

DNA-Peptide Nanotubes as Artificial Extracellular Matrices to Engineer Cell-Material Interfaces

Gujie Mi, Di Shi and Thomas J. Webster

Department of Chemical Engineering, Northeastern University, Boston, USA.

Introduction: The effective regeneration of complex orthopedic tissues relies on suitable biomaterial scaffolds bearing native structural and physiochemical properties to direct cell and tissue growth. In the body, a nanoscale extracellular matrix (ECM), which is a complex network composed of collagen, fibronectin and other macromolecules, is a natural web of intricate nanofibers that not only provides structural support but also relays crucial biochemical and biomechanical signals¹. The chemical and physical interactions between cells and an ECM play a central role in cellular processes as well as tissue development and regeneration. In this context, the construction of artificial matrices that mimic the structure of the naturally occurring ECM and display biological signals might prove to be highly promising in controlling tissue regeneration². DNA has emerged as one of the most promising building blocks for such nanoconstruction due to the fact that it is highly programmable and is easy to functionalize with nanometer precision³. In the present study, DNA nanotubes were constructed by thermal annealing based on double-crossover motifs⁴ and were further covalently functionalized with a BMP-7 derived peptide every 14 nm along the tube with the aim to mimic some of the structural and regulatory characteristics of the natural ECM. The structure of the nanotubes was inspired by collagen with similar size and stiffness, and the BMP-7 derived peptide was selected because of its role in regulating the proliferation, differentiation and apoptosis of bone cells. The objective of the present *in vitro* study was to design, synthesize and characterize bioactive DNA nanotube as well as to evaluate its potential to be used as artificial extracellular matrices to engineer cell-material interfaces.

Materials and Methods: All DNA strands used in this study were from Integrated DNA Technology (IDT) and dissolved at 500 μM in IDTE buffer (IDT), stored at $-20\text{ }^{\circ}\text{C}$ prior to use. For generating fluorescent tubes, the S3 sequence was ordered from IDT with a 3'-fluorescein (FAM) modification. To make DNA-peptide conjugates, the BMP-7 derived peptide was conjugated to a DNA strand by biosynthesis using a previously reported Huisgen 1,3-dipolar cycloaddition reaction between a strained cyclooctyne and an azide². The resulting conjugate was purified by multiple high-performance liquid chromatography (HPLC) and characterized by mass spectrometry methods. DNA nanotubes with or without functionalization were formed through a thermal annealing process and were characterized for their size and morphologies using transmission electron microscopy. The nanotubes were then deposited onto (3-Aminopropyl) triethoxysilane (APTES) activated glass coverslips through electrostatic interactions and were visualized under fluorescence microscope. To investigate the changes in cellular behavior (i.e., adhesion and proliferation), human osteoblasts (Promocell, C-12720) were seeded at a density of 20,000 cells/cm² for adhesion and a density of 10,000 cells/cm² for proliferation on glass coverslips with various coating conditions (25/10/5/2.5 μM). Osteoblast basal medium (Promocell, C-27015) supplemented with osteoblast growth medium supplement mix (Promocell, C-39615) and 1% Pencillin/Streptomycin was used to culture the cells and cells at passage numbers of 4-8 were used for the adhesion and proliferation experiments. MTS assays were used to determine cell density after predetermined incubation times (3 hours for adhesion and 3 days for proliferation) and cell density was then calculated with correlation to a standard curve. All experiments were conducted in triplicate and repeated at least three different times. Statistical differences were determined using analysis of variance (ANOVA) where $p < 0.05$ was considered statistically significant.

Results and Discussion: TEM images confirmed that the rationally designed DNA tiles can self-assemble and form long tubelike structures about 10-12 nm in diameter and often many micrometers in length with both walls and a central pore clearly visible (Figure 1a). Incorporating BMP-7 peptide into one of the strands yielded an almost indistinguishable structure (Figure 1b), indicating that modifications at selected positions do not affect the tube formation.

Furthermore, cell studies (Figure 2) showed dramatic increases in osteoblast adhesion and proliferation across all groups tested. Although further investigation is needed to fully elucidate the changes in cell functions as well as mechanisms of action responsible for such an increase in cell adhesion and

proliferation, it indicated the promising potential of bioactive DNA nanotubes to be used as artificial extracellular matrices in modulating cell-material interactions.

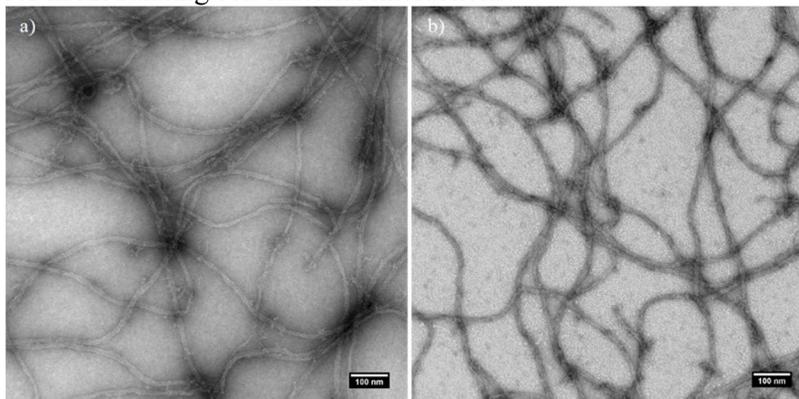


Figure 1. TEM images of (a) double-crossover tile based DNA nanotubes; (b) DNA nanotubes with peptide modification. Scale bar=100 nm.

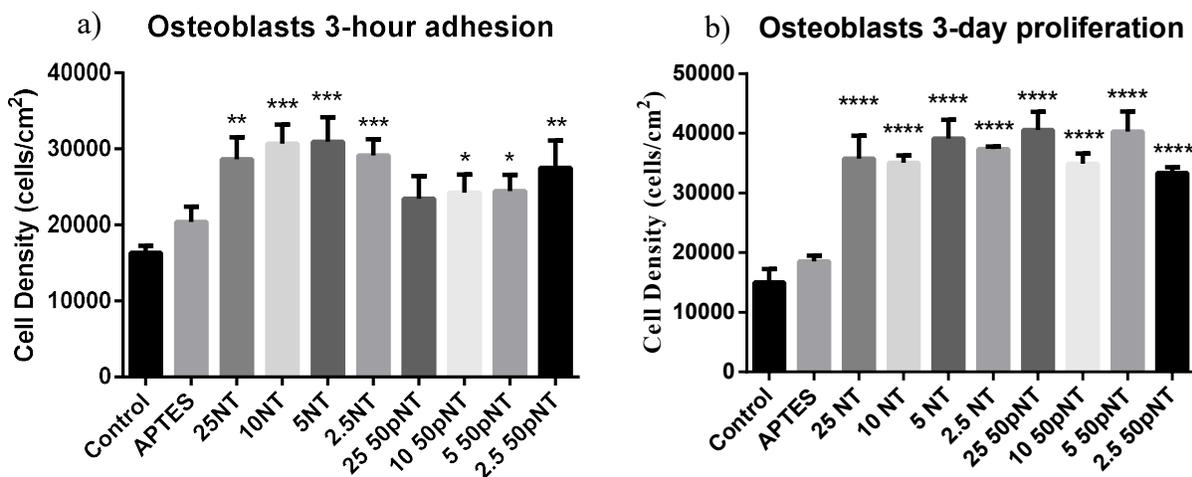


Figure 2. a) Osteoblast 3-hour adhesion study. Cells were seeded at 20,000 cells/cm². b) Osteoblast 3-day proliferation study. Cells were seeded at 10,000 cells/cm². Values are mean±SEM, N=3. *p<0.05, **p<0.01, ***p<0.005 and ****p<0.001 when compared to the control group. 25NT, DNA nanotubes at a concentration of 25 μM; 25 50pNT, peptide functionalized DNA nanotubes at a concentration of 25 μM.

Conclusions: Through the above experiments, DNA nanotubes self-assembled from DNA tiles were successfully designed, constructed and characterized. The ECM-like fibrillar morphology as well as the enhanced adhesion and proliferation of osteoblast cells indicated their potential to be used as improved artificial extracellular matrices.

Acknowledgement: The authors would like to thank Northeastern University for funding.

References:

1. Frantz, C., Stewart, K. M. & Weaver, V. M. The extracellular matrix at a glance. *J. Cell Sci.* **123**, 4195–4200 (2010).
2. Stephanopoulos, N. *et al.* Bioactive DNA-peptide nanotubes enhance the differentiation of neural stem cells into neurons. *Nano Lett.* **15**, 603–609 (2015).
3. Aldaye, F. A., Senapedis, W. T., Silver, P. A. & Way, J. C. A Structurally Tunable DNA-Based Extracellular Matrix. *J. Am. Chem. Soc.* **132**, 14727–14729 (2010).
4. Rothemund, P. W. K. *et al.* Design and Characterization of Programmable DNA Nanotubes. *J. Am. Chem. Soc.* **126**, 16344–16352 (2004).