

Development of Engineered Cartilage Tissue Construct Maintaining Healthy Function of Cartilage Cells

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INTRODUCTION: The degradation of cartilage tissue affects millions of individuals each year and can emerge from a variety of factors. Abnormal biomechanical loads can often lead to suboptimal physiological conditions for cartilage cells and their surrounding matrix tissue. While excessive biological load is the traditional focus of cartilage damage, a lack of gravitational load can also result in cartilage tissue degradation. In the absence of biomechanical loading, cartilage cell functions may be damaged and tissue homeostasis is likely to be lost. While there are many tissue engineering efforts to produce cartilage models on Earth, there is a lack of successful cartilage tissue engineering efforts in microgravity due to the unfavorable microenvironment for long term cartilage homeostasis. To overcome this challenge, we have developed a cartilage tissue construct, the Multiple-Compartment Tissue Engineered Construct (MTEC) comprised of our injectable solid scaffold named Janus Base Nano-matrices (JBNMs) with our innovative RNA delivery vehicle, Janus Base Nanopiece (JBNPs) (**Figure 1**). Our MTEC not only maintains cartilage cell chondrogenesis and long-term homeostasis, but also delivers mechanotheapeutic RNAs, such as miRNA140, to overcome abnormal biomechanical loading on cartilage including the microgravitational environment. To investigate the MTECs therapeutic efficacy, we will be testing chondrogenic differentiation of hMSC on the International Space Station (ISS), where microgravity may factor into cartilage health and homeostasis. We hypothesized that the MTEC can be utilized to maintain cartilage homeostasis under the influence of microgravity and evaluate the therapeutic potential of RNAs.

METHODS: JBNm and JBNps were synthesized separately, and characterization of these nanomaterials included transmission electron microscopy (TEM), bright-field microscope, UV-Vis, and zeta potential. Biological experiments were conducted *in vitro* on human mesenchymal stem cells (hMSC) and included cell viability, adhesion, proliferation, differentiation, and transfection studies.

RESULTS SECTION: Synthesis of JBNm and JBNp combinations were optimized to maximize therapeutic potential of the MTEC components. Cell proliferation and viability assays demonstrated that *in vitro* culture with the MTEC resulted in significantly improved cell numbers and health respectively. Furthermore, rt-PCR methods demonstrated that the delivery of miRNA140 using JBNps were shown to induce chondrogenic hMSC differentiation. Chondrogenic markers, including COL2, ACAN, and SOX9 were upregulated while expression levels of COL1, COLX, and IHH were unaffected or partially diminished (**Figure 2**). Furthermore, GAG staining of miRNA140 transfected hMSC revealed a significant increase in ECM production and chondrogenic differentiation after 1 week. MTECs also demonstrated the ability to transfect hMSC with fluorescence labeled RNAs over the course of multiple weeks, proving its potential to deliver functional RNAs to cells encapsulated within the MTEC.

DISCUSSION: Cartilage degradation in the presence of microgravity is understudied due to the lack of cartilage engineering efforts in unfavorable growth conditions and access to microgravitational environments. An MTEC capable of increasing cellular adhesion, proliferation, and differentiation while also delivering mechanotheapeutic RNAs is a promising platform to investigate the therapeutic potential of JBNm and JBNp for cartilage differentiation and homeostasis in microgravity. Furthermore, these MTECs will be tested on Earth as well as the ISS which presents a unique opportunity for us to analyze the MTECs ability to counteract cartilage degradation in the presence of a microgravitational environment. This project also allows us to utilize JBNps in combination with JBNm as a drug-loaded scaffold capable of both cell anchorage as well as RNA delivery. This way we may recruit cells to JBNm, allow proliferation and differentiation to occur while simultaneously influencing the transcriptomic activity over the course of many weeks.

SIGNIFICANCE/CLINICAL RELEVANCE: The development of our MTEC highlights the capabilities of JBNm and JBNp as a drug-loaded scaffold capable of influencing cell behavior, including proliferation, viability, and differentiation. By testing these MTECs on Earth and the ISS, we gain a unique opportunity to understand the effects of mechanotheapeutic RNA delivery and JBNm scaffolding on chondrogenic differentiation and homeostasis in microgravity and inadequate biomechanical loading.

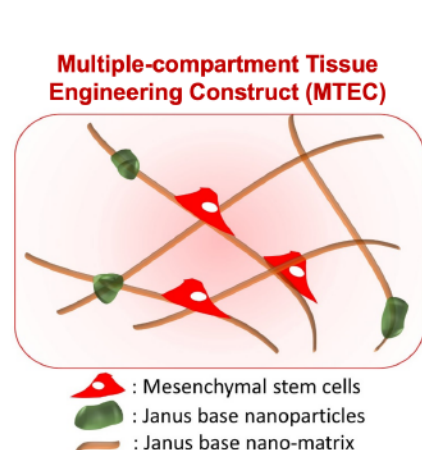


Figure 1 MTEC comprised of JBNm and JBNp scaffold to house hMSC growth.

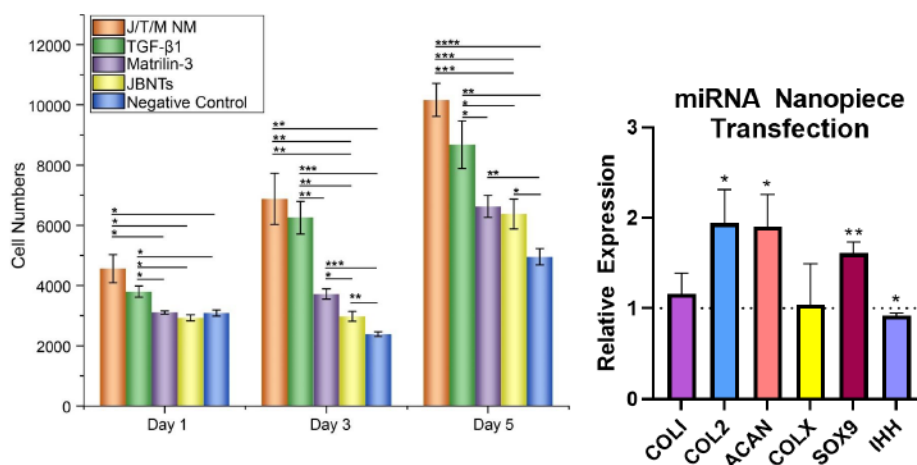


Figure 2 JBNm (left) supports significant cell proliferation over 5 days. JBNp (right) can effectively deliver and induce chondrogenic differentiation after just 48 hours.