

GsMTx4 reduces chondrocyte vulnerability to the injury *ex vivo*.

Rajkumar Govindan^{1,2}, Thomas Suchyna³, Whasil Lee^{1,2,4}

¹Department of Biomedical Engineering; ²Center of Musculoskeletal Research, University of Rochester Medical Center; ³University of Buffalo, NY;

⁴Department of Pharmacology and Physiology, University of Rochester Medical Center.

DISCLOSURES: No disclosures.

INTRODUCTION: Osteoarthritis (OA) is characterized by degradation of joint tissues over time. Mechanical stimuli applied to the knee joint plays a major role in the regulation of catabolism and anabolism axis of articular cartilage and in maintaining the homeostasis of the joint. Alterations to physiological loading such as injurious loading promotes the expression of catabolic enzymes which in turn destroy the extracellular matrix (ECM) of the articular cartilage and promote inflammation [1][2]. Mechano-vulnerability, an important factor for evaluating the wellness of the articular cartilage is defined as the ability of the cartilage to withstand the injurious mechanical loading [3]. Mechanosensitive channels such as Piezo1 and Piezo2 play a major role in the regulation of pain and subsequent progression of osteoarthritis by promoting inflammation through transducing the injurious mechanical loading [2][4]. These ion channels could be a potential drug target for treating osteoarthritis as the disease-modifying agents should address mechano-vulnerability as mechanical properties of chondrocytes and ECM directly influence this vulnerability. GsMTx4, a small peptide extracted from the spider venom, was shown to inhibit the activation of the Piezo1 channel. In addition, Piezo1 inhibition through GsMTx4 reduced chondrocyte apoptosis and alleviated OA in rats through the Calcineurin1/ NFAT1 pathway [5]. We previously developed a custom biopsy impact loader to study the mechano-vulnerability in mice through the confocal microscope. We modified the impact loader to study the vulnerability in porcine cartilage explants [4]. We also synthesized GsColD by modifying the GsMTx4 through the attachment of a peptide that targets the collagen type II of the articular cartilage. This study evaluates the alteration in the mechano-vulnerability of articular cartilage cultured *ex vivo* when it is treated with GsMTx4 and GsMTx4-coLD, pre-, and post-injurious loading.

METHODS: Cartilage explants were harvested from femoral condyles of the porcine knee using a 4-mm biopsy punch and cultured in DMEM supplemented with FBS (10%), HEPES, Non-Essential Amino Acids (NEAA), Antibiotics (100x) and Antimycotics. All the explants were used for the experiment within one day from the day of the harvest. The explants were randomly selected for all the groups. For the pre-treatment group, the explants were pre-treated with GsMTx4/GsColD and Calcein-AM (1:200) for 40 minutes at 37°C and injured using the impact loading device (ILD) equipped with 1-mm biopsy punch and imaged 3 minutes and 20 minutes post-injury. For the post-treatment group, the explants were first injured using the ILD followed by treatment with GsMTx4/GsColD and Calcein-AM for 40 minutes. After every injury, the explants were immediately imaged by a confocal microscope at the magnification of 10X. Propidium-iodide (1:50) was used to stain the dead cells following every injury step. Control explants followed the same experimental setup except for the incubation with GsMTx4 and GsColD. Expansion of thickness post-injury represents the vulnerability of cartilage to injurious loading. The maximum-intensity projection of all the samples was collected and the width/thickness of the hollow ring-shaped injury area of each sample was measured using ImageJ.

RESULTS: The results showed that the explants have reduced mechano-vulnerability when pre-treated with the GsMTx4 and GsColD as the thickness of injury is significantly low in the GsMTx4 group ($p < 0.005$) in comparison to the control. And similar observation was observed for the 20 minutes post-injury group. It is also observed that when the explants were treated with GsMTx4 and GsColD post-injury, the thickness of the injury is comparatively low in the GsColD group in comparison with the control while there is less reduction in the thickness of the injured area in GsMTx4 group in comparison to control.

DISCUSSION: We observed differences in the response of the efficacy of the drug when it is treated with pre- and post-injurious loading. The variation in response of the pre-treatment group and the post-treatment group could be attributed to the Piezo1 channel activation as the pre-treatment group had channel inactivation by GsMTx4 for 40 minutes which prevents the injurious signaling/inflammation mediated through Piezo1 upon injury. For the post-treatment group, GsColD has reduced mechano-vulnerability as the injured chondrocytes have higher Piezo1 activity as a result of mechanotransduction. These could be actively targeted by the GsMTx4 containing collagen II targeting peptide in comparison to native GsMTx4. We also observed the thickness of the injury after 20 minutes is relatively higher in control compared to 3 minutes while there is relatively less increase in the thickness of GsMTx4 and GsColD after 20 minutes when compared to 3 minutes. Regardless, the mechano-vulnerability was reduced when it was treated with GsMTx4 and GsMTx4 attached to collagen targeting peptide. The results show that GsMTx4 could be an ideal drug candidate for modulating mechanotransduction in the pathophysiology of osteoarthritis.

SIGNIFICANCE/CLINICAL RELEVANCE: Therapeutic options for OA are limited to addressing its symptoms and more efficacious treatments are currently explored for alleviating OA. GsMTx4 could be a potential drug that addresses not only the symptoms but also the underlying pathophysiology of the disease. Further in vivo and clinical studies might help elucidate the mechanism of action of GsMTx4.

ACKNOWLEDGEMENTS: NIH R35GM147054, R01AR082349 and P30AR069655.

REFERENCES: [1] Hodgkinson+ (2022). [2] Lee+ (2014). [3] Kotelsky+ (2022). [4] Lee+ (2021). [5] Ren+ (2023).

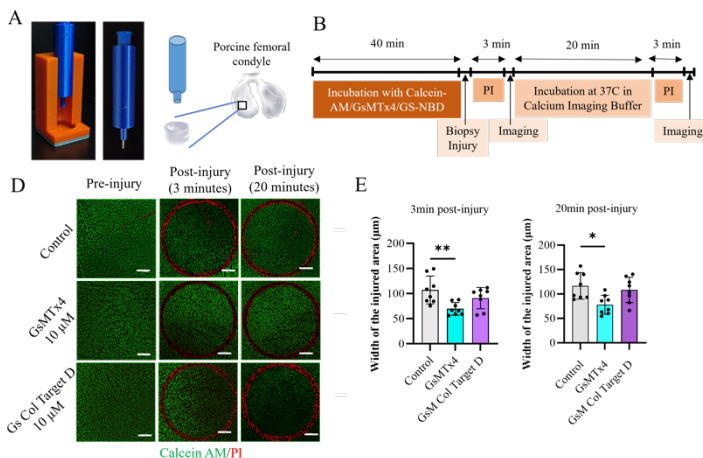


Fig 1. A) Impact Loading device (ILD). B) Schematic study design for the administration of drugs to the explants. C) Representative microscopic images of chondrocytes pre-treated with drugs. Scale=200μm. D) Effects on mechano-vulnerability of articular cartilage when pre-treated with GsMTx4 peptides. Width of the injured area was quantified by ImageJ. Data presented as Mean±SD; One-way ANOVA with Tukey's multiple comparisons test was used for statistics; * $P < 0.05$; ** $P < 0.02$

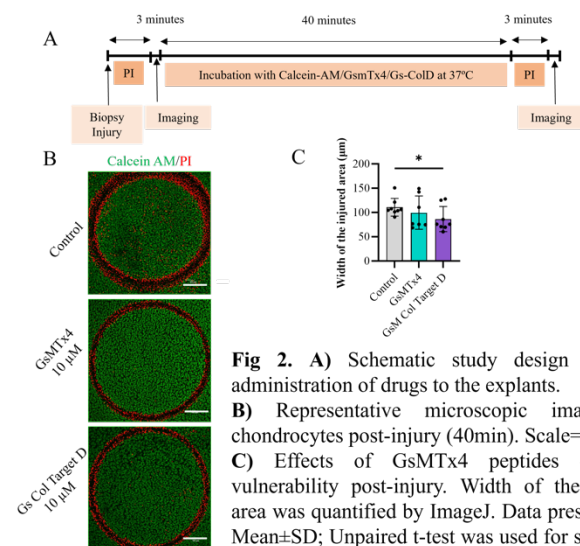


Fig 2. A) Schematic study design for the administration of drugs to the explants. B) Representative microscopic images of chondrocytes post-injury (40min). Scale=200μm. C) Effects of GsMTx4 peptides on cell vulnerability post-injury. Width of the injured area was quantified by ImageJ. Data presented as Mean±SD; Unpaired t-test was used for statistics; * $P < 0.05$