# Prolonged delivery of a P2X7R antagonist using an injectable nanostructured hydrogel improves bladder function after SCI

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**Statement of Purpose:** Spinal cord injuries (SCI) consistently impair sensory nerve function leading to devastating complications, including urinary incontinence associated with neurogenic detrusor overactivity (NDO). We developed a nanodelivery system for controlled and localized inhibition of P2X7 purinergic receptors (P2X7R) [1]. The goal of this study was to decrease deleterious secondary injuries using a novel nano-formulation for localized delivery of the P2X7R antagonist at the injury site, minimizing scar formation and neurodegeneration and leading to improved bladder function.

**Methods:** PLGA-Si nanocomposite microparticles loaded with the P2X7R antagonist brilliant blue-G (BBG) were synthesized using a water-in oil-in water suspension technique. The microspheres were encapsulated into themoresponsive Pluronic hydrogel. The release of antagonist from nanostructured hydrogel was measured in vitro at 37°C using UV Spectroscopy at predetermined time points and the degradation was characterized through SEM. The effect of P2X7R antagonist loaded nanocomposite hydrogel was evaluated in a partial-SCI rat model. The animals received a transection of the T8/T9 dorsal cord aspect (i.e. intact ventral cord) and were treated with either an empty or an antagonist loaded nanocomposite hydrogel. The changes in locomotor capacity using the BBB scoring system and standing capacity were recorded. Four weeks after treatment, a cystometric evaluation with a suprapubic catheter was performed; rats were euthanized after cystometry and spinal cord immunohistochemistry was performed to determine the effects of BBG on the dorsal horn expression of the neuronal marker Map2.

**Results:** We have standardized a fabrication protocol for P2X7R antagonist-loaded nanocomposite hydrogels. The formulation has been effectively characterized for the long-term release of antagonist in vitro and demonstrates constant release dosage (Figure 1A, B). This is a run-on. Perhaps try to break it up. "The functional evaluation for the changes in locomotor and standing capacities show an improvement after two weeks (Figure 1C, D), followed by a trend towards better recovery and improved bladder function at four weeks within the BBG treated group (Figure 1 E, F). The expression of Map2 was evaluated at a cord region as close as possible to the center of the dorsal transection. Neuronal immunostaining suggest that the dorsal horns from rats receiving the empty preparation have a more severe injury than rats treated with the P2X7R antagonist (Figure 1G, H).

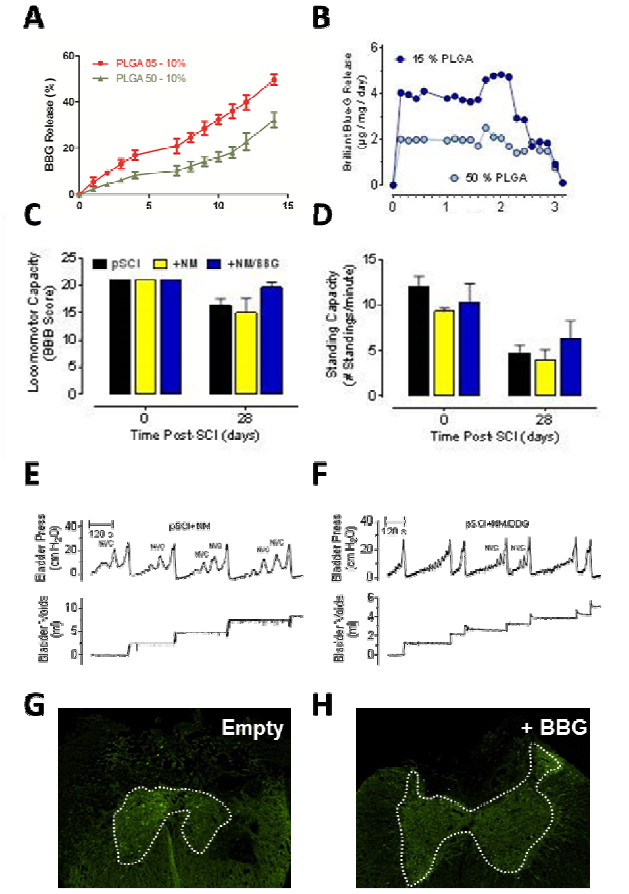


Figure 1. Cumulative release profile (A) and daily dose release (B) of BBG from 85-10% or 50-10% PLGA-Si nanocomposite particles. Locomotor capacity for pSCI (black), pSCI+NM(Empty) (yellow) or pSCI+BBG/NM (blue) rats (C). Standing capacity for pSCI, pSCI+NM and pSCI+BBG/NM rats (D). Typical cystometrograms of rats receiving empty nanomaterials (E) or microspheres with the antagonist (F). Dorsal horn expression of the neuronal marker Map2 in empty (G) or BBG (H) rats (sections taken as close as possible to the center of the injury, where the gel was applied).

**Conclusions:** Our results show a trend towards better locomotive recovery in the injured rats treated with the P2X7R antagonist loaded in the nanocomposite hydrogel. This injectable formulation could have storage characteristics suitable for long-term local delivery and inhibition of P2X7R for the treatment of partial SCI. This approach may offer several advantages to improve locomotion, bladder function and perhaps the micturition reflex.

**References:**

1. Peng et al. Proc. Natl. Acad. Sci 106 (2009): 12489-93