Drug Deliverable, Self-assembled Nanotubes for Bone Tissue Engineering

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Statement of Purpose: Rosette nanotubes (RNTs) are novel, biomimetic, synthetic, self-assembled drug delivery agents. Because of base stacking and hydrophobic interactions, the RNT hollow-tube structure can be used for incorporating drugs. Another advantage of using RNTs is their ability to be injected and become solid at body temperatures for orthopedic applications without the use of any external stimuli (such as UV light or crosslinking agents). The nano-features of RNTs create a favorable, biologically-inspired, cellular environment.

In this study, methods to incorporate dexamethasone (DEX, a bone growth promoting drug) into RNTs were investigated. The drug-loaded RNTs were characterized using Nuclear Magnetic Resonance (NMR), Diffusion Ordered Spectroscopy (DOSY), Ultraviolet-visible Spectroscopy (UV-vis), and Atomic Force Microscopy (AFM). Results showed that small molecular drugs with hydrophobic aromatic rings were incorporated into RNTs. Subsequent drug release experiments demonstrated that dexamethasone (DEX) was released from the RNTs and had a positive impact on osteoblast functions. Importantly, compared to other drug carriers, RNTs increased total drug loading and was the highest when DEX was incorporated during the RNT self-assembly process. Thus, this study offered a novel drug delivery device that itself is bioactive and can be used to deliver a variety of drugs for various orthopedic applications.

Methods: For drug-loading studies, RNTs with lysine side chains (RNTs-k1) were first dissolved in d-methanol. Then t-butanol (Sigma Aldrich) was added as a standard control for comparing any decrease in DEX integration on ^{1}H NMR. Lastly, excess DEX (Sigma Aldrich) was added for drug loading giving a 5mg/mL DEX to d-methanol solution. For this study, a ratio of 5:3 DEX to RNTs was studied. The percentage of the diminishing drug peak was recorded compared to the standard to determine the amount of drug loaded.

For controlled release DEX experiments, glass coverslips (circular; Dia: 18mm; Thick: 1mm; Fisher) were cleaned by methanol, acetone and water in a sonicator. Three groups were prepared in deionized water: 1. glass coverslips were dipped in 1mg/mL of a DEX solution 2. RNTs were dissolved and self-assembled in 1mg/mL of a DEX solution making a 0.1mg/mL RNT:DEX solution; then glass coverslips were immersed into such a solution 3. Glass coverslips were first soaked into a 0.1 mg/mL RNT solution. After air drying, they were then immersed into in a 1 mg/mL DEX solution. Then, all three groups were air dried and placed in 6-well plates with 3 mL of a PBS buffer per well inside a 37^{o}C incubator. The supernatant was then extracted on a daily basis for up to 10 days. A BCA assay was used at an absorbance of 562nm to determine released DEX.

For cell-bioactivity tests, osteoblasts (bone-forming cells, ATCC, CRL-11372, cultured in DMEM with 10% FBS and 1% penicillin/ streptomycin under standard cell culture conditions) were seeded at 1000 cells/cm^2 in well plates in the presence of released and fresh DEX at 10^{-8} M. At the end of 1, 3 and 5 days, osteoblasts were fixed, stained and counted under a fluorescence microscope. Statistics were performed using a one-tail *t*-Test.

Results: Due to their large molecular weight and long relaxation time, RNTs and the DEX incorporated RNTs were not visible on ^{1}H NMR spectra. Therefore, the percentage of drug loaded could be analyzed by deducting differences in peak heights of the drug compared with standards. At the DEX:RNT ratio of 5:3, about 33% of DEX was incorporated into the RNTs. To further provide evidence for the incorporation of DEX into RNTs, a change in the diffusion coefficient of the DEX peak from 5.6 to 4.85 was observed in DOSY spectra.

The drug release studies showed that the RNTs were able to load more drugs onto glass surfaces than unaltered glass surfaces (Figure 1). Lastly, from drug bioactivity cell studies, released DEX was not only as active as fresh DEX solutions, but the RNT:DEX solution also resulted in higher osteoblast density due to the bioactivity of the RNTs (Figure 2).

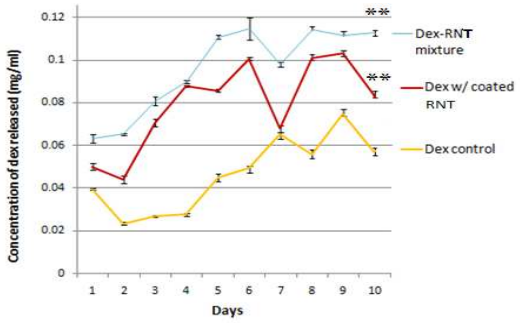

Figure 1. Comparison of dexamethasone release up to 10 days. Data are mean ±SEM (n = 12). **p < 0.05 compared to the controls (DEX only).

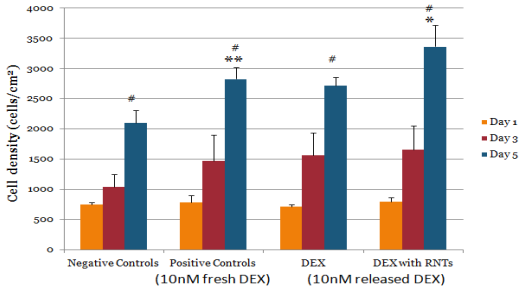

Figure 2. Osteoblast density cultured with released DEX. Data are mean ± SEM (N=3). **p<0.05 compared to negative controls (no additives). *p<0.05 compared to both controls and DEX. #p<0.05 compared to day 1 and 3 results.

Conclusions: This study demonstrated that RNTs were able to incorporate drugs (DEX) and increase drug loading when self-assembled at body temperatures. Moreover, DEX released from RNTs was still bioactive, even more so than fresh DEX alone. Thus, RNTs are promising drug delivery devices with biocompatible and biomimetic properties, which can be injected and self-assemble into bone defect sites to improve bone regeneration.

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