Improving Mesenchymal Stem Cell Homing by Self-Assembled Fibronectin/Janus-Base Nanotube Matrix

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Disclosure: Yupeng Chen (Nanode Therapeutics)

INTRODUCTION

Approximately 6 million people suffer fractures each year in North America; and 5 to 10 percent of those cases involve patients who either have delayed healing or fractures that do not heal. Mesenchymal Stem Cells (MSCs) can differentiate into bone forming osteoblasts to aid in fracture healing. To guide MSC homing into the fracture site, we developed a Nano-matrix which can be injected into the target location, then self-assemble into a cell growth scaffold. The Nano-matrix is formed by Janus base nanotubes (JBNTs) and Fibronectin (FN). JBNTs are nucleobase-derived nanotubes mimicking collagen fibers; and FN is one of the cell adhesive glycoproteins, is responsible for cell-extracellular matrix interaction by guiding the stem cells to migrate or to differentiate to desired cells types. Here, we will show the successful fabrication and characterization of the Nano-matrix as well as their excellent bioactivity in encouraging human mesenchymal cells (hMSCs) adhesion and migration. This work lays a solid foundation for using the Nano-matrix as an injectable approach to improve hMSC homing and function for bone fracture healing.

MATERIALS AND METHODS

For material development and characterization, different ratios of JBNT and FN are assembled together at the physiological environment. The self-assembled Nano-matrix was characterized using light and electron microscopes. For stem cell homing, cell adhesion and migration experiments are conducted. Briefly, negative controls (no additives), JBNTs alone, FN alone and the JBNT/FN Nano-matrix are prepared and air-dried in a cell culture plate. hMSCs are cultured in stem cell growth media with 10% FBS and 1% P/S solution and seeded into the cell culture plate. After 4 hours adhesion or 24 hours migration experiments. The cells are fixed, stained and then taken in 10x20 magnification microscope with image capture equipment. The images are obtained and analyzed with a computer and a software for analysis (IMAGEJ)

RESULTS

Material characterization results showed the biomimetic process between the JBNT and FN. As shown in **Fig. 1**, transparent JBNT and FN solutions are mixed, they were able to self-assemble into the nano-matrix in ~16 seconds. **Fig. 2** demonstrated the morphology of the Nano-matrix compared to the FN alone which does not form any matrix structure. The stem adhesion results showed that the Nano-matrix dramatically increases stem cell adhesion. It also encourages stem to present an elongation morphology that represents stem cells' nature (**Fig. 3**). Furthermore, we also observed the significant increase of cell migration with on the Nano-matrix (the figure was not shown). With the results seen in stem cell migration and adhesion, it demonstrated that the Nano-matrix facilitates stem cell homing to the desired the site.

DISCUSSION

We were able to successfully fabricate and characterize the self-assembled nano-matrix. Important to note, this self-assembly process is based on the negative/positive charge interaction without the aid of chemical initiators or UV light. Therefore. it is a biomimetic and biocompatible process similar to the binding between collagen and FN during ECM formation. Moreover, the JBNT/FN Nano-Matrix was shown to enhance MSC migration and adhesion onto it. In this manner, the Nano-Matrix may serve as an advanced scaffold to improve stem cell homing at the injury site for tissue healing. Especially, our results also demonstrated that the Nano-Matrix may be able to promote MSC growth and differentiation. Therefore, the JBNT/FN Nano-Matrix is very promising for improved fracture healing.

SIGNIFICANCE

Based on our experiment, we can conclude that the JBNT/FN Nano-matrix can be used to help guide MSCs homing into a target site (such as the bone fracture site) due to the increase in stem cell migration and adhesion. This development can possibly be the first step to aid in bone healing the fracture sites through this injectable and self-assembled Nano-matrix.

ACKNOWLEDGEMENT

This study was supported by NIH grants (R03AR0693831 and R01AR072027) and NSF CAREER award (1653702).

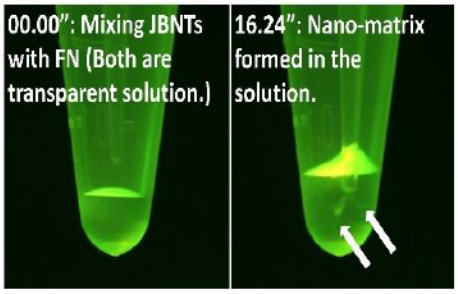

Figure 1. A video screenshot showing when JBNT and FN mixed in water, they self-assembled into the Nano-Matrix in a few seconds.

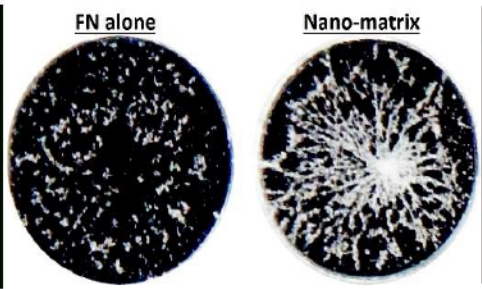

Figure 2. Image of FN alone compared to Nano-Matrix after the addition of JBNT in light microscope.

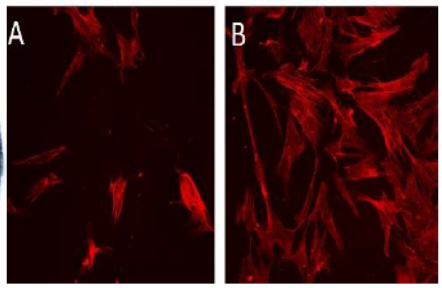

Figure 3. This is a cell adhesion experiment where figure 3(A) is negative control and figure 3(B) is the Nano-Matrix.