

# Losartan sustained-release nanofibers promote cartilage regeneration for osteochondral defects

Kohei Yamaura<sup>1</sup>, Nicholas Sather<sup>2</sup>, Haruki Nishimura<sup>1</sup>, Kiminari Kataoka<sup>1</sup>, Anna Metlushko<sup>2</sup>  
Chelsea S. Bahney<sup>1</sup>, Marc J. Philippon<sup>1,3</sup>, Samuel I. Stupp<sup>2</sup>, Johnny Huard<sup>1</sup>

<sup>1</sup> Center for Regenerative Sports Medicine, Steadman Philippon Research Institute, Vail, Colorado, USA

<sup>2</sup>Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, Illinois, USA; <sup>3</sup>The Steadman Clinic, Vail Colorado USA.

**Disclosures:** CSB (1 Iota Biosciences; 8 ORS ISFR, TERMIS, OTA, AO Foundation; 9 Associate Editor Journal of Tissue Engineering & Regenerative Medicine), MJP (1 Smith & Nephew, Arothsurface, Conmed; 9 AJSM Editorial Board); JH (1 Cooke Myocyte)

**INTRODUCTION:** Microfracture is an effective treatment for relatively small cartilage defects due to bone marrow stimulation. However, the cartilage tissue obtained by this repair method is primarily fibrocartilage, which often results in poor long-term outcomes. We have previously reported that oral and intra-articular administration of losartan, a TGF- $\beta$ 1 inhibitor, promotes hyaline cartilage regeneration in a rabbit osteochondral defect model.<sup>1,2</sup> However, systemic administration of losartan, an antihypertensive drug, raises concerns about side effects, and intra-articular administration presents the problem of short half-lives of low molecular weight compounds in the joint. Therefore, we have fabricated the injectable liquid-type peptide amphiphile (PA) nanofibers with sustained release of losartan as a solution to these problems and reported their efficacy *in vitro* for chondrogenic differentiation in an osteoarthritis chondrocyte model.<sup>3</sup> The purpose of this study is to evaluate the efficacy of intra-articular injection of losartan sustained-release PA nanofibers for cartilage regeneration in rabbits undergoing microfracture after osteochondral defects. We hypothesized that losartan nanofibers would promote cartilage regeneration in osteochondral defects.

## METHODS:

***In vitro* evaluation:** Based on our previous study, positively charged PA nanofibers with 5 mg/ml losartan were used in this study. Cell proliferation and toxicity in bone marrow mesenchymal stem cells (BMSCs) were evaluated *in vitro* according to losartan dose (-, 0.5, 5, 50, or 500 $\mu$ M, n=9 for each group). Subsequently, cell proliferation in BMSCs of losartan PA nanofibers was evaluated using the following four groups: i) control group (without losartan and PA nanofiber), ii) losartan alone group (without PA nanofiber), iii) PA nanofiber group (without losartan), and iv) losartan PA nanofiber group (n=9 for each group). Referring to the previous report that the most effective losartan concentration for human chondrocytes is 5  $\mu$ M, 1.2  $\mu$ l of PA nanofibers dissolved in 1 ml of milli-q water was implanted on a semi-permeable membrane due to the sustained release properties from PA nanofibers. The concentration of losartan solution equivalent to the amount of losartan contained by these losartan PA nanofibers was 25  $\mu$ M. Next, three-dimensional BMSC pellets were produced in two weeks and transferred to Transwell plates. Those pellets were then treated for one week in the above four groups. Those pellets were treated for one week in the above four groups. Those pellets were treated for one week in the above four groups, following which mRNA expression of chondrogenesis-related markers (Col IIa1, Acan, SOX9, and Col X) was compared. ***In vivo* evaluation:** Osteochondral defects (5 mm diameter and 2 mm depth) and microfracture were created in the distal femoral trochlear groove of 25 New Zealand White Rabbits aged 4 months (Fig. 3A). The rabbits were classified into the following five intra-articular injection treatment groups (n=5 for each group): i) control group (PBS 0.5ml), ii) losartan group (losartan solution 0.5ml, 1 mg losartan content), iii) PA nanofiber group (without losartan, 0.5ml), iv) early losartan PA nanofiber group (0.5 ml, 1 mg losartan content), and v) late losartan PA nanofiber group (0.5 ml, 1 mg losartan content). Only the late losartan PA nanofiber group was administered at 3, 6, and 9 weeks postoperatively, while the other groups were administered immediately after surgery and at 3, 6, and 9 weeks postoperatively. The rabbits were sacrificed at 12 weeks postoperatively and the distal femur was harvested. Quantitative evaluation of cartilage regeneration was performed using the International Cartilage Repair Society (ICRS) macroscopic cartilage evaluation score to compare between five groups.

**Statistical analysis:** All *in vitro* experiments were repeated at least three times. All plots and statistical comparisons were made using GraphPad Prism version 9.4.0 (GraphPad Software, La Jolla, CA, USA). Bar plots represent the mean  $\pm$  standard deviation with each individual replicate plotted as a dot on the bar graph. A prior power analysis (power 0.8, p<0.05) using G\*Power (v 3.1; Universitat Dusseldorf) based on a previous study calculated the number of sample sizes required for each group *in vivo* to be five. The groups were compared by analysis of variance (ANOVA) followed by Tukeys HSD post-hoc testing with statistical significance set at p < 0.05.

**RESULTS:** Losartan reduced BMSC cell proliferation at 14 days in a dose-dependent manner (Fig. 1A). Losartan nanofibers showed no significant inhibition of cell proliferation at 14 days compared to the control group and significantly increased cell proliferation compared to losartan solution containing the same dose (Fig. 1B). In addition, losartan nanofibers significantly increased aggrecan in chondrogenesis of BMSCs compared to the other groups (Fig. 2). Subsequently, losartan nanofiber treatment group significantly promoted macroscopic cartilage regeneration in a rabbit osteochondral defect model (Fig. 3B-C).

**DISCUSSION:** The key results of this study showed that losartan nanofibers promote cartilage regeneration in a microfracture model after osteochondral defect. This result suggested that positively charged, high molecular weight losartan nanofibers could release losartan more efficiently by allowing it to remain in the joints longer due to its electrical stability against negatively charged glycosaminoglycans and its molecular weight properties. They also allow for sustained release of losartan at effective concentrations that are not cytotoxic and promote chondrogenesis of BMSCs. Furthermore, the scaffolding effect of nanofibers was also suggested to efficiently proliferate BMSCs in the joints by bone marrow stimulation. The *in vitro* results shown in this study support these findings. We will further evaluate the histology, immunohistology, and  $\mu$ CT bone microstructure of the harvested femur in the future.

**SIGNIFICANCE:** Losartan sustained-release nanofibers may be a novel and clinically useful treatment for promoting cartilage regeneration after osteochondral defects.

**REFERENCES:** 1. Utsunomiya, H., et al. Am J Sports Med. 2020; 48: 974-984. 2. Logan, C.A., et al. Am J Sports Med. 2021; 49: 2509-2521. 3. Yamaura, K., et al. Front Bioeng Biotechnol. 2023.

**ACKNOWLEDGMENTS:** We gratefully thank the Shannon Foundation for their generous support of this collaborative research.

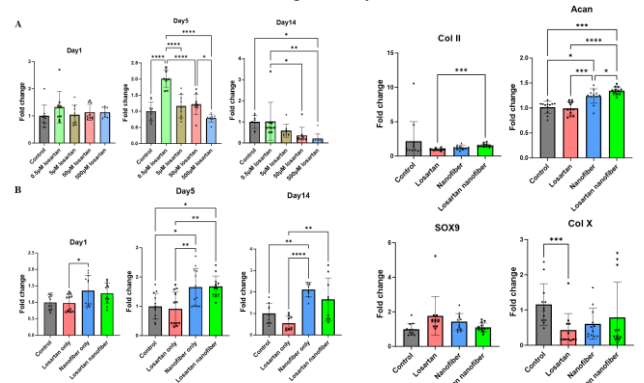


Figure 2. Effect of losartan nanofibers on mRNA expression of chondrogenesis-related markers in BMSCs

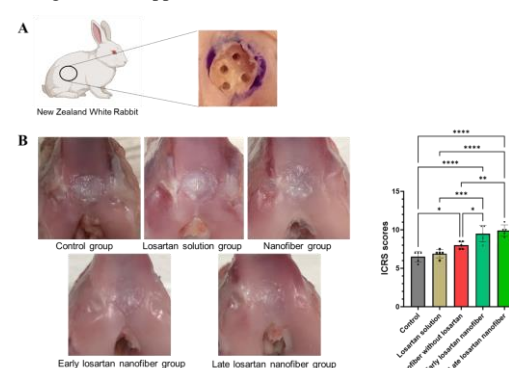


Figure 3. Effect of losartan nanofibers on cartilage regeneration for osteochondral defect  
A: Osteochondral defect and microfracture in trochlear groove of distal right femur in rabbits  
B: Comparison of macroscopic findings and ICRS scores among the five groups