Polyplexes for RNA Silencing: A New Frontier in Anti-Inflammatory and Neuroregenerative Therapies After Spinal Cord Injury

Roberta Censi

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Chronic Inflammation

- Prolonged
- Inflammatory response
- Damages healthy tissues
- Immune cell recruitment
- Futile attempts at repair
- **Induces chronic diseases**

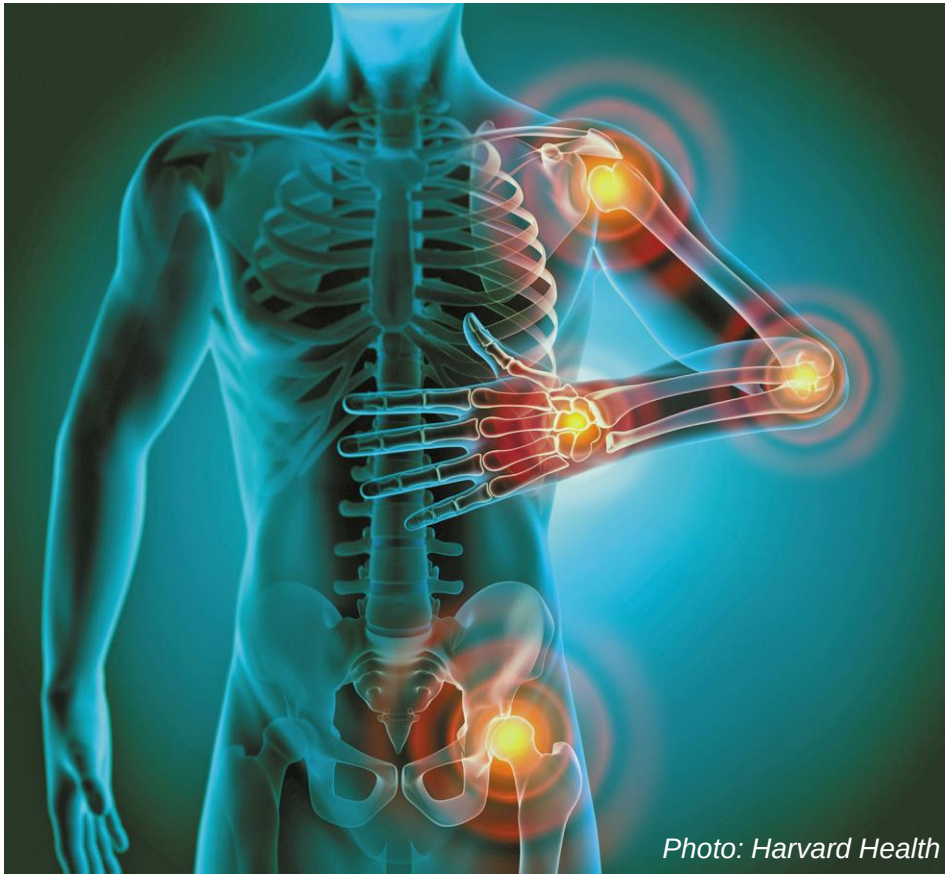


Photo: Harvard Health

Chronic Pain

- Persists >3 months
- Constant and intermittent, without cause
- Injuries, infections, **nerve damage**
- Affects daily life and emotional well being

Photo: Harvard Health

Chronic Pain and Inflammation

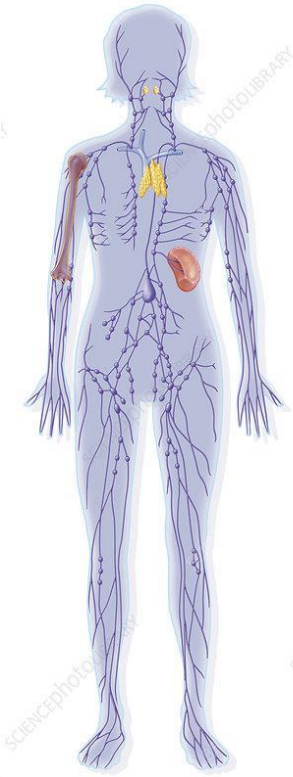
20% of global population

Global **Health** Issue

Induces chronic diseases

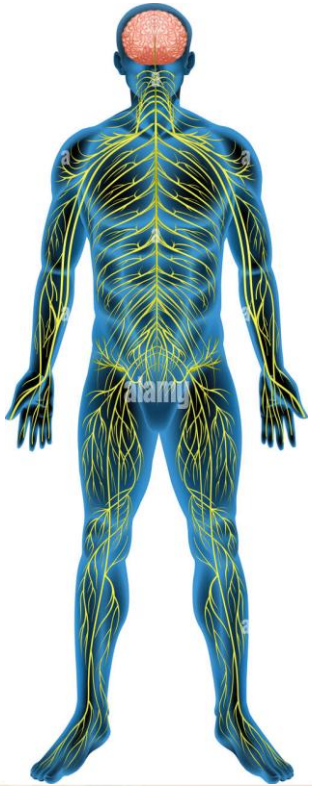
Pain and Inflammation: Immune and Nervous Systems

Immune System



Inflammation

Pain



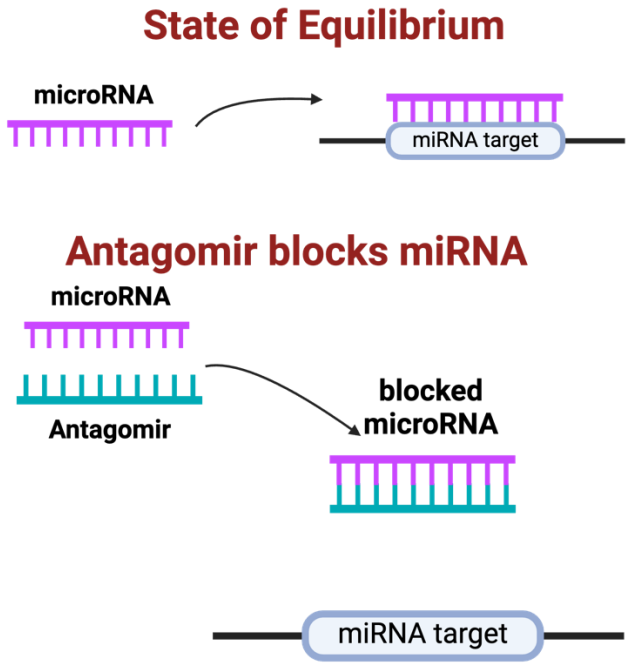
Nervous System

Pain and Inflammation: Immune and Nervous Systems

Nervous System

Pain

Antagomir: Mechanism of Action

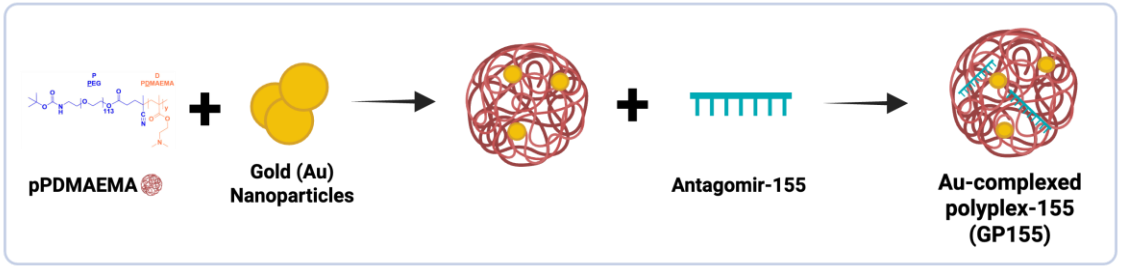


Unfavorable antagomirs physiochemical properties

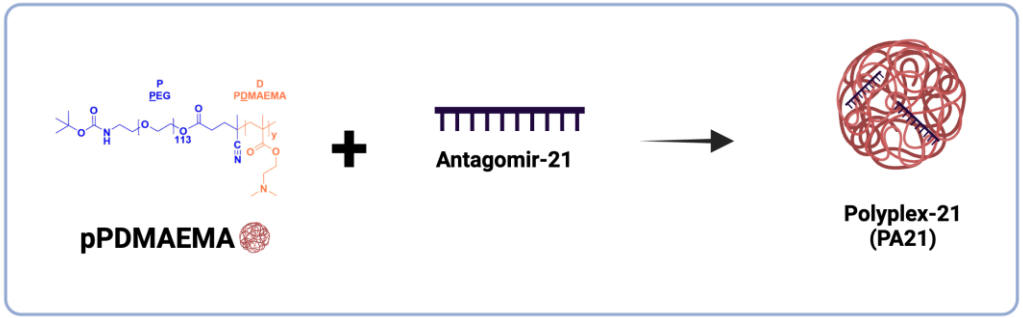
- High negatively charged density and high MW
- Poor cellular uptake
- Degradation by nucleases

NEED FOR DELIVERY SYSTEMS

Synthesis of Polyplexes



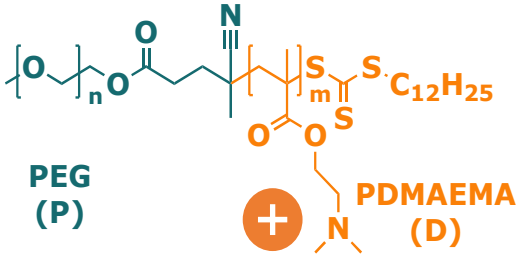
(a) Gold Complexed Amir-155 Polyplexes (GP155) for Targeting Neurons in SCI



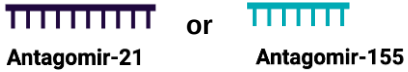
(b) Amir-21 Polyplexes PA21 for Targeting Macrophages in Peripheral Neuropathy

Synthesis of Polyplexes

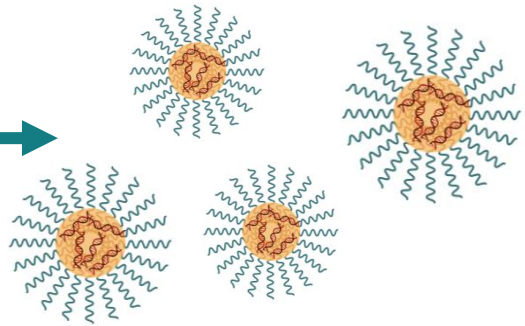
PD diblock copolymer

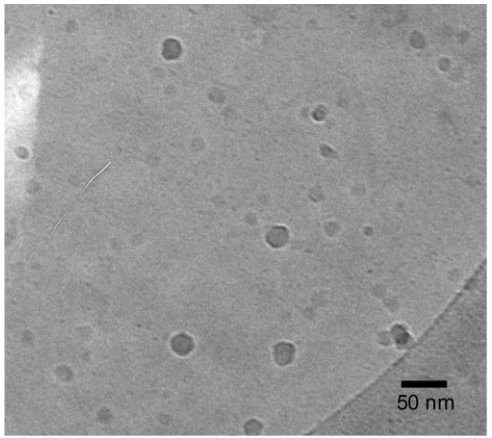


Synthetized via one-step
RAFT polymerization



Polyplexes



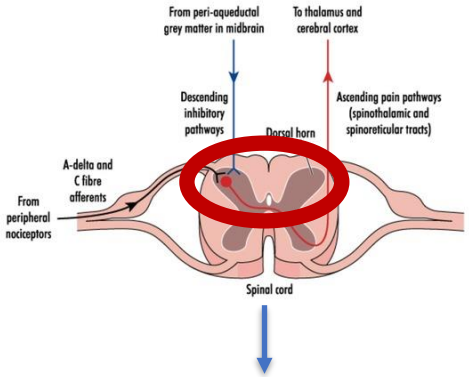


PEG-pDMAEMA MW 35kDa
PEG MW 5kDa
pDMAEMA MW 30kDa

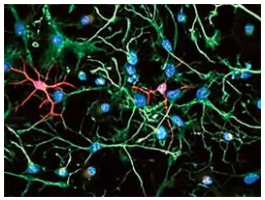
pDNA-Based Size and Zeta Potential Screening of Polyplexes

Table 1: Size, PDI and Zeta Potential of Au NPs, and Polyplexes (with and without Au NPs)				
	NP ratio	Size (d.nm.)	PDI	ZP (mV)
PEG-pDMAEMA-eGFP-pDNA polyplexes	1	550±65	0.75±0.12	3.81±2.26
	5	338±24	0.26±0.3	21.4±1.47
	10	118±2	0.26±0.20	30.79±1.72
	20	138±0.62	0.24±0.02	36.19±1.52
	30	276±20	0.23±0.01	28.50±1.27
Au-PDMAEMA polyplexes	10	377±7	0.28±0.01	11.52±0.37
	30	305±29	0.31±0.06	14.16±0.28
Au NPs	n.a.	42±22	0.24±0.05	-16.85±0.55

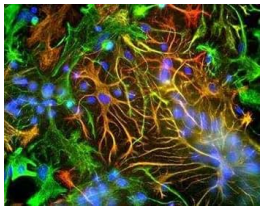
In vitro Study: Cell Internalization in dorsal horn culture from prenatal mice



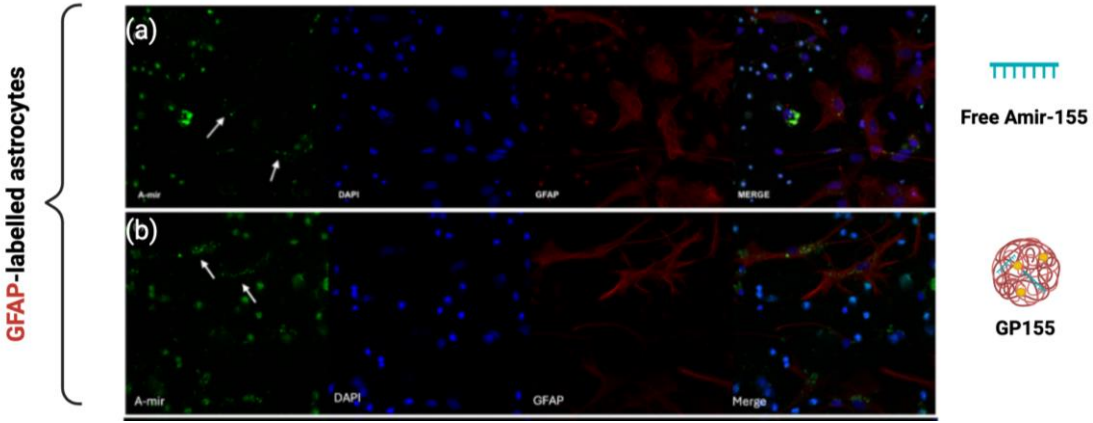
Beta Tubulin III (BIIIT): neuronal marker

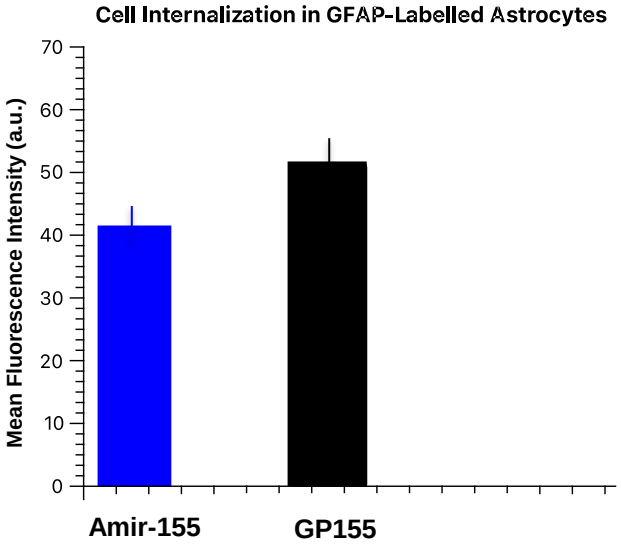


Glial Fibrillary Acidic Protein (GFAP): astrocyte marker



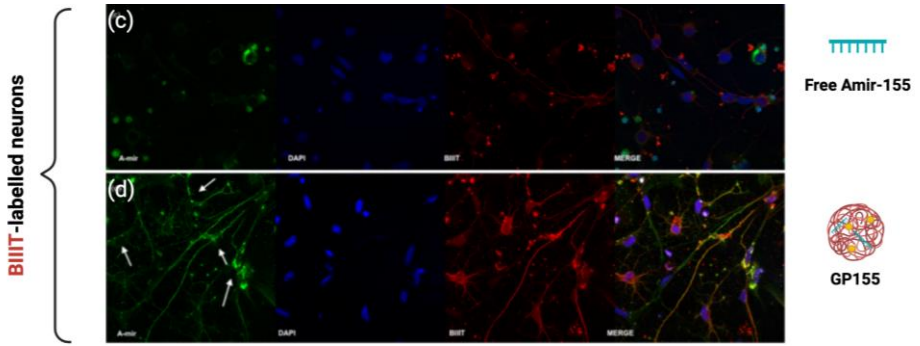
Cell Internalization in GFAP-labelled astrocytes



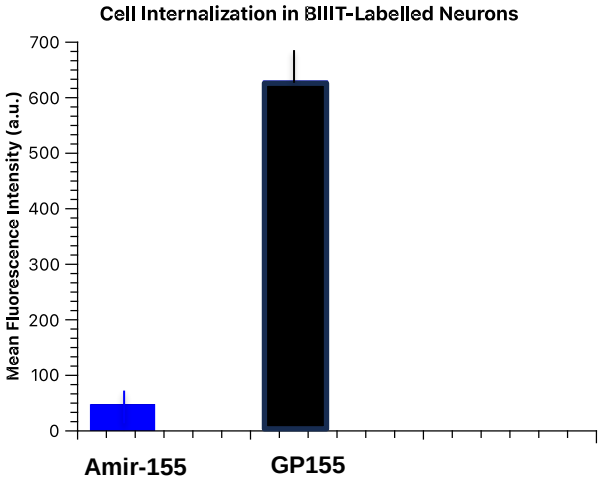


23% increase in MFI in astrocytes with GP155

Cell Internalization in BIIIT neurons

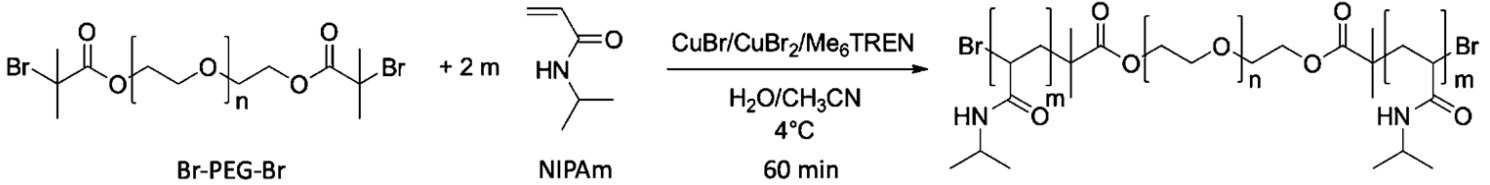


Z-Stack of GP155: shows planar position of cells



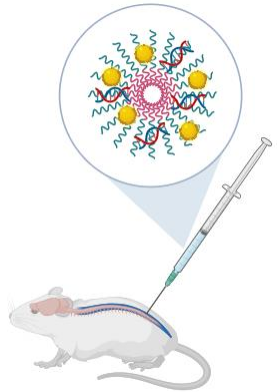
12x increase in MFI in neurons with GP155

In Vivo Administration of GP155 in SCI Rat Model



Synthesized by ATRP
MW 35 kDa

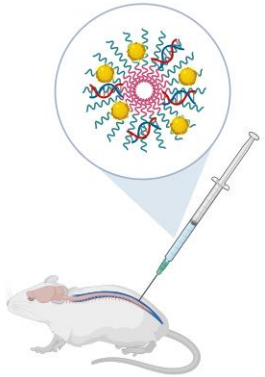
Benefits of Hydrogel Administration

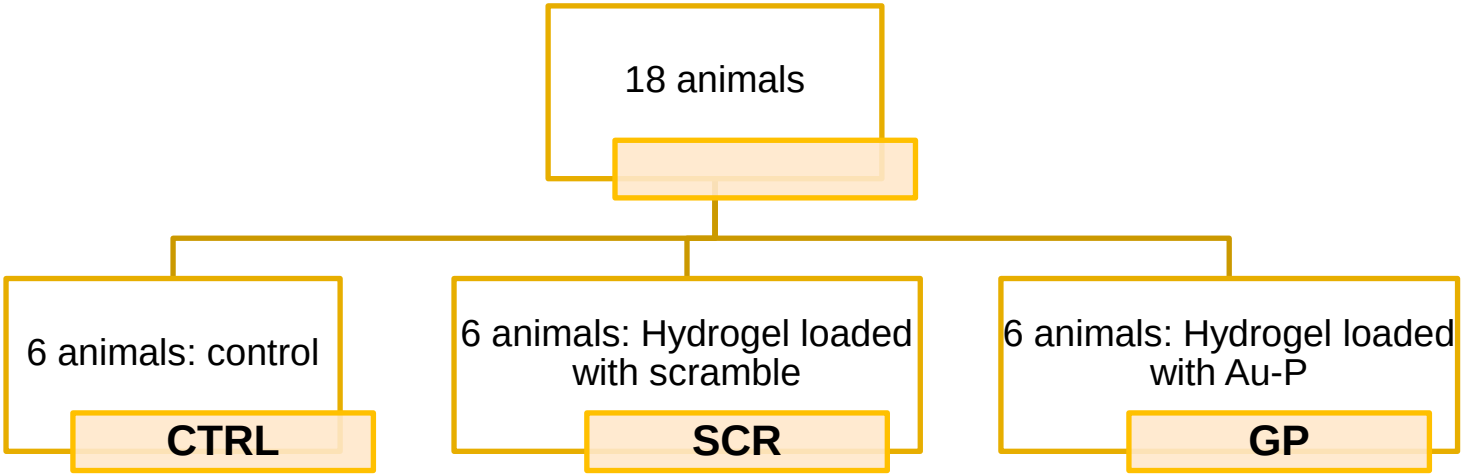


- **Controlled Release:** hydrogel encapsulation ensures slow release of GP155
- **Targeted Delivery:** hydrogel injections ensures targeted delivery
- **Reduced Off-Site Toxicity:** targeted delivery at site of injection ensures drug release at the injury site

5µl of the therapeutic GP155 injected at the site of injury

In vivo Study: Female Sprague Dawley rats

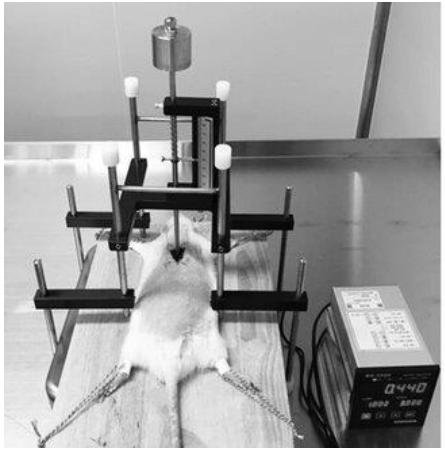


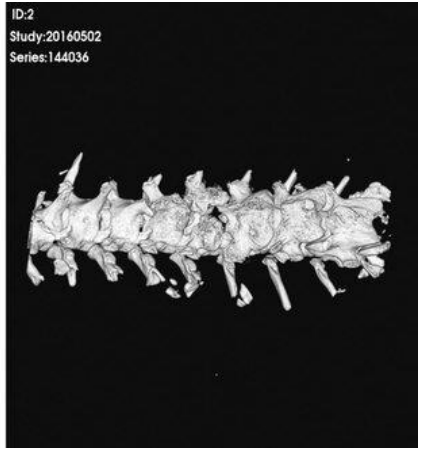


Surgery

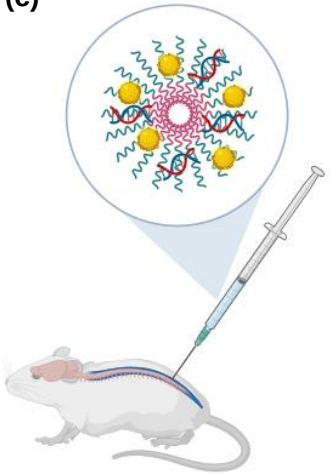
(a)

(b)





(c)

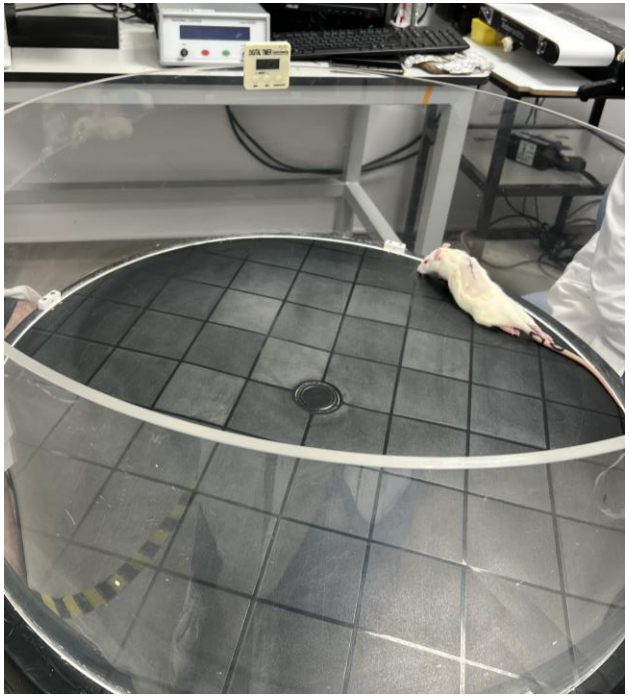


(a) Infinite Horizon (IH) impactor system. 200kdyn force was used to impact the **(b)** T9-T10 region of the spinal cord*. **(c)** the lesion site was then injected with 5µl of CTRL/SCR/GP

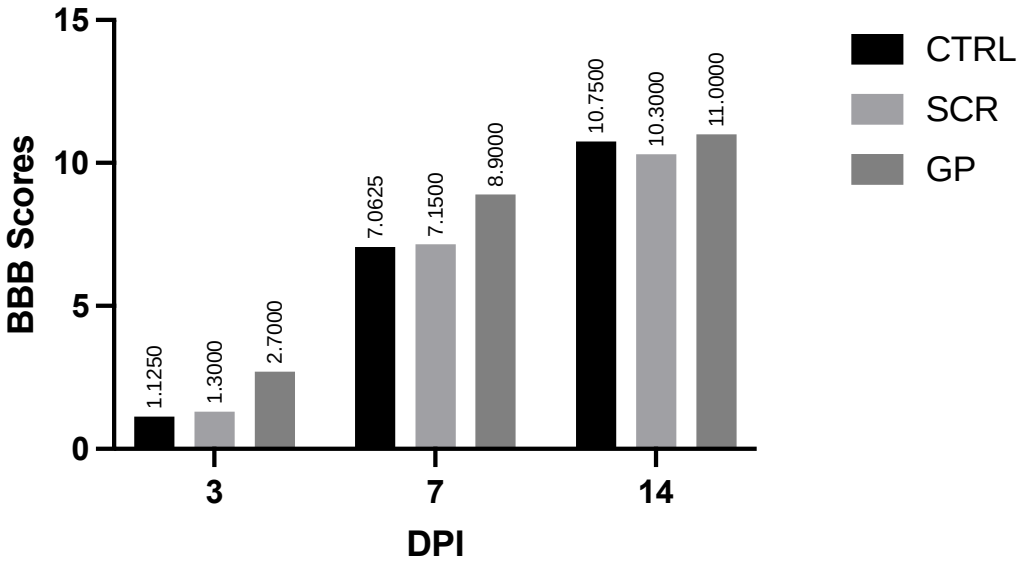
*Sohn, H. M., *J Korean Soc Spine Surg.* 2018

Basso Beattie and Bresnahan (BBB) Scores: 21-point locomotor scale

(a)



(b)

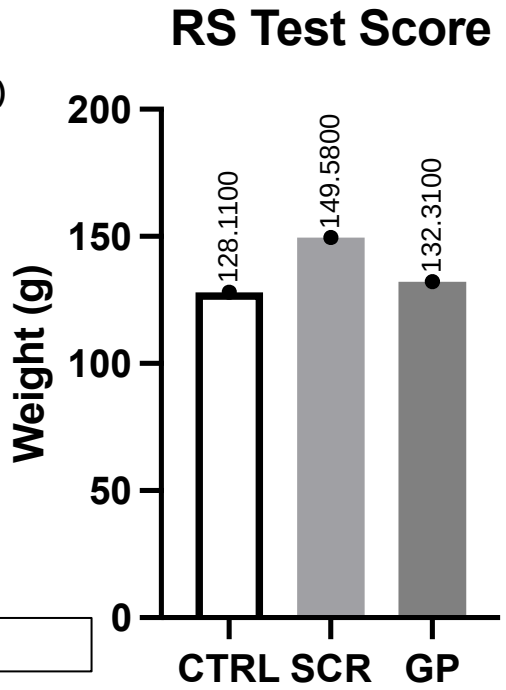


(a) Post-SCI animals in a round chamber for locomotive BBB test
(b) A major increase in locomotion for GP animals at 3dpi

Randall-Selitto (RS) Pressure Test Scores

(a)

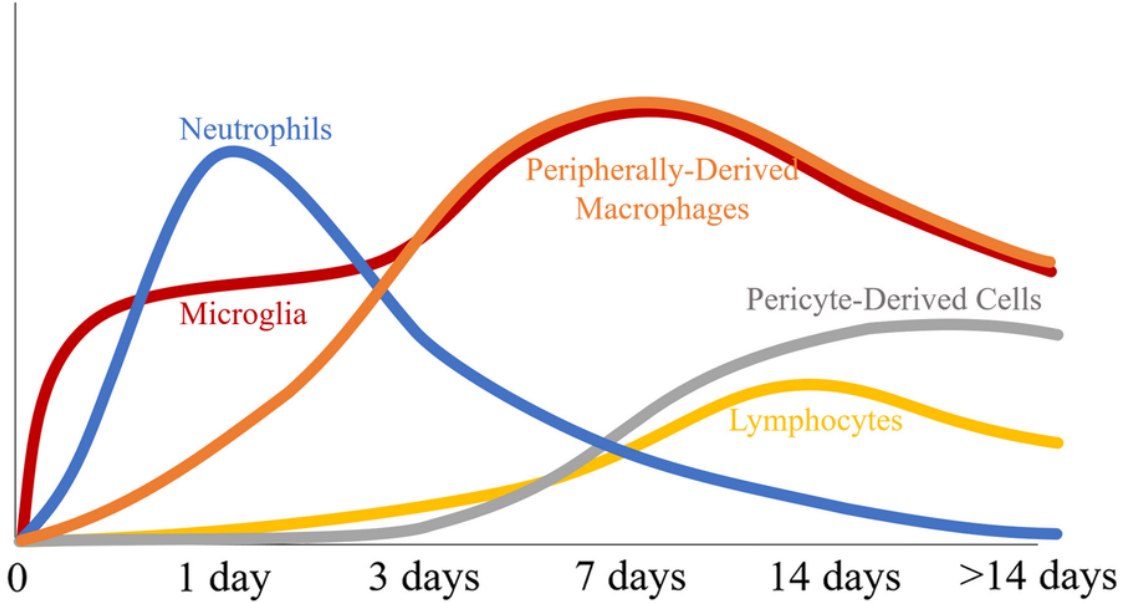
(b)



Mechanical hyperalgesia not detected

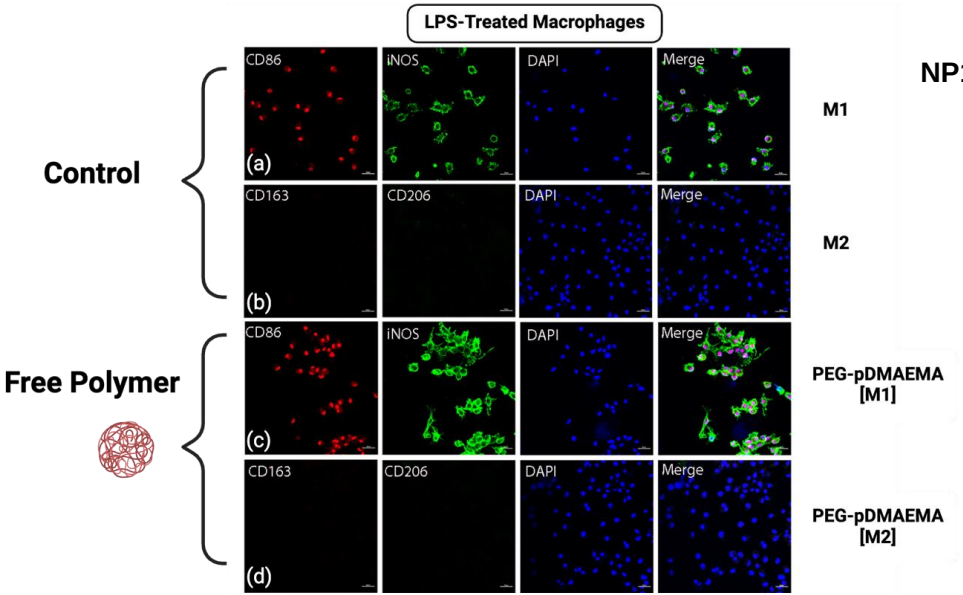
(a) Paw pressure RS test machine
(b) The RS test scores showed more ambiguity between the three groups and were deemed inconclusive

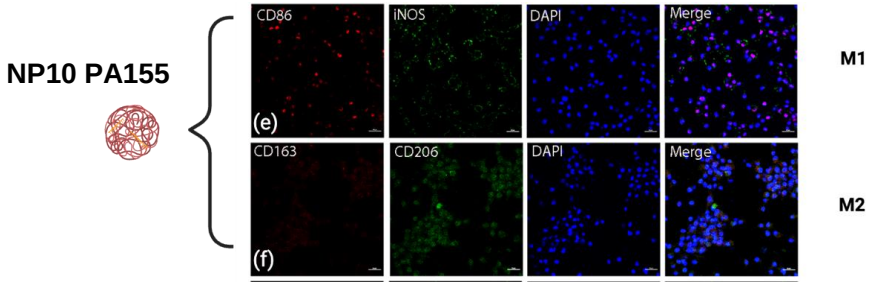
Inflammation after spinal cord injury: a review of the critical timeline of signalling cues and cellular infiltration



Hellenbrand, D.J., *J. Neuroinflammation*. 2021

PA155 on LPS-Treated Bone Marrow Derived Macrophages



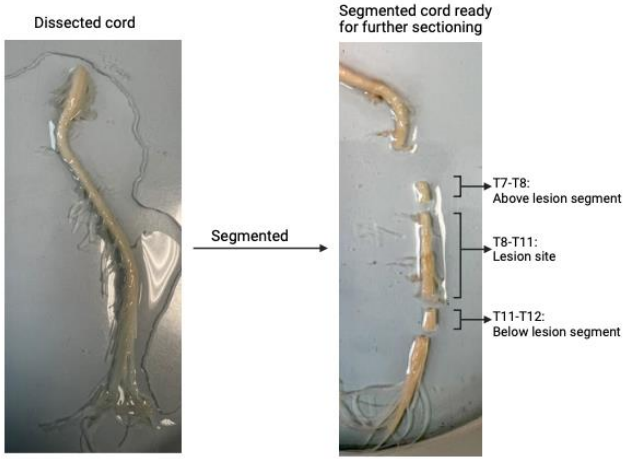


30% increase in M2-Marker CD206
20% decrease in M1-Marker iNOS

Ongoing

Histology

Spinal cords collected and cut into 20-40 µm sections with cryostat



Second *in vivo* study

3-day *in vivo* study for better understanding a-mir mechanism

Other miRNA Targets

Exploring other miRNA targets for inflammation and pain like:

- **miR-21**: common inflammation promoter in peripheral nervous system

Thanks for listening!

Acknowledgments

University of Camerino
Dr. Cristina Casadidio
Dr. Lakshmi Sathi Devi
Dr. Qisan Ma
Dr. Stefan Jackson
Martina Fronduti
Orion Jucja
Divishth Gupta
GiacomoDi Giambattista

University of Leeds
Prof. Ronaldo Ichiyama
Prof. Nikita Gamper
Sofia Figoli

King's College University
Prof. Marzia Malcangio
Dr. Lynda Zeboudi

University of Chieti Pescara G. D'Annunzio
Prof. Piera Di Martino
Dr. Maria Rosa Gigliobianco

Dr. Saniya Salathia

Nanoparticle-Based Imaging and Therapy of Chronic Pain in the Dorsal Root Ganglia

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 956477