A bionic layer-by-layer drug-released Nano-Matrix for mesenchymal stem cells chondrogenic differentiation promotion and chondrocytes hypertrophy prevention

Libo Zhou[1], Jinhyung Lee[1],Wuxia Zhang[1], Yupeng Chen[1]
[1]Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269, USA.
Email: libo.zhou@uconn.edu; jinhyung.lee@uconn.edu; wuxia.zhang@uconn.edu; yupeng.chen@uconn.edu

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INTRODUCTION: Biocompatible scaffolding materials, bioactive molecules and stem cells have been widely utilized in cartilage engineering to mimic the properties of native cartilage tissue. However, there is still a major challenge in how to accurately induce stem cells to chondrogenic differentiation at the desired location. Moreover, conventional cartilage tissue engineering methods usually focused on promoting chondrogenesis while ignored to inhibit hypertrophy and other differentiations. Additionally, bioactive molecules released with conventional drug release strategies usually leak into undesired areas and cause adversary effects. To overcome those these limitations, we designed an innovative biomaterials platform with the ability to promote the chondrogenic differentiation of stem cells and inhibit hypertrophy of chondrocytes at the target location. The scaffold was formed based on novel organic nanomaterials, Janus base nanotubes (JBNTs). We have assembled Matrilin-3 (an anti-hypertrophic cartilage protein) and Transforming Growth Factor Beta-1 (TGF- β1, a chondrogenesis growth factor) into JBNTs to form a Nano-Matrix (abbreviated as J/M/T Nano-Matrix). Different from semisolid hydrogels, the J/M/T Nano-Matrix is a solid mesh which provides better cell anchorage via its fibrous structure. Moreover, the J/M/T Nano-Matrix is also different from conventional pre-made solid scaffolds. It can be injected in a solution into "difficult-to-reach" deep tissues. For example, it can be injected into irregular-shaped fractures or cavities to form a scaffold for cell and tissue regeneration. Furthermore, we have developed an innovative layer-by-layer strategy to create different layers in each nanometer-sized fiber of the Nano-Matrix. In this manner, we can confine TGF- β1 into a specific location inside the matrix to achieve precisely controlled release of growth factors. In short, the J/M/T Nano-Matrix presents many innovations in materials and it is very promising to be applied for cartilage injure repair.

METHODS: Nano-Matrix was formed by the self-assembled of JBNTs, Matrilin-3, and TGF-β1. Transmission electron microscope (TEM) was used to observe the morphology of Nano-Matrix and JBNTs. The layer-by-layer structure of Nano-Matrix was characterized with confocal microscopy after labeling protein with fluorescent dyes. Nano-Matrix and other control groups of solutions were added into a chambered coverglass and freeze-dried for cell adhesion experiment. Human mesenchymal stem cells (hMSCs) were seeded into the coverglasses and cultured for 4 h. The morphology of hMSCs was characterized by a confocal microscopy. Nano-Matrix and other four groups of control solutions were added into a none-treated 96-well plate and freeze-dried for cell proliferation experiment. The hMSCs were seeded into the plate and incubated with materials. After 2 days. CCK-8 method was used to calculate the numbers of cells. hMSCs were wrapped in agarose gel and cultured with different mediums. After 14 days, cell differentiation was explored with RT-PCR.

RESULTS SECTION: J/M/T Nano-Matrix formed very quickly, in a matter of seconds. As shown in Figure 1A, the JBNTs are single and thinner nanotubes with diameters around 3.5 nm. While assembled with proteins, obvious scaffold structures formed by the thick bundles of JBNTs. When zooming in one section of the scaffold, the layer by layer structures can be observed, which was considered assembled by the JBNTs and proteins. As shown in Figure 1B, proteins are distributed along the nanotube and formed long bundles. From the merged image, it is discovered that TGF-β1 (green fluorescence) were wrapped by Matrilin-3 (red fluorescence). Cells co-cultured with Nano-Matrix looks much stretcher than other groups, indicating that Nano-Matrix makes it easier for cells to adhere on the chambered coverglasses (Figure 2A). As shown in Figure 2B, after only one day of incubation, the cells of Nano-Matrix and TGF-β1 groups showed a distinctly higher proliferative ability than the Matrilin-3, JBNTs, and negative control groups. Cell differentiation experiment showed that Nano-Matrix promote mesenchymal stem cells chondrogenic differentiation and prevent further hypertrophy of chondrocytes obviously.

DISCUSSION: We fabricated a bionic layer-by-layer drug-released Nano-Matrix with DNA based nanotubes JBNTs, TGF-β1, and Matrilin-3. The biomimetic extracellular matrix can provides skeletal support and biomolecule stimulation for hMSCs. We have demonstrated that the J/M/T Nano-Matrix improved the adhesion, proliferation and chondrogenic differentiation of the hMSCs without any additive. Although J/M/T Nano-Matrix has been proved a big promising candidate for promoting chondrocytes regeneration, in vivo study should be carried out to further explore its treatment effect for cartilage injuries.

SIGNIFICANCE/CLINICAL RELEVANCE: J/M/T Nano-Matrix has shown great promising as an injectable solid scaffold for stem cell treatment, especially for those injuries happened in deep tissues.

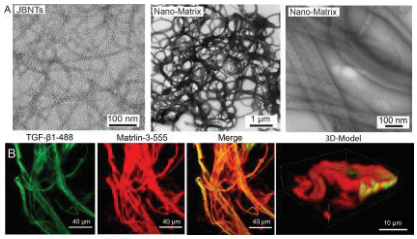

Figure 1.（A) TEM images of JBNTs and J/M/T Nano-Matrix at different magnification. (B) Confocal images of J/M/T Nano-Matrix formed with JBNTs and fluorescent dyes proteins.

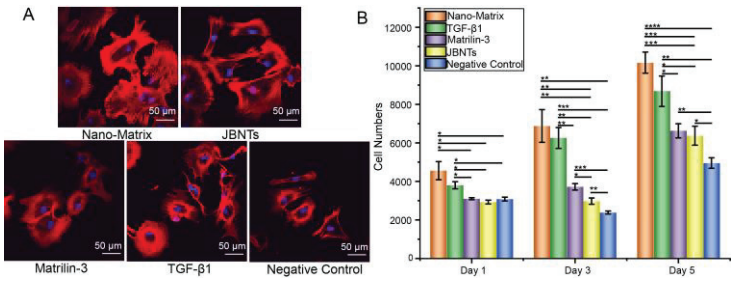

Figure 2. (A) Confocal images of hMSCs cultured on chambered coverglass precoated with different materials. (B) Statistical analysis of hMSCs proliferation. *P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. n=6