INTRODUCTION:
New porous metals have been developed for medical applications in order to improve the interface between the implant and the adjacent biological structures. As opposed to coated materials, porous nitinol (PNT) represents a clear advantage for biological tissues, which can fully integrate throughout its complete network of interconnected fenestration. Potential biomedical applications of PNT include soft tissue attachment and bone carrier devices for guided bone regeneration. For example, PNT intervertebral fusion devices for degenerative disc diseases meet the conditions that represent a clinical application that may become an alternative to traditional interbody cages, which otherwise require autologous bone grafting and longer surgery time. Since PNT fusion devices are intended for implantation at the spinal level, potential wear debris (if any) must be evaluated for the possibility of developing adverse reactions in adjacent spinal nervous tissues. To our knowledge, the implantation of PNT particles has never been evaluated specifically on nervous tissue of the spinal cord. A spinal subchronic assay was therefore performed on PNT particles using a rabbit model for 1-, 4- and 12-week post-surgical recovery periods.

MATERIALS AND METHODS:
Animal model: New Zealand white female rabbits (2.5-3kg) were implanted with PNT particles at the level of the spinal cord tissue and more specifically the dura mater. The rabbits were allocated into two main groups: PNT (N=4/recovery period) and sham (N=1/recovery period) animal test groups. The operations were performed at Ste-Justine Hospital Research Center (Montreal, QC, Canada) and the study was approved by its Institutional Committee for Good Animal Practices. Animal care complied with the Canadian Council on Animal Care (CCAC) guidelines for care and use of experimental animals.

Biomaterials: Porous nitinol powders consisted in small PNT particles (5-300µm in diameter; 46.0at.%; Ni, 54.0at.%; Ti; Actipon®; Biorhemi Inc., Montreal, QC, Canada). Powders were sterilized via autoclave at 121°C for 1h and prepared for local injection (10-12mg) on the spinal cord.

Surgical technique: Animals were first allowed to acclimatize to laboratory conditions for 1 week, then premedicated with an intramuscular injection mix of ketamine hydrochloride (30mg/kg), xylazine (5mg/kg) and acepromazine (0.5mg/kg) for 30min. Following endotracheal intubation and general anesthesia (1.5% halothane and 1.5L O₂), animals were positioned prone with their backs shaved, scrubbed clean with providine and sprayed with a mix of iodine/alcohol. Following Rivard et al. [1], a 4-cm incision was made along the spine at mid-lumbar region. Two spinous processes were exposed and one spinous process excised at lumbar level L3. The ligamentum flavum was cut in order to facilitate access to the spinal canal. The particles were blown directly into the spinal canal using a Pasteur pipette. The incision was closed in 3 layers with absorbable sutures. The sham rabbits underwent the same surgery without any spinal canal invasion.

Necropsy and histology: The PNT and sham rabbit groups were euthanized using an intravenous solution of sodium pentobarbital. Each individual spinal cord was exposed by cutting the vertebral arch on both sides of the implanted segment. The spinal cord was carefully removed by excision of the nerve roots. A magnifying glass, specimens were examined macroscopically in order to observe general tissue reaction (if any) to PNT particles. The spinal cord segment was then fixed in 10% buffered neutral formalin for 1 week before being processed for histology. The lumbar spinal cord segments were sliced in three 5-mm blocks (implantation site, caudal and cranial sides) and then embedded in paraffin. Three 4-µm histological sections (per block) were then prepared using a rotary microtome. The sections were then placed in a warm water bath (37°C), mounted on slides and stained with Hematoxylin-Phloxin-Saffran (HPS) for light microscopy examination. Histological evaluation of the dura mater inflammatory reaction to the particles was based on the ASTM standard F981-91 [2], where a score of 0 to 3 was based upon the number of the inflammatory cell types in high power field. Necrosis and toxicity to the material were also evaluated.

RESULTS:
The macroscopic analysis revealed that all particles remained on site, clung to the adipose and soft tissue. A minimal inflammatory reaction was observed after 1 week: a mild redness was observed but it was limited to the implantation site (Fig. 1A). The reaction included the presence of residual foci of haemorrhage and adipocyte necrosis, histiocytes, and haemosiderin. Some granulocytes, lymphocytes and plasma cells were also present at 1 week. Some inflammatory cells extended to the epidural space adjacent to the implantation site both cranially and caudally (Fig. 1B). Remote spinal cord tissue and nerve roots seemed normal compared to those of sham rabbits.

At 4 weeks post-surgery however, only slightly oedematous fibrotic tissue remained with a mild non-specific inflammatory reaction being limited to the adjacent soft tissue. The inflammatory process had already become more localized and no invasion of the cranial or caudal sections was observed. Moreover, a compact acellular fibrotic tissue (collagenous capsule) associated with a minimal non-specific inflammatory reaction took over after 12 weeks of implantation time.

Most importantly, no necrosis of the dura mater was observed around the particles and no inflammatory cells had colonized the dura mater regardless of implantation time. In the epideral space, the nerve root and spinal cord were free of reaction at all time as well. According to the ASTM standard scoring system [2], a normal reactivity was observed at 1 and 4 weeks post-surgery (score 0-2). The inflammation further resorbed by 12 weeks (score 0-0.5).

REFERENCES:

Figure 1. Spinal cord observation after 1 week post-surgical recovery time. Macroscopic view of PNT particles clung to spinal cord tissue (A). Microscopic view of mild inflammatory reaction at the implantation site (B).

DISCUSSION:
This local subchronic implantation study is one of its kind since nitinol particles have never been tested in vivo on the nervous tissue, especially the spinal cord: certainly the most adjacent soft tissue to an intervertebral device and one of the most sensitive and fragile tissue of the human body. Of course, this in vivo assay represented a worst-case scenario since PNT intervertebral fusion device debris are not expected to normally occur following interbody implantation. Nevertheless, this macroscopic and histologic evaluation confirmed the local compatibility of PNT with the spinal cord and its nerve roots in a rabbit model. Indeed, the spinal cord, dura mater and nerve root tissues did not show adverse reactions such as severe inflammation and necrosis. Conversely, the PNT particles were kept entrapped in a soft tissue capsule at the posterior side of the spinal cord: a normal biological tissue encapsulation process that takes place against foreign bodies. These local subchronic results further support previous reports showing high biocompatibility of nitinol alloys [3]. Long-term implantation of PNT interbody fusion devices is currently under development in order to further characterize the biofunctionality and biocompatibility of porous nitinol as a spinal implant.

SPINAL EVALUATION OF POROUS NITINOL PARTICLES: A SHORT-TERM STUDY IN RABBITS

*Rhalmi, S; **+Assad, M; **+Leroux, M A; *Charette, S; *Rivard, C H
*Ste-Justine Hospital, Paediatric Research Center, Montreal (QC) Canada. **+Biorhemi Inc., R&D Dept., Montreal (QC) Canada