A Library of Janus Base Nano-Matrices for Tissue Engineering

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INTRODUCTIONS: Scaffolds are designed to create a microenvironment triggering expected biochemical cues for musculoskeletal tissue regeneration. Although various biomaterial scaffolds have been developed to promote cell adhesion and subsequent functions, conventional scaffolds usually focused on their biocompatibility and bioactivity on one specific type of cells while largely overlooked the influence on other types of cells in the same tissue or neighboring tissues. Therefore, conventional scaffolds have limited selectivity on influencing cell functions. Here, we developed a library of Janus base nano-matrices (NMs) based on DNA nanotechnology. The NMs are self-assembled from DNA-mimicking Janus base nanotubes (JBNTs) and extracellular matrix (ECM) proteins. JBNTs, morphologically mimicking collagen fibers, been shown to improve cell adhesion and proliferation. Importantly, JBNTs have no selectivity on different types of cells, but after they were assembled with ECM proteins into Janus base NMs, the NMs presented selectively bioactive on different types of cells. For example, we have showed that the JBNT/Matn1 NM can promote human mesenchymal stem cell (hMSC) and chondrocyte adhesion while simultaneously suppress bone and vascular cell adhesion. In this manner, the JBNT/Matn1 NM may be used as a smart scaffold to specifically enhance cartilage regeneration while inhibit bone growth. As a summary, we have developed a library of Janus base NMs. They are versatile scaffolds that can selectively promote different cell lineage adhesion and functions, overcoming the limitation of low selectivity of conventional scaffolds.

METHODS: Four types of NMs with different proteins, matrilin-1 (Matn1), matrilin-3 (Matn3), cartilage oligomeric matrix protein (COMP) and fibronectin (FN) along with JBNTs, were prepared as per previously published standardized protocols, observed and characterized with ZOE Fluorescent Imager, transmission electron microscope (TEM), zeta potential, and UV-Vis. HMSCs, chondrocytes cells (C28/I2), human umbilical vein endothelial cells (HUVECs), osteoblasts are seeded into slides coated with NM for four hours before fixing, stained, and observed in confocal microscopy.

RESULTS SECTION: JBNTs and proteins self-assembled to form NMs bundles (JBNT/Matn1, JBNT/Matn3, JBNT/COMP, and JBNT/FN) can be observed in ZOE fluorescent Cell Imager imaging seen in **Figure 1(b-e)**, respectively. JBNTs can also be observed in **Figure 1 (a)**. **Figure 2** shows the quantitative heatmap of NMs against negative control (NC) without any additives on cell numbers after measurement and calculation taken with ImageJ.

DISCUSSION: Our NMs scaffold are advantageous due to the fibrous nature of our JBNTs, morphologically and biologically mimicking collagen, a major component found in the ECM of the human body seen in **Figure 1 (a)**. Though lacking in selectivity, JBNTs are served as a backbone, can self-assemble with different ECM proteins forming variety of NMs with different morphologies as seen in **Figure 1 (b-e)**. The versatility of JBNT allows for the fabrication of different NM resulting vastly different bioactivities. **Figure 2** shows that different NMs influenced different cell functions. For example, the JBNT/Matn-1 NM promoted hMSC and C28/I2 functions while inhibiting osteoblasts and HUVECs. This proves the JBNT/Matn-1 NM is to be suitable for cartilage tissue engineering showing anti-hypertrophy properties and promotes chondrogenesis, supporting the results of our study. On the other hand, JBNT/FN NM promoted hMSC and HUVEC functions but inhibited osteoblasts and chondrocytes, which may be used for angiogenesis and soft tissue repairs. We also further demonstrated that our biocompatible and injectable NM-based JBNTs can be customized for different microenvironments depending on various needs. The potential application of our NM library as an injectable scaffold would be highly beneficial for future therapeutic applications.

SIGNIFICANCE/ CLINICAL RELEVANCE: The NM library has shown great promising as an injectable solid scaffold for different treatment especially for those hard-to-reach injured sites. Our biocompatible and injectable NMs is a versatile platform where different microenvironments can be achieved.

IMAGES AND TABLES:

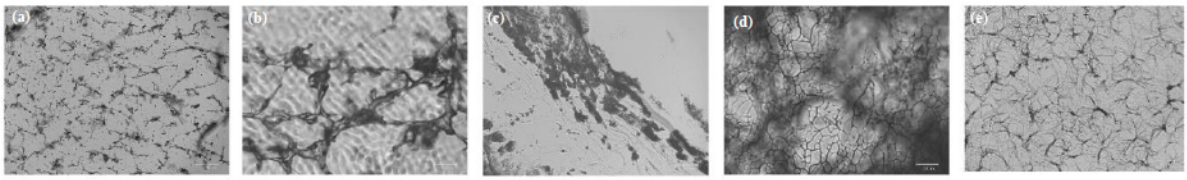

Figure 1. Morphologies of different NMs taken in ZOE Fluorescent Cell Imager, (a) JBNTs only, (b) JBNTs/Matn-1, (c) JBNTs/Matn-3, (d) JBNTs/COMP (e) JBNTs/FN

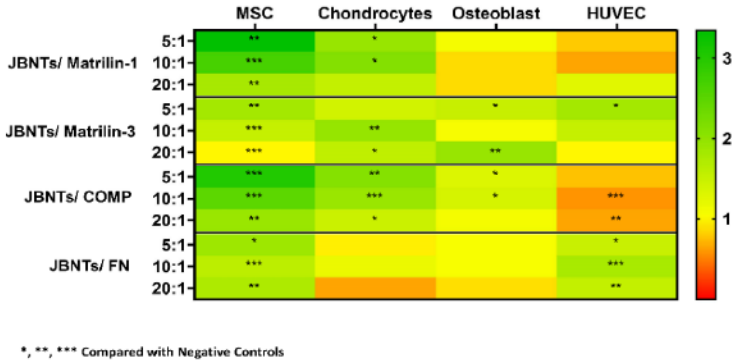

Figure 2. Qualitative cell adhesion compared to negative control without additives heatmap. Significant differences are shown where *p<0.05, **p<0.001, ***p<0.0001 and ****p<0.0001.