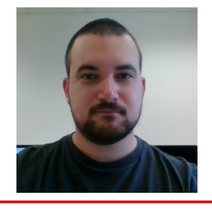
# Characterization of rat contusive spinal cord injury model and treatment with local medicated scaffold

Alma Mater Studiorum Bologna, Dottorato in Scienze Veterinarie, XXXIII Ciclo
Dottorando: Andrea Bighinati
Tutor: prof.ssa Luciana Giardino



**INTRODUCTION**

Spinal cord injury is a invalidating condition primarily caused by preventable accidents such road traffic crashes, falls or violence. Up to 90% of spinal cord lesion are caused by trauma, though the proportion of non-traumatic spinal cord injury appears is growing. Symptoms of spinal cord injury depend on the severity of the injury and its location in the spinal cord. Symptoms may include partial or complete loss of sensory function or motor control of arms, legs and/or body. The most severe spinal cord injury also involves bowel or bladder control, breathing, heart rate and blood pressure regulation. Most people with spinal cord injury experience chronic pain. There are no treatments for the acute phase of the spinal cord injury, being the steroid therapy almost virtually abandoned.

**The general aim of this three-year project is to develop novel therapeutic solutions for the acute phase of the spinal cord injury. The specific aims of the first year are:**

**1. The morphological and functional characterization of the model of contusive spinal cord injury in the rat at different post-lesion times;**

**2. The study of the synaptic plasticity in the spinal cord and brain motor circuits after spinal cord lesion at different post-lesion times;**

**3. Pilot experiments aimed to the implantation of medicated PLLA scaffold in lesioned animals are also presented.**

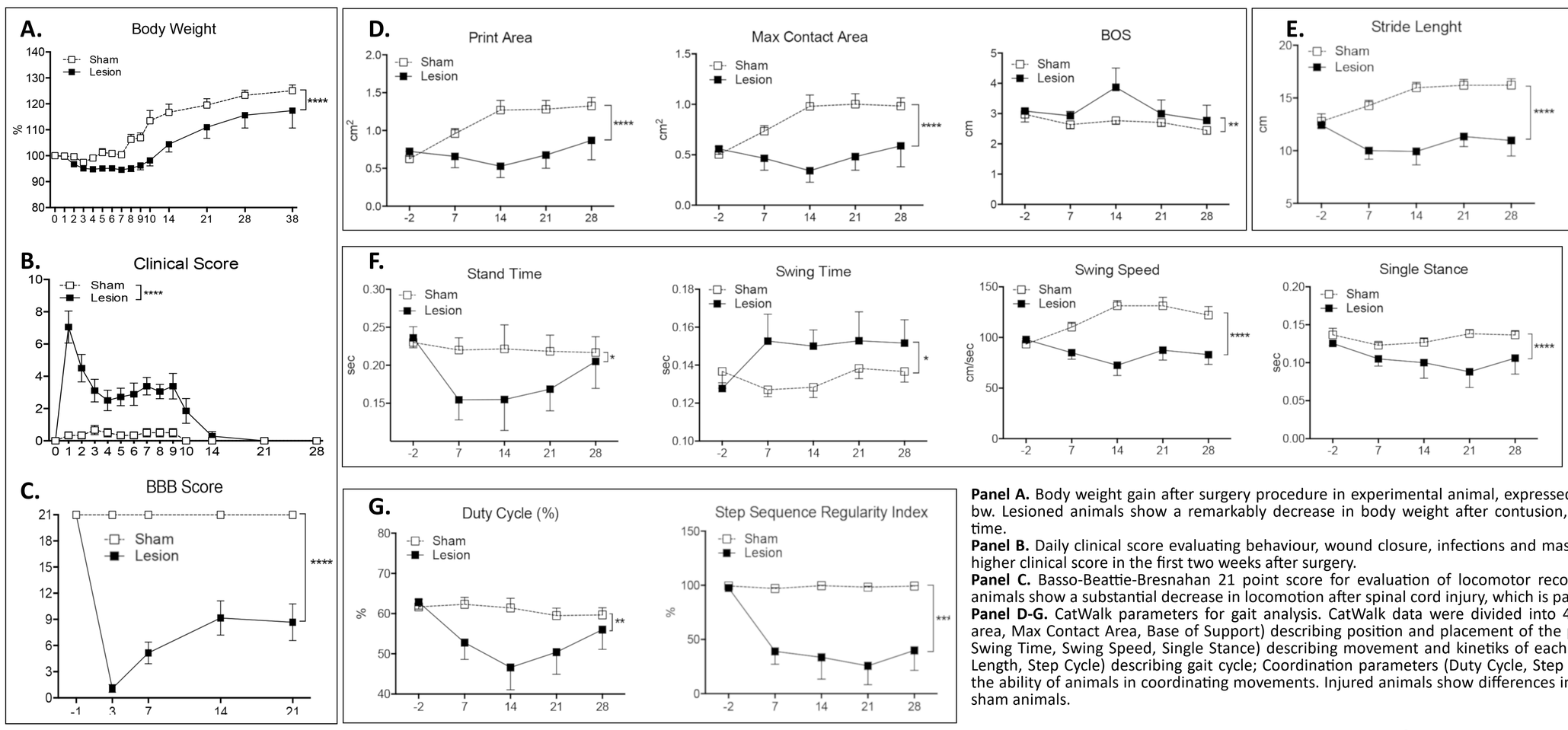
**METHODS**

CD-Sprague Dawley (Charles River, Italy) female rats, 225-300 g body weight, were used in this study. Spinal cord lesion was performed at T8-T10 level after laminectomy with Impact One impactor (Leica BioSystems, Germany) using a force of 1 N (0.75 m/s) and 0 s of stance time, the depth of impact was 2 mm in order to reach the ventral horns of gray matter. For functional characterization we used 10 animals per group, for evaluation of lesion volume area, synaptic plasticity analysis and implantation of medical scaffold were used 5 animals per group. An equal number of sham-operated animal (laminectomy, only) were used as control.

Evaluation of hind limb functional locomotor loss was assessed with Basso-Beattie and Bresnahan) and gait analysis was performed with CatWalk (Noldus, The Netherlands) automatized system. For the definition of lesion area Toluidine blue and Hematoxilin/Eosin staining were performed on serially sectioned spinal cord. Lesion area was then determined for each reconstructed section with ImageJ (NIH) as number of pixels occupying the lesion site and expressed as ratio compared to total area.

mRNA real-time PCR was performed to study gene expression. The relative expression of all studied genes was calculated through the 2^(-ΔΔCT) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value. Graphs show mean values±SEM, statistical analysis was performed by 2-way ANOVA (*) or T-test (#). Significant differences have been indicated.

## 1. The morphological and functional characterization of the model of contusive spinal cord injury in the rat at different post-lesion times

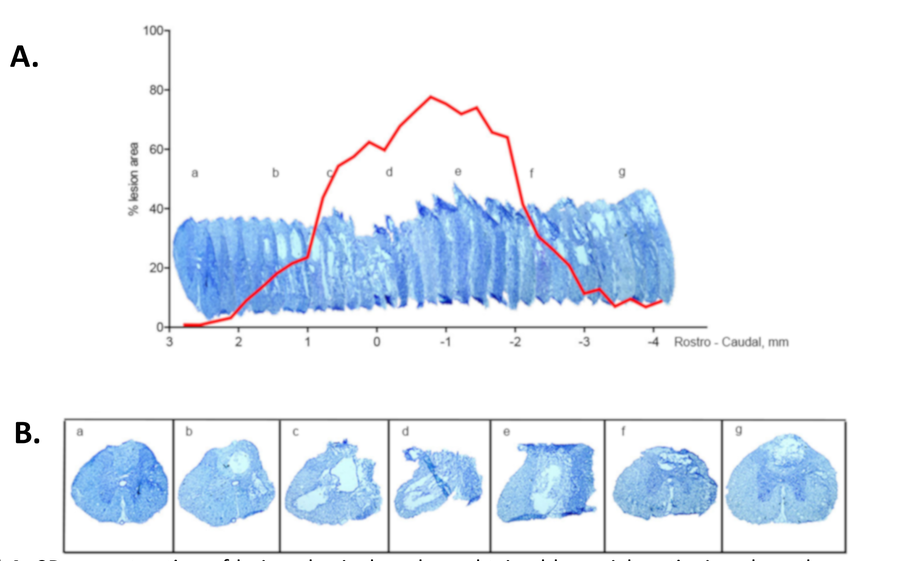


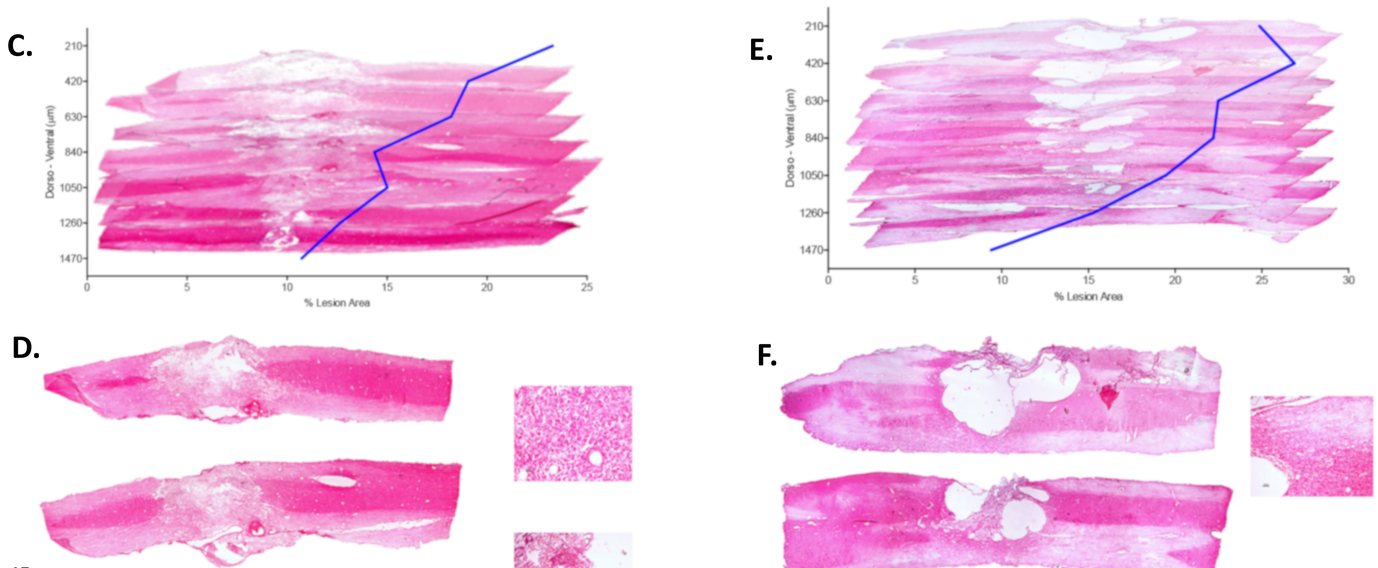

**Panel A.** Body weight gain after surgery procedure in experimental animal, expressed as % changes respect to pre-surgery bw. Lesioned animals show a remarkably decrease in body weight after contusion, which is partially recovered over the time.

**Panel B.** Daily clinical score evaluating behaviour, wound closure, infections and mass index (ref). Lesioned animals display higher clinical score in the first two weeks after surgery.

**Panel C.** Basso-Beattie-Bresnahan 21 point score for evaluation of locomotor recovery after spinal lesion. Lesion group animals show a substantial decrease in locomotion after spinal cord injury, which is partially recovered over the time.

**Panel D-G.** CatWalk parameters for gait analysis. CatWalk data were divided into 4 categories: Spatial parameters (Print area, Max Contact Area, Base of Support) describing position and placement of the paws; Kinetic parameters (Stand Time, Swing Time, Swing Speed, Single Stance) describing movement and kinetiks of each paw; Comparative parameters (Stride Length, Step Cycle) describing gait cycle; Coordination parameters (Duty Cycle, Step Sequence Regularity Index) describing the ability of animals in coordinating movements. Injured animals show differences in all parameters analysed compared to sham animals.



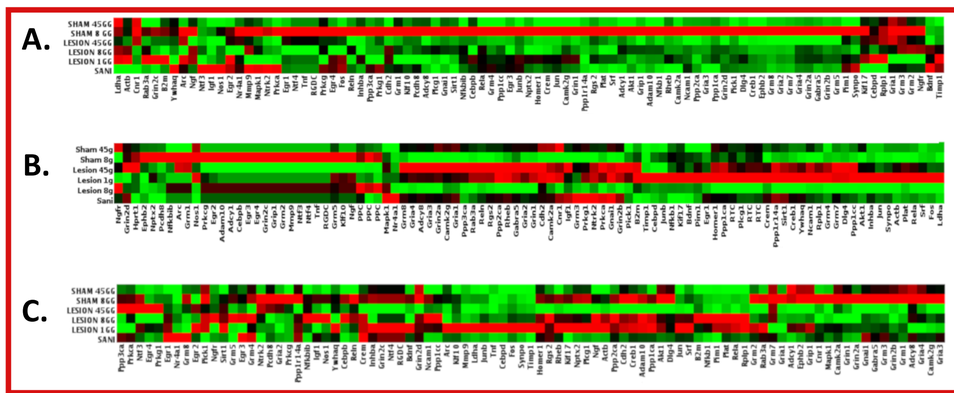

**Panel A.** 3D reconstruction of lesioned spinal cord, as obtained by serial sectioning along the coronal plane, 45 days post injury. The red line reports the lesioned areas, evaluated as ratio between intact and damaged tissue.

**Panel B.** representative low-mag microscopic images at different rostro-caudal levels along the analysed spinal cord.

**Panel C-F.** Longitudinal 3D reconstruction of lesioned spinal cord at 8 days (C, D) and 45 (E, F)days post injury. Lines in C and E represent the percentage of lesioned tissue.

**Panel D.** Micrographs of the spinal cord slices, also showing the high cellularity due to the acute inflammation at 8 days post injury.

**Panel F.** Micrographs of the spinal cord slices, also showing the structured lesion cavity, at 45 days post injury.



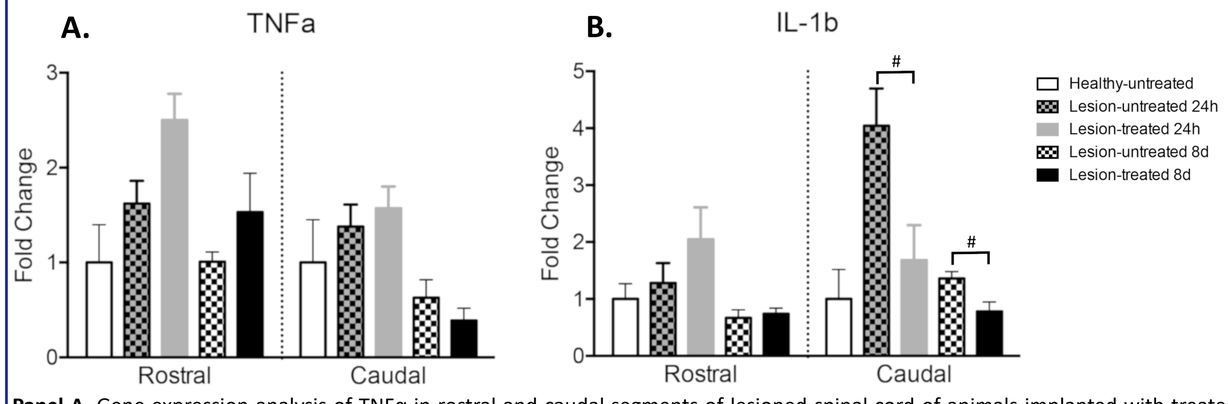

## 2. The study of the synaptic plasticity in the spinal cord and brain motor circuits after spinal cord lesion at different post-lesion times

**Panel A.** Clustergram of gene expression for the array “synaptic plasticity” in the motor cortex at different time point, e.g. 24 hours, 8 days and 45 days. Injured animals show reduced expression in genes involved in different cellular mechanism (IEGr, LTP, Extracellular Matrix), conversely sham animals did not show marked gene expression regulation after surgical procedure.

**Panel B.** Clustergram of gene expression for the array “synaptic plasticity” in the rostral segment of injured spinal cord. Lesioned animals show a massive upregulation in gene expression at 24 hours, followed by a consistent downregulation at 8 days post injury, after 45 days gene expression was upregulated again (LTP, LTD pathways). Interestingly sham animals present the same diffused gene expression downregulation at 8 days post surgery.

**Panel C.** Clustergram of gene expression for the array “synaptic plasticity” in the caudal segment of injured spinal cord. Gene expression was upregulated at each time point (IEGs, Extracellular Matrix). Sham animals did not show gene expression modification compared to control group.

## 3. Implantation of medicated scaffold in lesioned animals: preliminary data (patent application presented)




**Panel A.** Gene expression analysis of TNFα in rostral and caudal segments of lesioned spinal cord of animals implanted with treated or untreated scaffolds. Expression of TNFα in rostral segment was increased by treated scaffold at both time points, while in caudal segment TNFα was decreased at 8 days post lesion.

**Panel B.** Gene expression of IL-1b in rostral and caudal segment of lesioned spinal cords. IL-1b expression was unchanged in rostral segment while in caudal segment treated scaffold decrease expression of IL-1b at both 24 hours and 8 days post injury.

## Concluding remarks and future perspective

Our animal model shows all clinical characteristic of spinal cord lesion (neurological bladder, clinical recovery and hind limb paralysis) and present a high reproducibility and reliability in clinical symptoms and behavioral parameters.

The biological response to the SCI is divided into three phases: acute (a few seconds or minute after the injury), secondary (from a few minutes to a few weeks after the injury) and chronic (some months to years after the injury). In the acute phase vascular events are prevalent such as edema and serious alterations of the chemical microenvironment (ionic homeostasis, accumulation of neurotransmitters, plasma membrane compromise, etc.). Many of these events are also present in the secondary phase, including oxidative stress, an and immunological inflammatory reaction, the initiation of astroglial scarring, and demyelination leading to the electrophysiological collapse. In the chronic phase the demyelination, the astroglial reaction and the central cavitation continues, while the regeneration attempts, for example the sprouting by some neurons, determines alterations in the anatomy and physiology.

The aim of this three year project is to develop implantable scaffolds to prevent/limit secondary neurodegeneration, targeting inflammation and remyelination. The first year activities provided the model, related variability, reliable end-point for efficacy studies, and resolution of all technical problems related to the scaffolds implantataion (PLLA elecrospun scaffold medicated with two drugs, patent application presented). Local treatment with medicated scaffold implanted immediately after the spinal cord lesion reduces inflammation in the segment below the lesion site during the acute phase of inflammation.