# Multifunctional Matrix Self-Assembled from Matrilin-3 and Rosette Nanotubes for Cartilage Repair

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**Statement of Purpose:** Numerous biomaterials have been designed for cartilage repair. However, significant obstacles still remain including the adhesion of neo-cartilage tissue constructs with native tissues and the release of catabolic matrix metalloproteinase during wound healing. Here, an advanced biomimetic matrix were developed from the self-assembly of rosette nanotubes (RNTs) and a native cartilage specific extracellular matrix protein, matrilin-3 (MATN3). Particularly, RNTs improve chondrocyte adhesion by forming collagen-fibril-like nanotubes; and MATN3 enhances chondrogenesis and inhibits expression of catabolic genes such as MMP-13. We proposed that through biomimetic self-assembly, RNTs and MATN3 can form a multifunctional chondroprotective matrix for cartilage repair. Results showed that in only a few seconds, RNTs and MATN3 assembled to a cartilage-like matrix under the physiological environment. Especially, such novel bioactive matrix possessed multifunctional advantages from both, thereby enhancing chondrocyte adhesion and matrix integration as well as inhibiting catabolic gene expression. Therefore, we have developed multifunctional self-assembled matrix for advanced cartilage repair.

**Methods:** Material characterization of MATN3/RNT matrix was completed via transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS). Cell adhesion study was performed by seeding chondrocytes (ADTC5) with or without MATN3/RNT. After 4 hours, adherent cells were then fixed, stained and counted. Biomechanical test of the adhesion strength of cartilage plugs coated with the MATN3/RNT was performed on articular cartilage from young adult pigs. MATN3/RNT was applied to 4-mm-diameter explants of 10-mm-diameter full thickness cartilage specimens. After healing for 3 days, a push-out test was performed and the maximum load was recorded. Gene expression was quantified using real-time RT-PCR for aggrecan and MMP-13 mRNA levels, which was normalized to 18S rRNA.

**Results and Discussion:** TEM analysis showed that while RNTs independently assembled into collagen fibril-like structures (Fig. 1, left), in only a few seconds in water, MATN3 self-assembled with RNTs to form much wider and larger matrix (Fig. 1, middle). MATN3 (white dots confirmed by EDS, Fig. 1, right) adhered in between RNTs. Naturally, MATN3 is a connective molecule binding between various cartilage matrix proteins. Therefore, such result may due to a biomimetic process that MATN3 bind with collagen-fibril-like RNTs to form large bundles and then the whole matrix, as shown in Scheme 1.

Furthermore, cell adhesion analysis showed that the MATN3/RNT matrix drastically increased chondrocyte adhesion. In addition, compared to the cells cultured on a regular culture plate, chondrocytes on the MATN3/RNT generated more cell filopodia and showed a more spreading morphology, indicating a stronger cell adhesion.

Then, *ex vivo* biomechanical test showed that MATN3 and MATN3/RNTs enhanced the mechanical loading to push explants out, indicating a better bio-integration and healing with such two groups.

Lastly, gene expression analysis of cell functions showed that MMP-13 mRNA levels were decreased more than 60% after chondrocytes were cultured on the MATN3/RNT hybrid matrix for 24 hours while the mRNA levels of aggrecan increased 60% after cells were cultured on the hybrid nanotubes for 72 hours (Fig. 2).

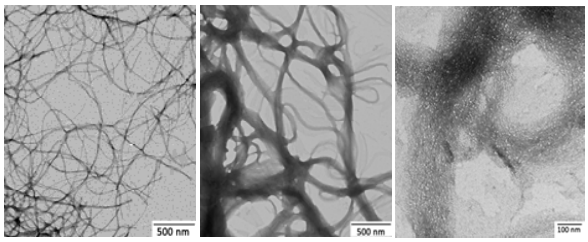
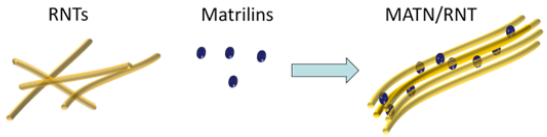



*Fig. 1. Morphologies of RNTs only (left) and RNTs assembled with MATN3 (middle) and MATN3/RNT in a higher magnification (right).*




*Scheme 1. Chondrocyte morphology on a regular culture plate (controls, left) and on RNT/MATN3 matrix (right).*

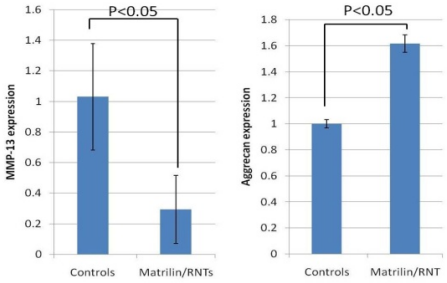



*Fig. 2. MMP-13 and aggrecan expression of chondrocytes on a tissue culture plate (controls) and on RNT/MATN3.*

**Conclusions:** Our data showed that the hybrid MATN3/RNT matrix enhanced chondrocyte adhesion in vitro and cartilage explant biointegration to the native tissues ex vivo. Therefore, the novel matrix is not only chondroconductive by enhancing chondrocyte adhesion and tissue integration, but is also chondroprotective by stimulating chondrocyte anabolic gene expression and inhibiting catabolic gene expression. These synergistic effects achieved by the hybrid nanomaterails may be highly beneficial to enhancing the tissue repair process for advanced cartilage repair.