Modulation of macrophages on pathogenesis of degenerative disc disease

Jesse Wang, Zhang Yi, Grace Oh, Chi Li, Li Xiao, Jin Li, Xudong Li.

Introduction:

Low back pain (LBP) is a major contributor to disability with significant socioeconomic burden, and degenerative disc disease as a common cause. Inflammatory cells and cytokines mediate the progression of the disease with macrophages (MΦ) as a central modulator. MΦ can be classified into a pro-inflammatory (M1) and anti-inflammatory (M2) phenotype, serving distinct functions in various pathological contexts. We hypothesize that degenerated disc activates MΦ to the pro-inflammatory phenotype, which, in turn, exacerbates disc degeneration while the anti-inflammatory MΦ tames the inflammation and retards disc degeneration. The aim of this project is to determine the phenotypes and therapeutic potential of MΦ in disc degeneration.

Methods:

A novel mouse disc-on-a-chip microfluidic platform was previously established (**Fig 1A**), allowing continuous nutrients supply to permit long-term (up to 3 weeks) mouse disc culture. We customized the microfluidic co-culture device to study disc-MΦ interaction.

To establish a disc degeneration model (**Fig 1B**), freshly isolated lumbar discs from C57BL/6 mice were cultured in growth medium with varying concentration (0, 1, and 10 ng/ml) and duration (0, 1, and 5 days) of interleukin-1 (IL-1), 3 discs were cultured in each experimental group. Next, to study the molecular pathway of M1 activation during co-culture, quiescent MΦ (Raw 264.7 cells) were seeded onto an optical bottom plate overnight and co-cultured with degenerated discs (**Fig 1C**). At the conclusion of the treatment period, the culture medium and discs were assayed for nitrite concentration, amount of released glycosaminoglycans (GAG), and expression of various anabolic and catabolic genes via quantitative polymerase chain reaction (qPCR). *t*-test and one-way ANOVA with multiple comparisons were performed to compare the difference with 2 and 3 groups, respectively. For each condition, 3-4 independent experiments were performed.

Results:

We have established a disc degeneration model by pre-treating mouse lumbar discs with IL-1. Discs treated with increasing concentration of IL-1 demonstrated increased degradation of disc extracellular matrix as shown by increasing glycosaminoglycan in the growth media. Also demonstrating increasing serum nitrate level with increasing IL-1 concentration (**Fig 2A**). Increasing treatment duration from 1 day to 5 days at 1ng/ml of IL-1 also increases levels of GAG and nitrite in the media (**Fig 2B**).

Discs and macrophages were co-cultured on the on-chip microfluidic device and discs and growth media were analyzed. Discs co-cultured with MΦ increased levels of pro-inflammatory gene expression including IL-1, IL-6, TNFa and iNOS. This inflammatory gene expression was further increased when discs were pretreated with 1ng/ml of IL-1 (**Fig 3**).

Conclusion:

In this disc-MΦs microfluidic chip co-culture model, degenerated discs polarized M0 to M1 macrophages, and the M1 macrophages in turn worsened the disc degeneration, suggesting a vicious feedback cycle. Next steps will focus on role of anti-inflammatory (M2) macrophages on possibly slowing or reversing disc degeneration.

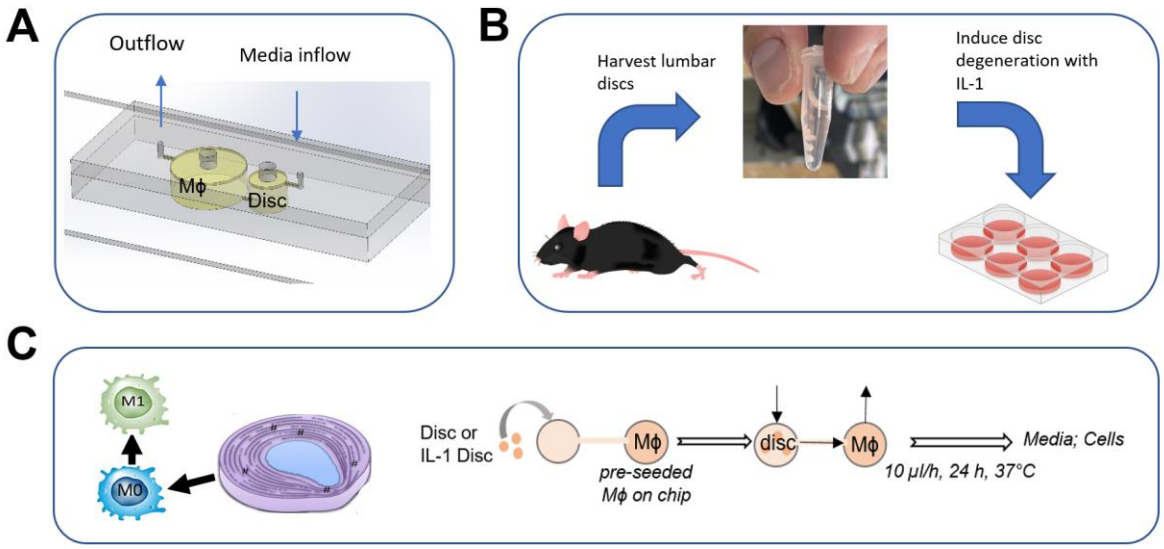

Figure 1. (A) Chip-on-disc culture device. (B) Disc degeneration model. (C) MΦ activation with degenerated disc.

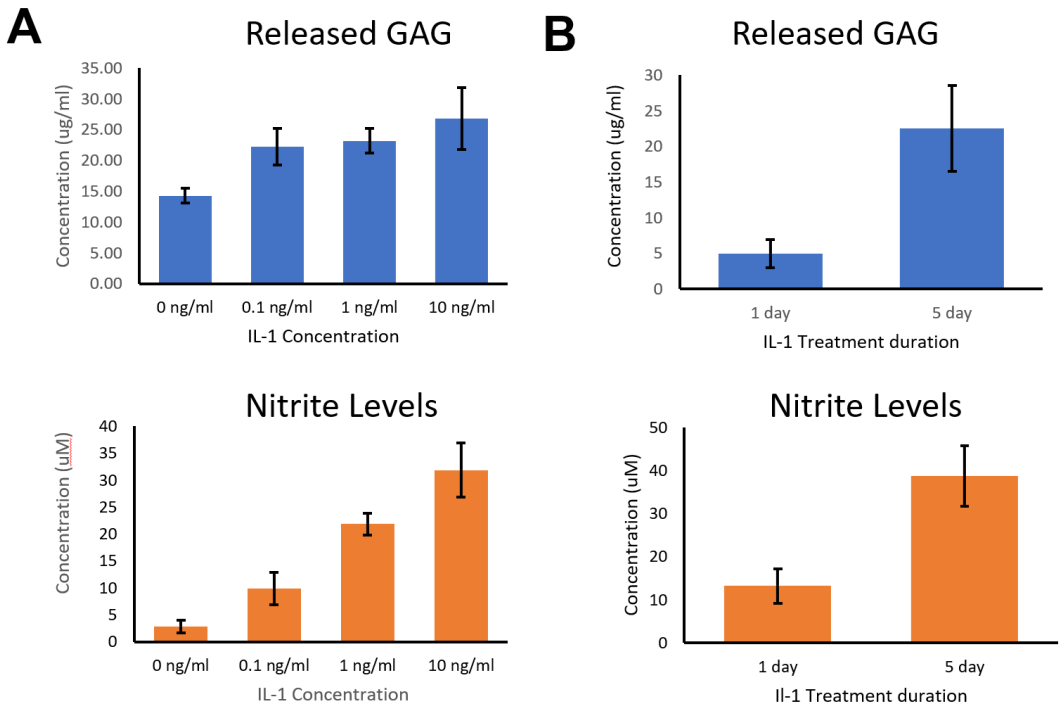

Figure 2. Increasing the (A) concentration and (B) duration of IL-1 treatment of mice discs increases release of GAG and nitrite in the culture media.

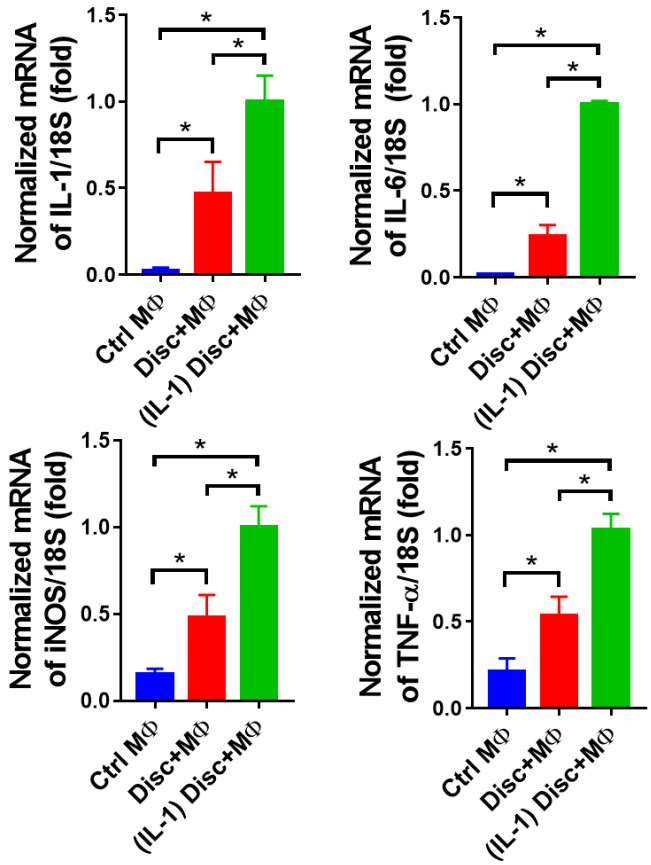

Figure 3. control discs promotes MΦ macrophage into pro-inflammatory (M1) phenotype, and IL-1 pretreated discs further increases inflammatory gene expression.