

## **THE IMMUNOMODULATORY EFFECT OF SILVER NANOPARTICLES IS CRITICAL FOR PROMOTION OF TENDON REPAIR**

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**INTRODUCTION:** Full recovery of tendon injury still remains difficult to achieve in modern medicine. Scarring and reduced mechanical properties of the repaired tendon are always the issues for tendon injury. Previously we demonstrated that local injection of silver nanoparticles (AgNPs) in a rat tendon rupture model successfully alleviated inflammation, improved collagen alignment and the tensile property of the repaired tendon at an early time point of 6 weeks [1]. In this study, we would like to investigate the mechanism behind. In the literatures, it was proven that there is a strong link between inflammation and tissue fibrosis in healing [2-6]. In tissue injury, the release of danger-associated molecular patterns (DAMPs) stimulates the inflammatory response which is often too vigorous for the actual situation and leads to the formation of fibrotic tissues [7]. It is therefore hypothesized that the immunomodulatory effect of AgNPs plays a key role in achieving the improved healing.

**METHODS:** Rat tendon injury model and tensile test: 8 weeks-old female Sprague Dawley rat was used for the experiment. The Achilles tendon was transected at 0.5cm from its insertion and the wound was closed. AgNPs (1mM) were injected locally every 5 days. The treatment started on either day 0, 7 or 14 post operation (n= 4). The group without any treatment act as the control (Table 1). The tendons were harvested on day 42. The tensile property of the tendons was measured using the MTS 858 Mini Bionex with 100N load cells at a strain rate of 1mm/min. Immunofluorescence (IF) staining: Tendon injury was performed as described. AgNPs were injected from day 0 post operation and the rats were euthanized on day 1, 3, 7 and 10 post operation (n=3). The tissue was fixed, dehydrated, wax embedded and sectioned to 5µm thick sections. The sections were dewaxed, microwave heat retrieved, blocked and incubated at 4 oC overnight with rabbit anti-Ly6G (Santa Cruz, US; 1:200), anti-F4/80 (Bioss, US; 1:200) and anti-CD3 (Bioss, US; 1:200) antibody, followed by Alexa Fluor 488 goat anti- rabbit IgG secondary antibody (Invitrogen, US; 1:1000) for 1 hour and mounted with Vectashield mounting medium with DAPI (Vector, US).

**RESULTS:** Tensile test on the tendons showed a decreasing tensile modulus with a delay in AgNPs treatment (Fig.1). The tensile modulus of AgNPs\_D7 group was comparable to that of the AgNPs\_D0 group while that of the AgNPs\_D14 group has significantly reduced. The untreated group had the lowest reading among the groups.

For the IF staining, it was observed that neutrophils (Fig.2) and macrophages (Fig.3) mainly migrated from the peripheral tissue. For neutrophils, the peak of infiltration was on the first day after injury and decreased overtime while number of macrophages sustained over the first week and increased on day 10 post injury. When comparison is made between the untreated and AgNPs treated samples, it was observed that there were less neutrophils and macrophages infiltrated into the wound at all time points. On the other hand, the number of T-cells remained similar in both groups.

**DISCUSSION:** Inflammation is the first event in the healing process which usually takes place in the first two weeks after injury [4]. It is believed that a regulated inflammatory process gives a permissive environment for the subsequent healing events, such as tenocyte proliferation, proteoglycan and collagen deposition, and the remodelling process [1]. Fibrosis, which is a pathological state of accumulation of excessive collagen, would also be suppressed. The tensile test suggested that AgNPs treatment during the inflammation period is critical in achieving good healing in terms of mechanical property. A delayed treatment started after 14 days resulting in significantly lower tensile property. This is a proof that AgNPs have an effect on the inflammatory process.

To further understand how AgNPs influence the process, their impact on the inflammatory cells was investigated. Surprisingly, the infiltrations of neutrophils and macrophages were suppressed but not that of T-cells. Macrophages and neutrophils are the two types of cell that are heavily involved in the innate immune response. Upon injury, resident macrophages in the tissue are activated and secrete cytokines such as tumor necrosis factor and interleukin-1 to attract neutrophils and to amplify the inflammation loop. The reduction in both cells suggested that there was a suppression on the inflammatory response. T-cells, which are activated differently from macrophages, were not affected indicating the possibility that AgNPs specifically act on macrophages in the tendon healing model.

**Significance:** This study gave us new insights on the mechanism of AgNPs in promoting tendon healing. It clearly demonstrated that AgNPs possess immunoregulatory property and that an early application of AgNPs, thus reduced inflammatory response, is essential for improving the functionality of repaired tendon.