Novel bio-scaffolds Janus Base Nano-Matrix for Mesenchymal Stem Cell homing

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INTRODUCTION: Bone fractures that occur in patients with osteoporosis are common and frequently, which is not only cause great pain but also bring a heavy economic burden to the patients. Bone fracture healing is difficult especially for older, as well as osteoporosis patients because the bone loss is mainly based on age and disease. Activation and migration of human mesenchymal stem cells (hMSCs) have been shown to play an important role in fracture healing, because hMSCs have the ability to promote tissue repair, angiogenesis, and reduce inflammation. Variously engineered scaffolds have been utilized to attract hMSCs to accumulate in a targeted fracture location, however, some locations are not easy to access for conventional pre-fabricated scaffolds. In this study, we fabricated an injectable novel scaffold composed of DNA derived nanotubes-Janus Base Nanotubes (JBNTs) and extracellular matrix adhesive glycoprotein Fibronectin (FN). The JBNTs are designed to morphologically mimic collagen fibers, so FN can self-assemble with them via non-covalent bonding into a Nano-Matrix. The injectable Nano-Matrix enhances hMSCs migration and adhesion in vitro and holds great promise to serve as a scaffold for tissue regeneration.

METHODS: JBNTs were synthesized by an effective approach published previously. FN (Gibco) aqueous solution was added into H_2O and pipetted for several times. JBNTs were pipetted into the FN aqueous solution to form Nano-Matrix. Transmission electron microscope (TEM) was used to observe the morphology of JBNTs and Nano-Matrix. Cell adhesion density was determined by counting the number of cells co-cultured with negative controls, JBNTs, FN and the JBNT/FN Nano-Matrix under the fluorescent microscope. A transwell method was used to determine cell migration on the Nano-Matrix.

RESULTS SECTION: The Nano-Matrix of JBNT and FN formed very quickly, in a matter of seconds. Under neutral conditions, JBNTs and FN are positively and negatively charged, respectively driving their complexation via charge interactions. As shown in the TEM images, JBNTs bonded with FN tightly and formed long fibroid Nano-Matrix (**Figure 1A, B**). When pH is dropped below the isoelectric point of the FN (pI=5.5-6.0), the Nano-Matrix bundles disassembled due to the positively charged FN (**Figure 1C**). Cell adhesion and migration density is one of the parameters used for analysis for the experiment. In vitro experiments demonstrate that Nano-Matrix enhanced hMSCs adhesion and migration significantly compared to control groups (**Figure 2A, B**). This may due to the JBNT/FN Nano-Matrix enhanced the focal adhesion between the substrate, cell membrane, and filopodia, which is critical for cell migration and adhesion.

DISCUSSION: We fabricated a novel self-assembled biomimetic Nano-Matrix with DNA based nanotubes JBNT and glycoprotein FN. The Nano-Matrix was composited of JBNTs and FN by self-assembled in aqueous solution. The biomimetic extracellular matrix can provide skeletal support for hMSCs cytoskeletal driven activities. In our experiments, we have demonstrated that the Nano-Matrix improved the migration and adhesion of the hMSCs without any additive.

SIGNIFICANCE/CLINICAL RELEVANCE: The injectable Nano-Matrix has shown great promise as an injectable scaffold for hard to reach injury sites to provide a target location and a scaffold for hMSC homing. Based on the pH dependent release behavior and the hydrophobic core, the Nano-Matrix also has potential to serve as a drug delivery platform, which will be explored in subsequent experiments.

IMAGES:

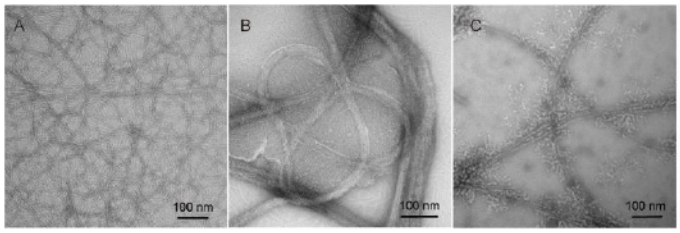

Figure 1. (A) TEM image of JBNTs. (B) TEM image of JBNT/FN Nano-Matrix. (C) TEM image of dis-assembled JBNT/FN Nano-Matrix.

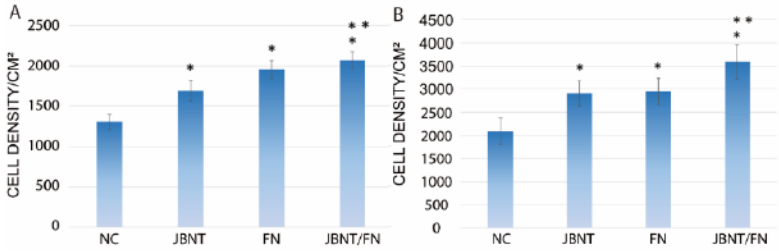

Figure 2. (A) Statistical analysis of cellular adhesion. Cell adhesion density was recorded in this experiment. *p<0.01 compared to negative controls. **p<0.05 compared to JBNT alone. N=3. (B) Statistical analysis of cell migration. *p<0.01 compared to negative controls. **p<0.05 compared to JBNT or FN alone. N=3