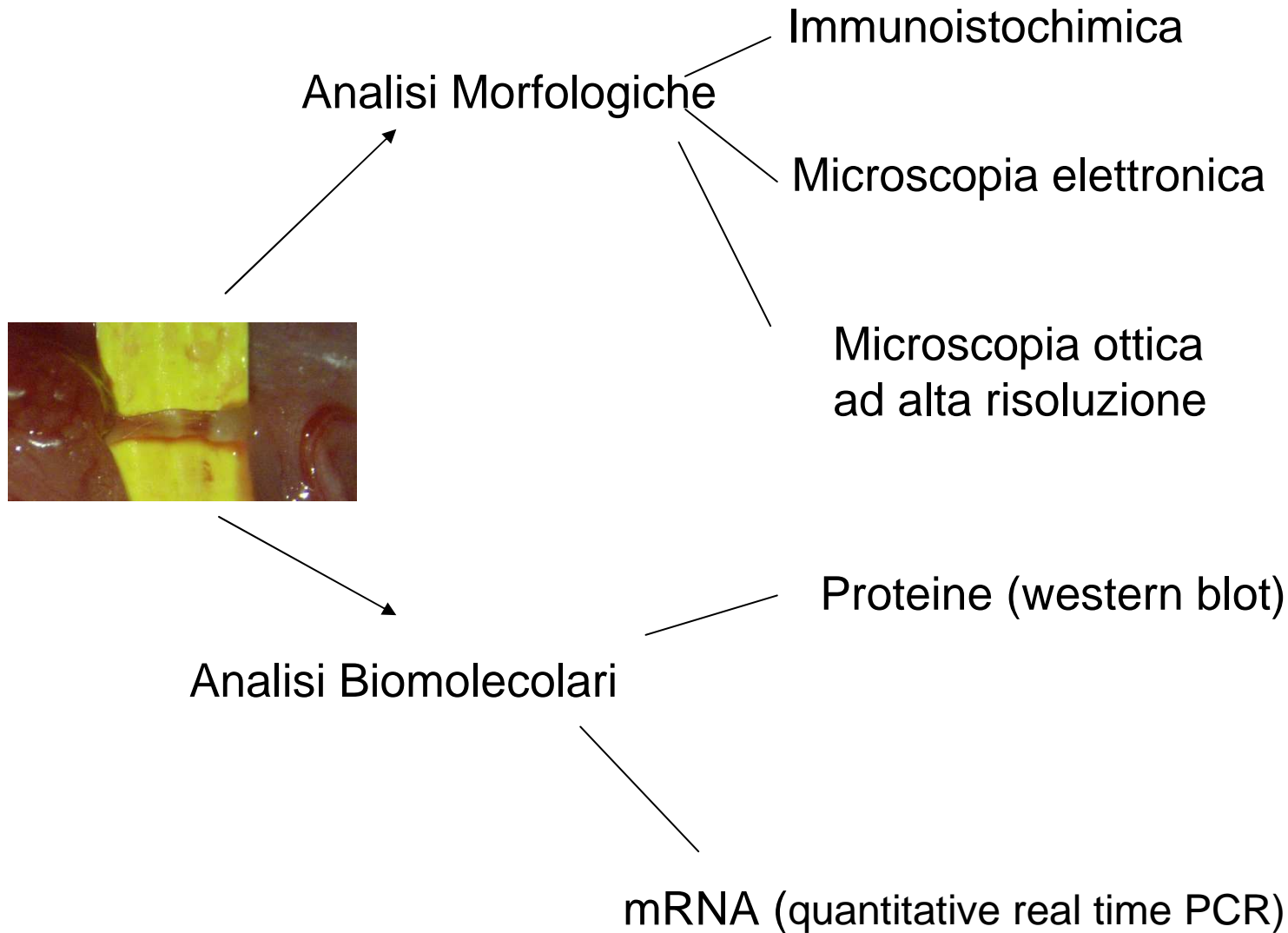
COLTURE PRIMARIE  
DI CELLULE DI  
SCHWANN



LINEE CELLULARI  
IMMORTALIZZATE DI  
CELLULE di  
SCHWANN o di  
NEURONI SENSITIVI O  
MOTORI



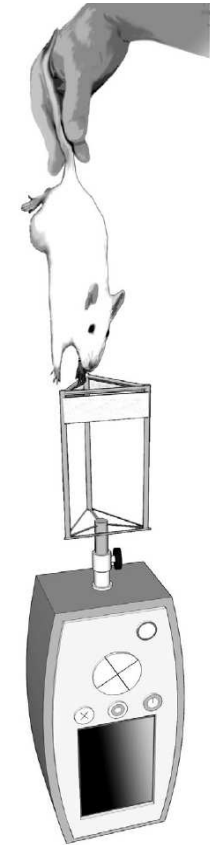
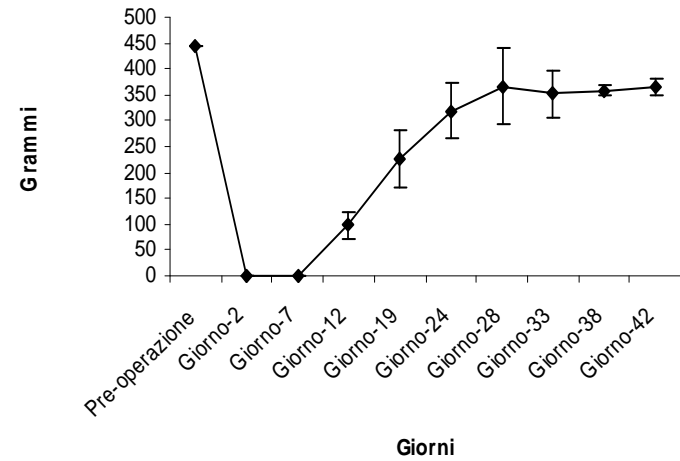
# Tecniche di analisi utilizzate



# Tecniche di analisi utilizzate

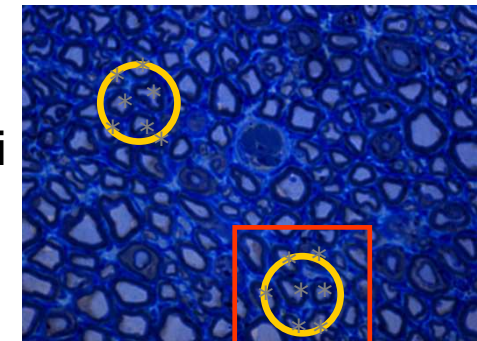
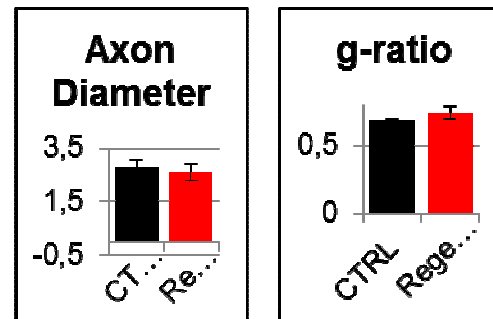
Analisi Funzionali

valutazioni del recupero funzionale del nervo mediano mediante Grasping Test

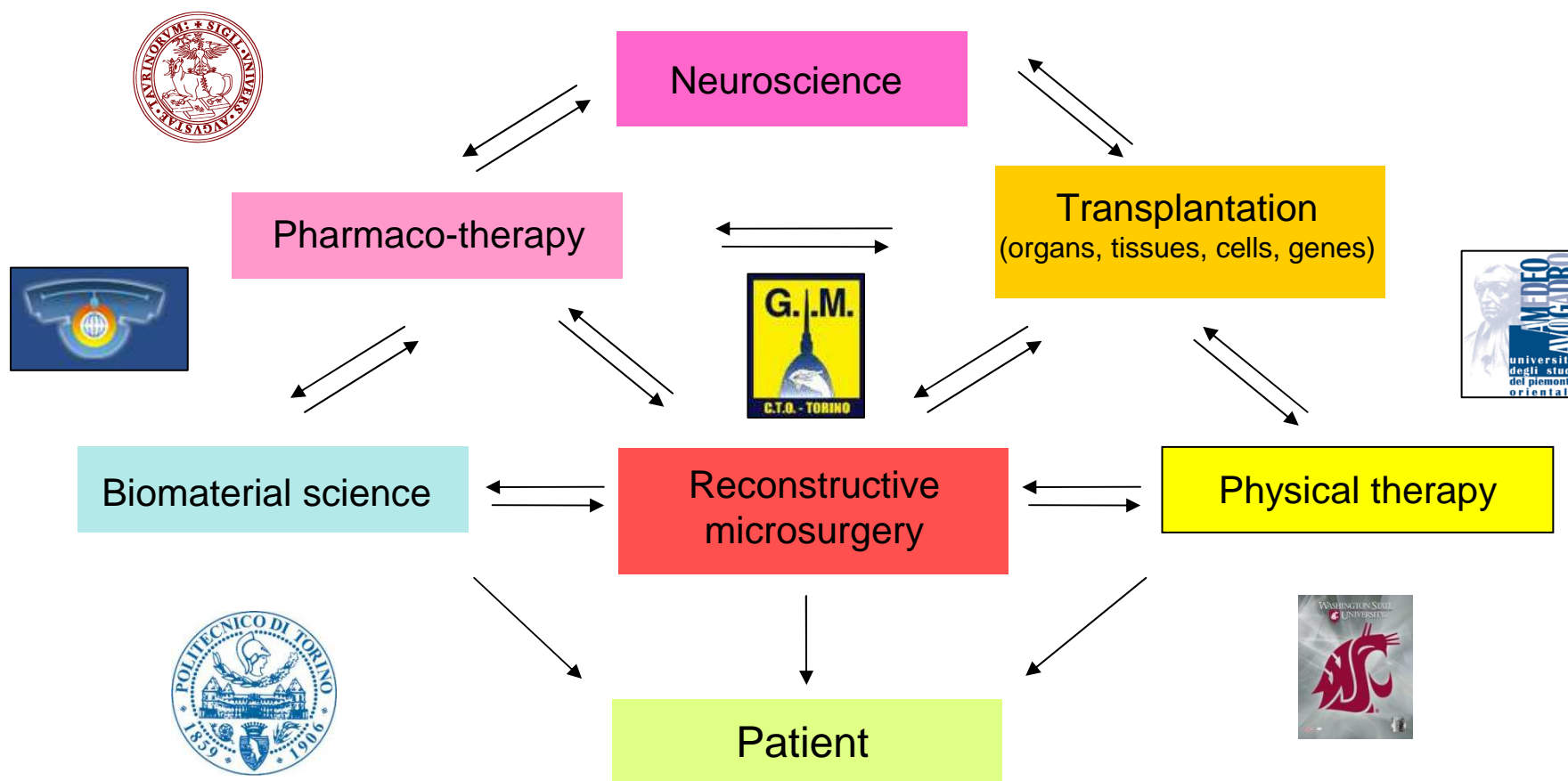


Analisi Morfometriche

valutazione del numero e delle dimensioni delle fibre rigeneranti



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



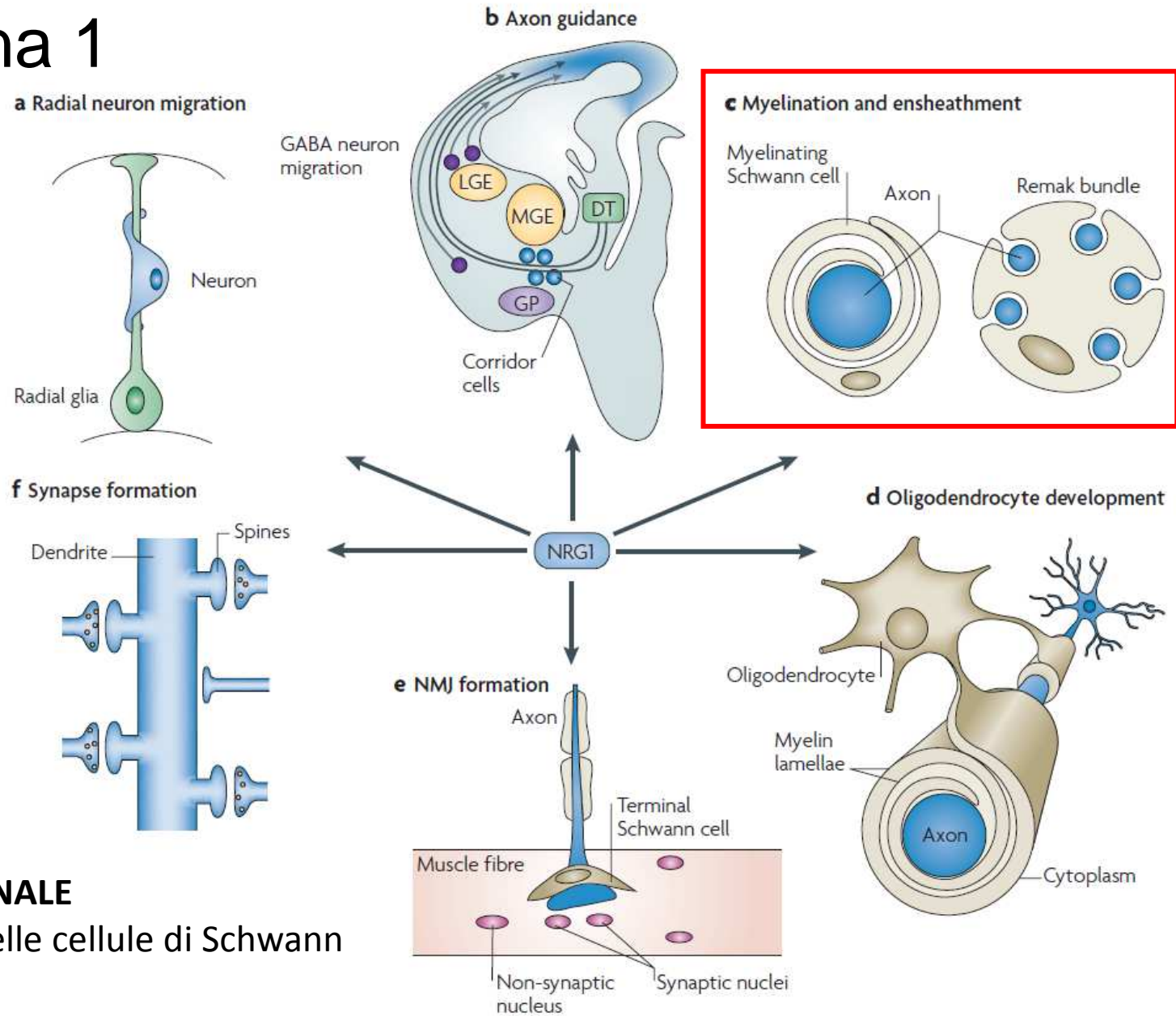
Quali proteine sono espresse durante la rigenerazione del nervo periferico?

Quali geni sono regolati durante la rigenerazione del nervo periferico?

Quali fattori possono favorire la rigenerazione nervosa?

Quali biomateriali possono essere utilizzati per favorire la rigenerazione nervosa?

# Neuregulina 1



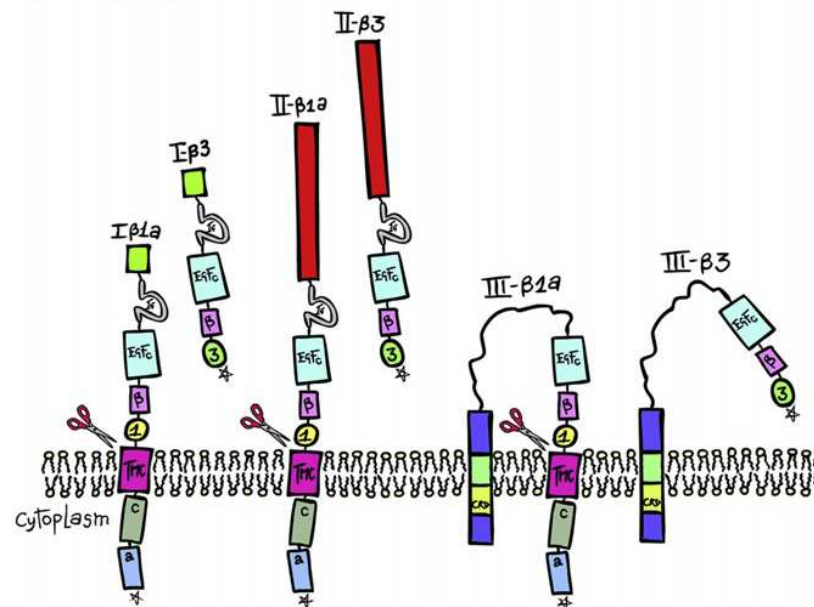
## NELLA VITA EMBRIONALE

- Differenziamento delle cellule di Schwann
- Mielinizzazione

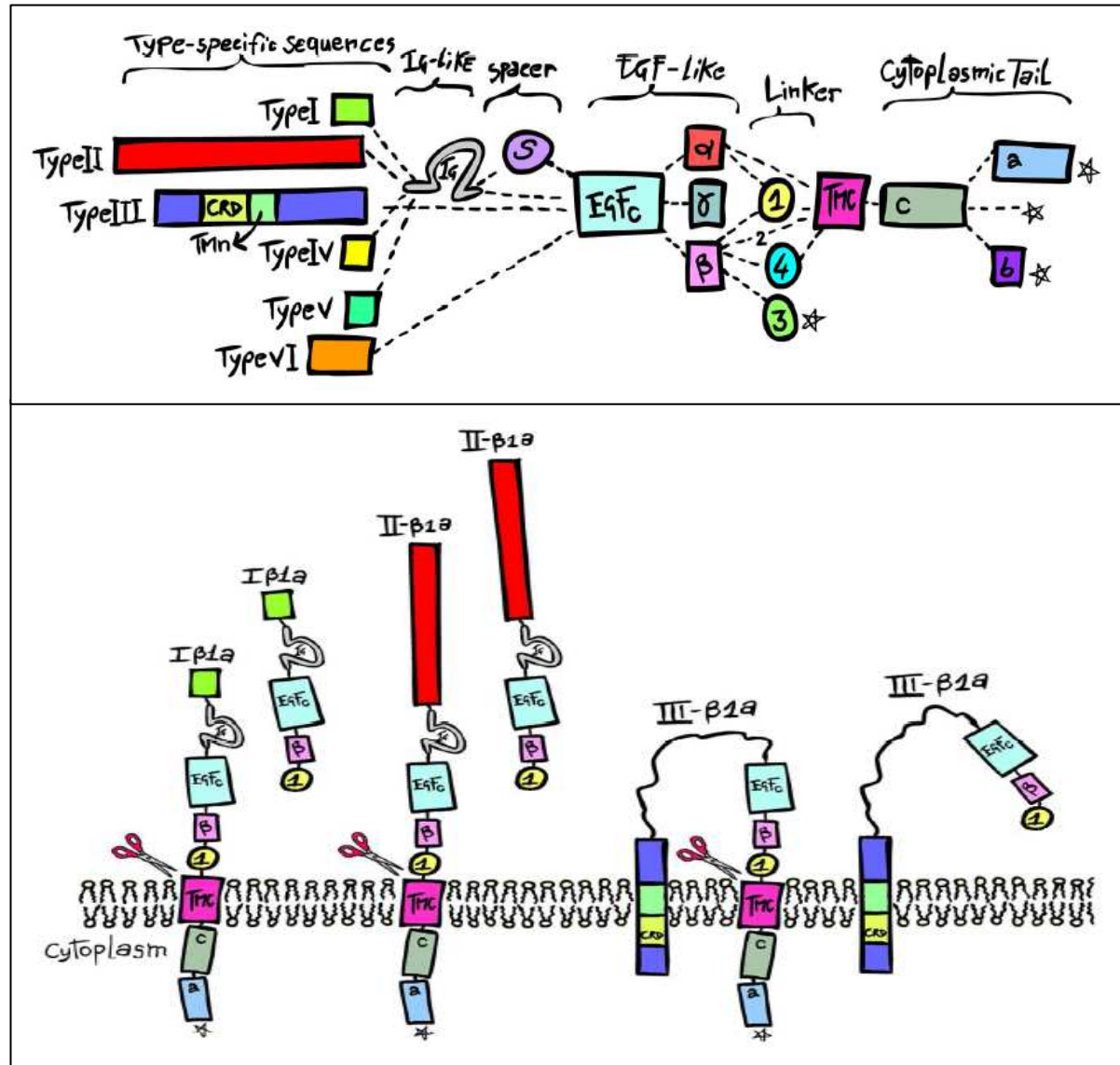
## NELLA VITA ADULTA

- Azione nelle diverse fasi del processo rigenerativo

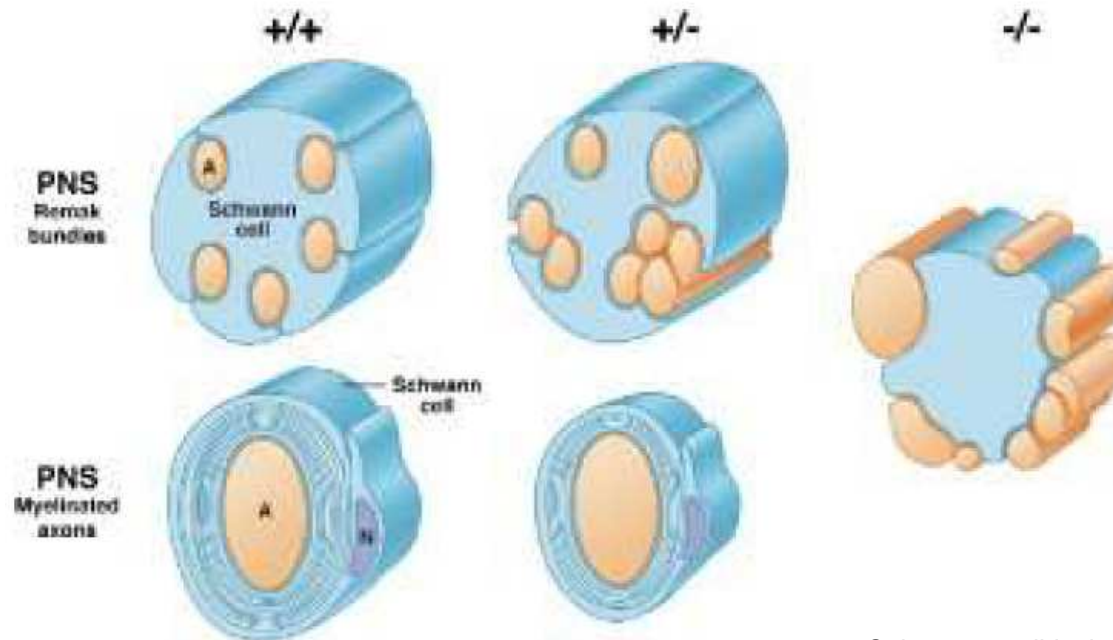
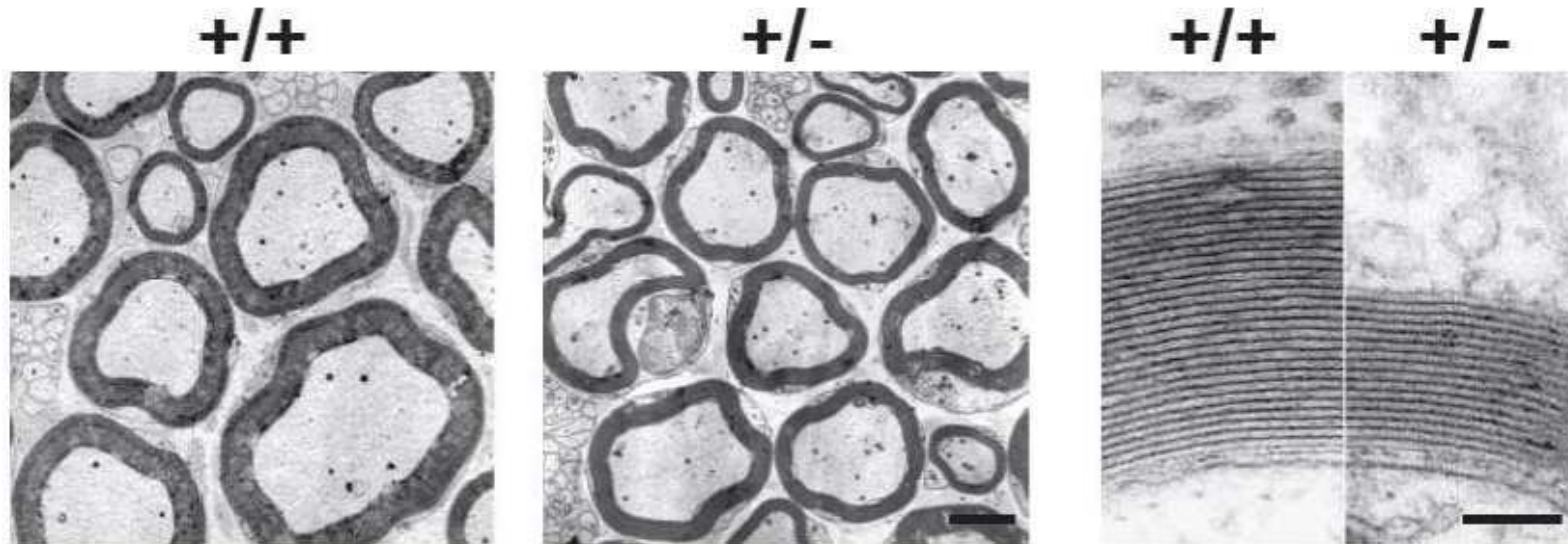
# Regulation of NRG1/ErbB system in peripheral nerve repair and regeneration



# Struttura della NRG1



# Ruolo della NRG1 nella mielinizzazione





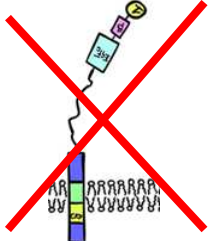
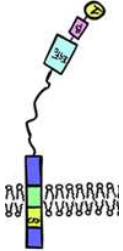
Neuregulins & remyelination

## TRANSGENIC MICE MODELS



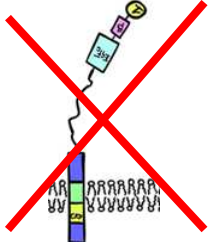
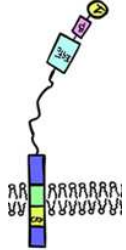
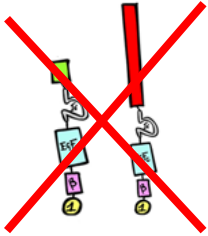
# Neuregulins & remyelination

## TRANSGENIC MICE MODELS

		myelination	remyelination
type III		-	-
type III		+	+

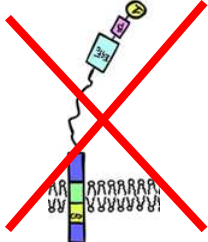
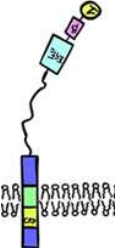
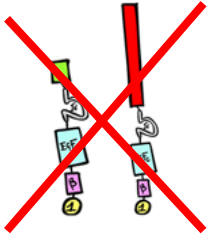

# Neuregulins & remyelination

## TRANSGENIC MICE MODELS

		myelination	remyelination
type III		-	-
type III		+	+
type I-II		/	-

# Neuregulins & remyelination

## TRANSGENIC MICE MODELS

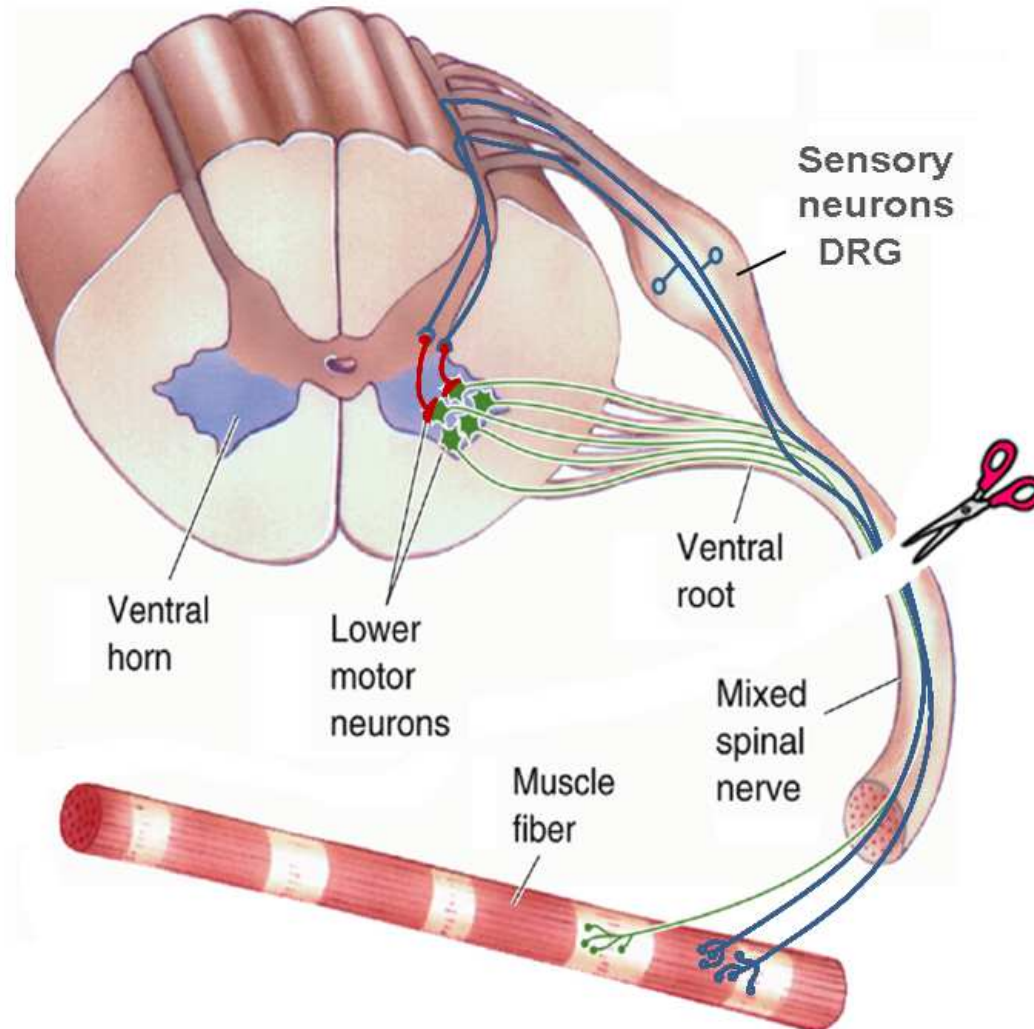
		myelination	remyelination
type III		-	-
type III		+	+
type I-II		/	-
type I-II		/	+

## MYELINATION ≠ REMYELINATION

- remyelination is not a simple recapitulation of nerve development
- both **NRG1 type III** expressed by neurons and **NRG1 type I-II** released by Schwann cells **contribute to the remyelination** after peripheral nerve injury

# Neuregulins & remyelination

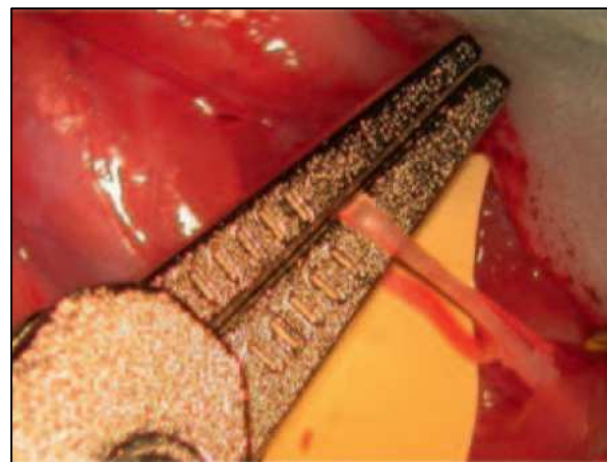
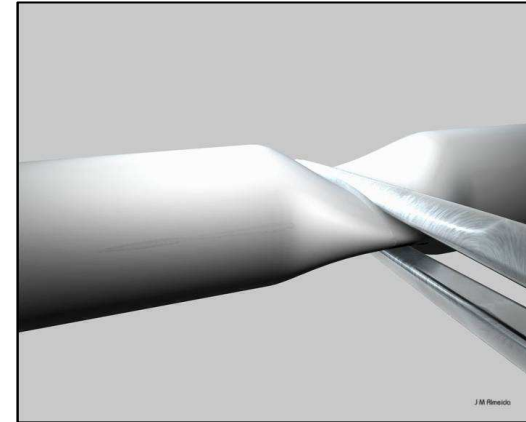
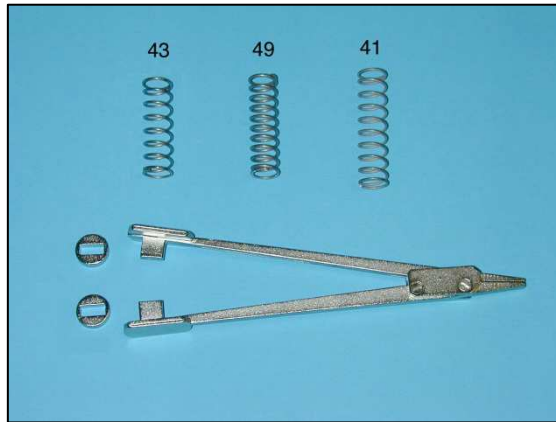
## NRG1-ErbB EXPRESSION ANALYSIS after NERVE INJURY

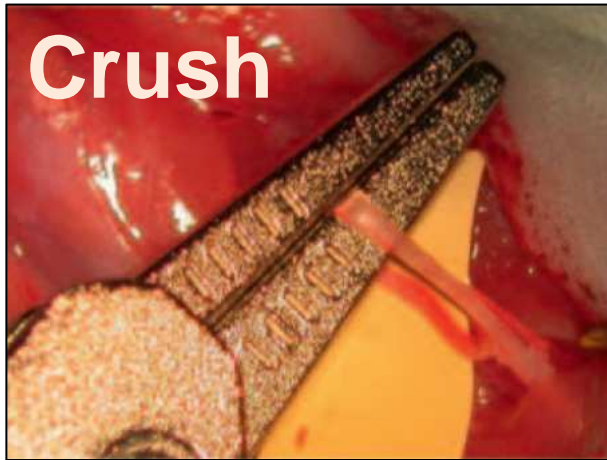


- Confronto tra due modelli sperimentali di rigenerazione nervosa
  - Assonotmesi (*crush*)
  - Neurotmesi ++ seguita da ricostruzione microchirurgica termino-terminale (T-T)
  - + Neurotmesi non seguita da ricostruzione microchirurgica (nervo degenerante)
- Analisi dell'espressione di geni coinvolti nella rigenerazione nervosa periferica (Neuregulina1 ed ErbB)
- Ricerca di nuovi geni housekeeping (HKG)

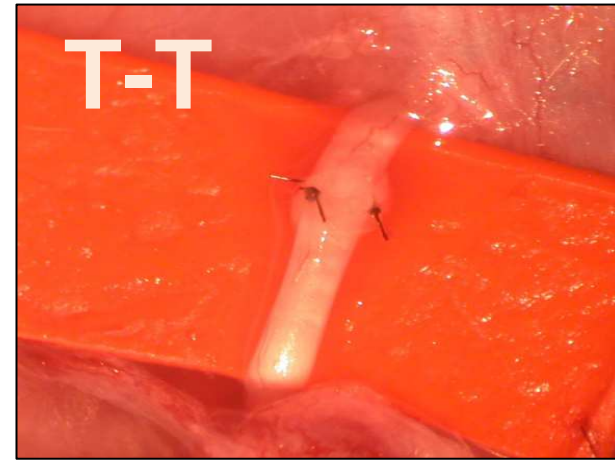


# *In vivo* model crush injury

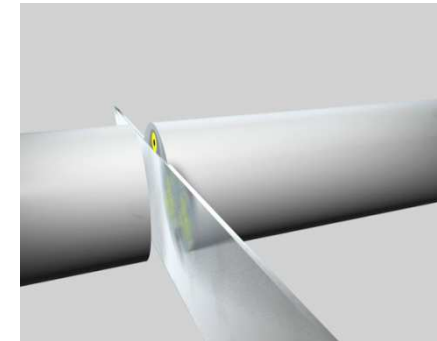
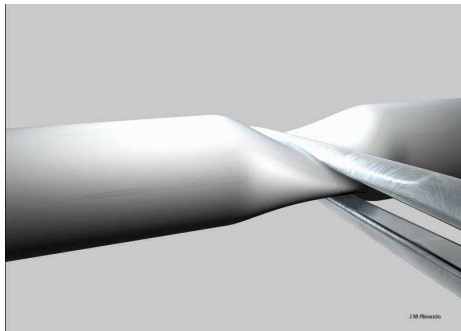




1, 3, 7, 14 giorni,  
4 settimane



1, 3, 7, 14 giorni,  
4, 8, 12 settimane



Estrazione RNA

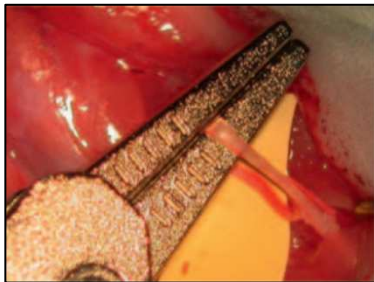
Retrotrascrizione

Real-Time PCR  
quantitativa

# Neuregulins & remyelination

## MBP mRNA EXPRESSION MIRRORS MYELINATION LEVEL

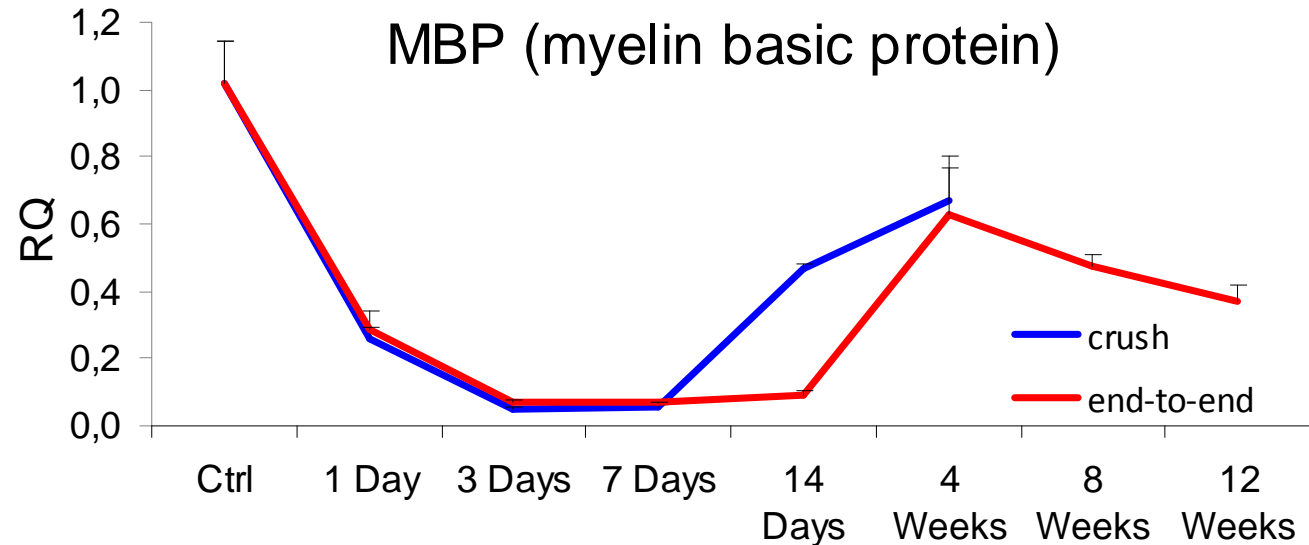
rat median nerve



nerve **crush** lesion  
(*axonotmesis*)



nerve complete cut lesion  
followed by **end-to-end** repair  
(*neurotmesis*)



1-nerve degeneration

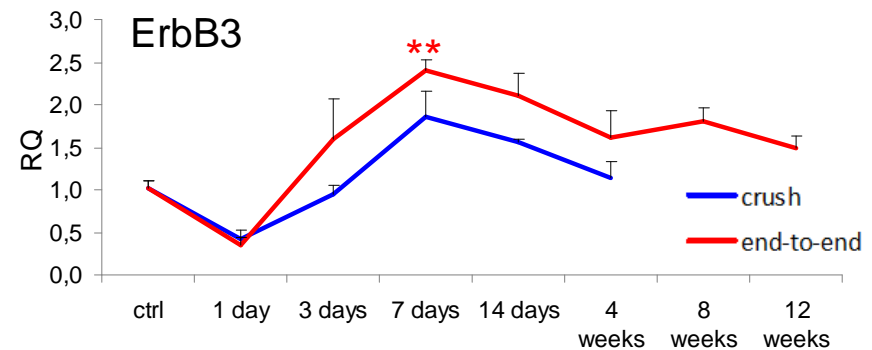
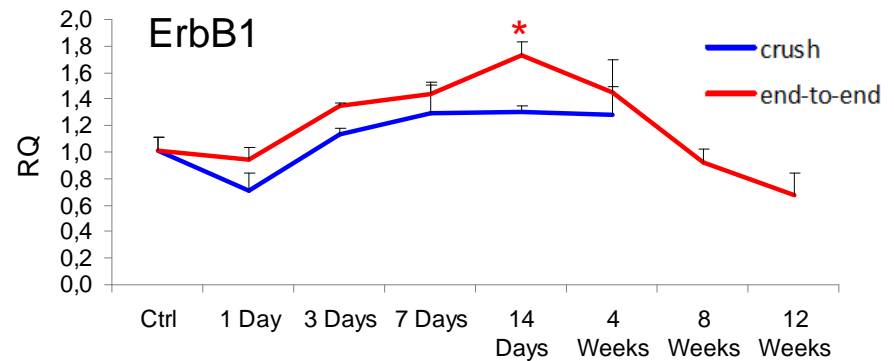
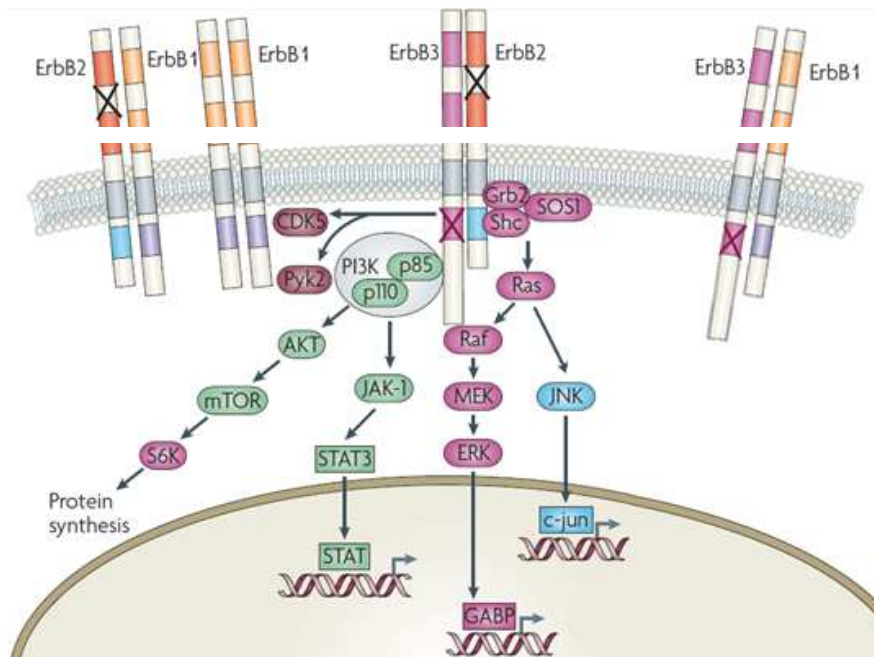
2-axon regeneration

3a-remyelination in the crush model

3b-remyelination in the  
end-to-end model

# Neuregulins & remyelination

## ErbB EXPRESSION IN THE INJURED NERVE

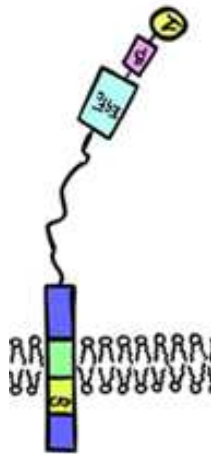


# Neuregulins & remyelination

## NRG1 ISOFORMS ANALYSED BY qRT-PCR



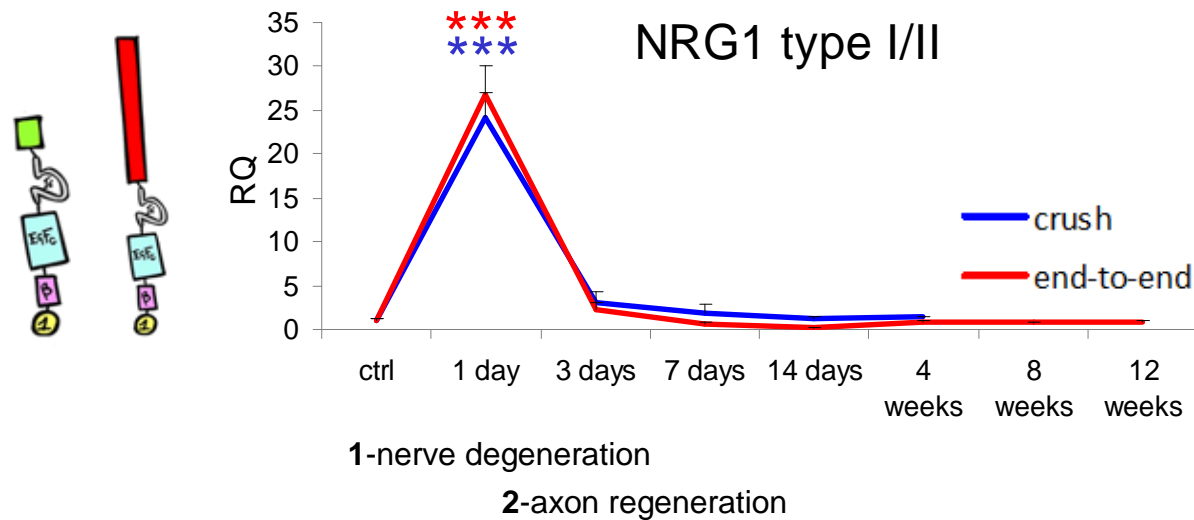
soluble NRG1 type I-II



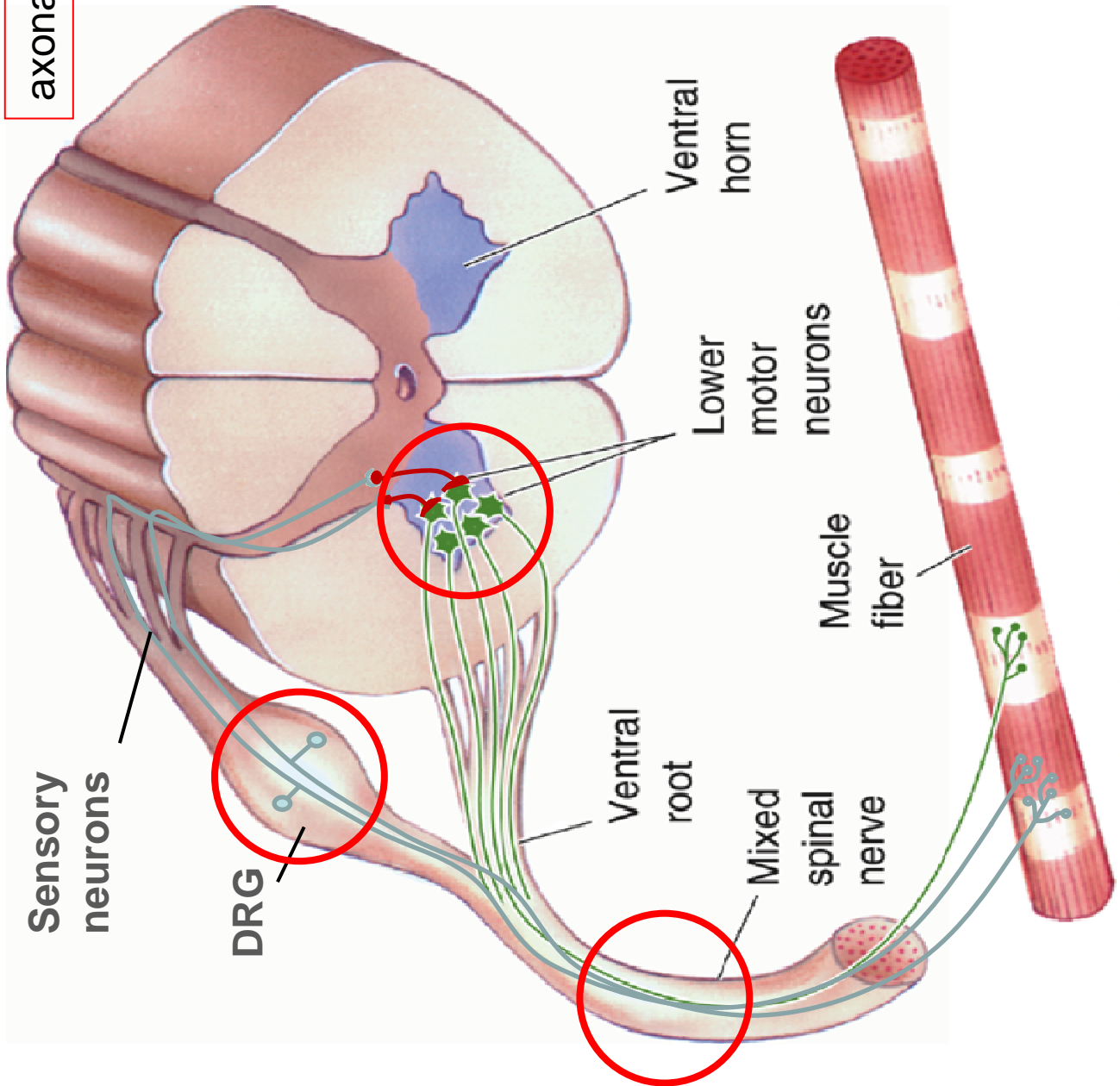
axonal transmembrane NRG1 type III

# Neuregulins & remyelination

## soluble NRG1 mRNA EXPRESSION in the INJURED NERVE

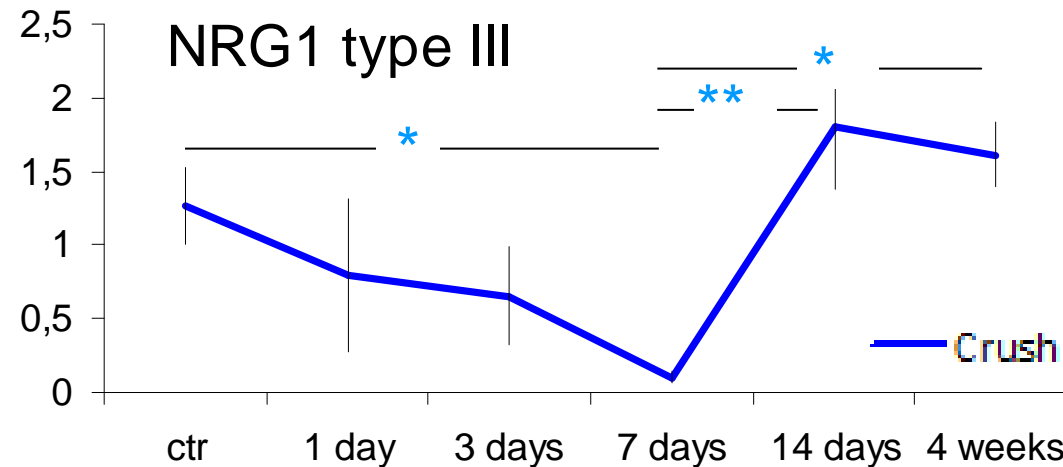
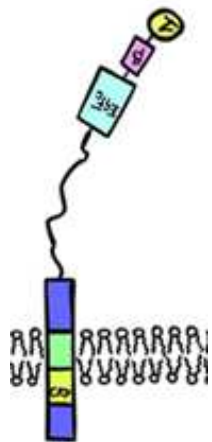


axonal transmembrane NRG1?



# Neuregulins & remyelination

## axonal transmembrane NRG1 EXPRESSION IN DRG



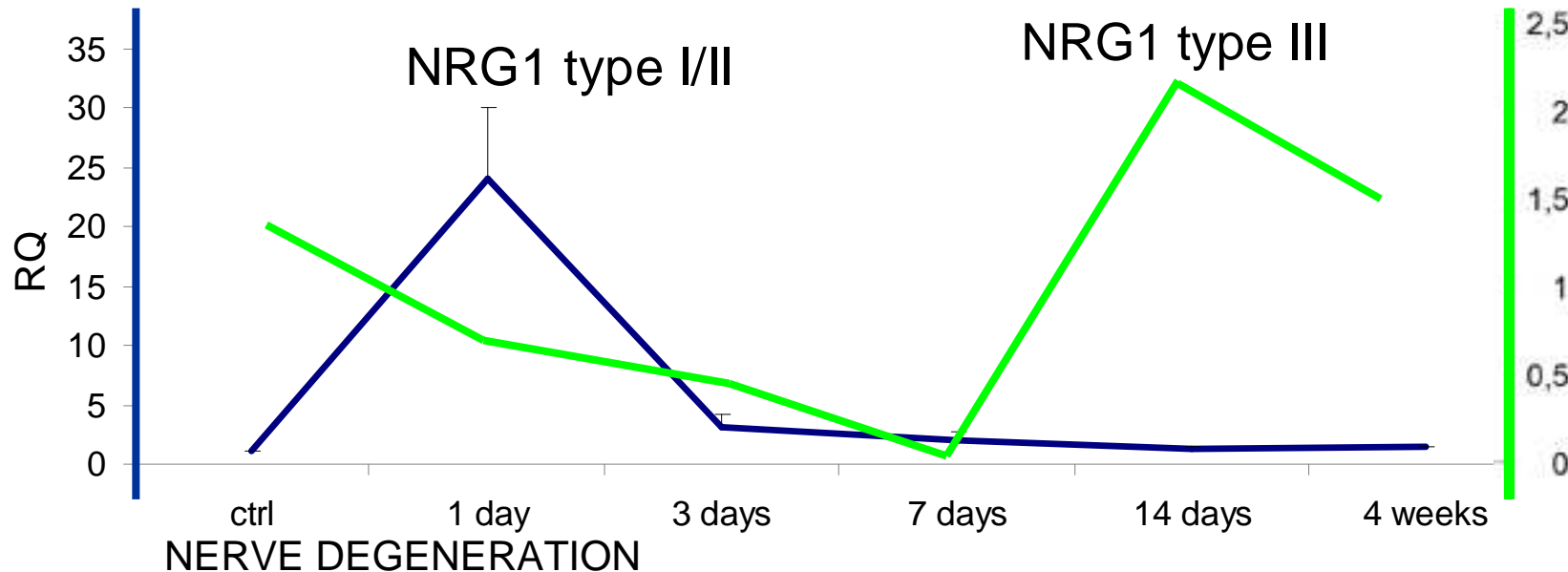
1-nerve degeneration

2-axon regeneration

3-remyelination



# Neuregulins & remyelination



NERVE INJURY



- SC dedifferentiate and proliferate
- SC produce NRG1 type I-II (both  $\alpha$  and  $\beta$ )



- SC survival and migration

- axons produce NRG1 type III



- SC redifferentiation and remyelination

## CONCLUSION AND FUTURE PERSPECTIVE

To promote nerve regeneration and functional recovery

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➔ expression of recombinant **NRG1 type III** in axons during **later phases** following nerve injury, could improve remyelination

- to express transmembrane isoforms, the use of viral vectors would be necessary; to bypass this step, manipulation of the processing of endogenously expressed NRG1 (e.g. TACE inhibition) could increase its promyelinating activity.