Printing Therapeutics in 3D using Nanoengineered Bioink

Charles W. Peak[1], Jeffrey Chen[1], Mu'ath Adlouni[1], Kanwar Abhay Singh[1], Akhilesh K. Gaharwar[1,*]
Biomedical Engineering, Dwight Look College of Engineering, Texas A&M University, College Station, TX 77843.

Statement of Purpose: Three-dimensional (3D) extrusion based bioprinting, is an emerging technology to mimic complex tissue structures. However, most of the bioinks used for 3D bioprinting lacks bioactive characteristics. Here, we have developed a nanoengineered bioink loaded with therapeutic proteins to direct cell function. We propose to utilize high surface area and charged characteristics of two-dimensional (2D) nanosilicates to sequester and deliver biologically active therapeutics.

Methods: Nanosilicates (Laponite XLG), procured from BYK Additives and Instruments. The synthesis of poly(ethylene glycol)-dithiothreitol (PEG-DTT) via a Michael-like step growth polymerization results in acrylate terminated degradable macromer. By stoichiometrically imbalancing the reaction towards PEG-diacrylate, the acrylate functionality of the resulting macromer was preserved and the resulting hydrogel solutions were UV curable. Bioink was obtained by additing 4% nanosilicates and polymer into DI water and vortexed vigorously for at least 2 minutes. Therapeutic proteins such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) were incorporated into bioink for *in vitro* testing. Bioink was used to fabricate 3D tructure using a HYREL System 30M 3D printer.

Results: The bioink is developed from a hydrolytically degradable polymer and two-dimensional (2D) synthetic nanoparticle. The addition of 2D nanosilicates to PEG-DTT results in formation of shear-thinning bioinks with high printability and structural fidelity (**Fig. 1**). The mechanical properties, swelling kinetics, and degradation rate of 3D printed constructs can be modulated by changing

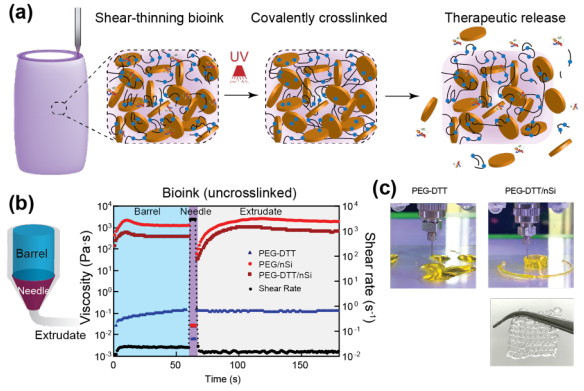

Figure 1. Synthesis and characterization of bioink loaded with protein therapeutics. (a) PEG-DTT/nanosilicate bioink is design. (b) Peak-hold viscosity test is performed to evaluate the shear-thinning and shear-recovery of bioink formulation. (c) PEGDTT by itself remained fluid, while PEG-DTT/nSi can be printed in 3D constructs with high mechanical stability.

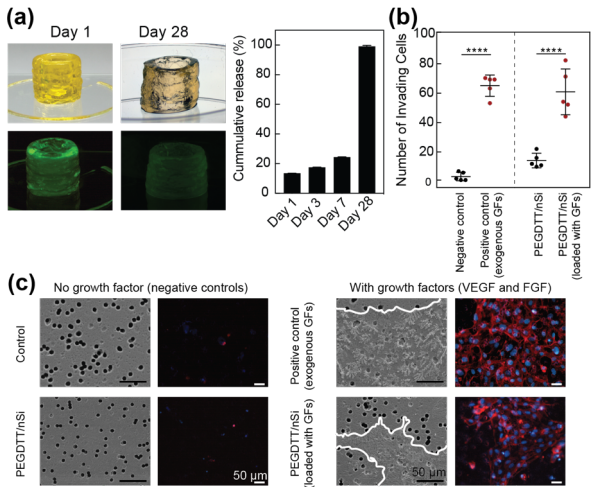

Figure 2. Bioink loaded with protein therapeutics. (a) The release of fluorescently labeled protein from 3D printed structure was monitor over 28 days. (b) The activity of released protein from 3D printed structure was determined via invasion assay. (c) Actin and nuclei staining of migrate HUVECs across transwell. Addition of growth factor to PEG-DTT/nSi influences cell migration.

the ratio of PEG:PEG-DTT and nanosilicates concentration. Due to high surface area and charged characteristic of nanosilicates, protein therapeutics can be sequestered in 3D printing structure for prolong duration. Sustained release of pro-angiogenic therapeutics from 3D printed structure, promoted rapid migration of human endothelial umbilical vein cell (HUVEC) (**Fig. 2**). The transwell insert suggest extracellular matrix deposition caused by migratory HUVECs on the growth factor containing scaffolds. This approach to design biologically active inks to control and direct cell behavior can be used to engineer 3D complex tissue structure for regenerative medicine.

Conclusions: Shear-thinning bioink with the ability to sequester and release therapeutic proteins from 3D printed structure have been introduced. The bioink consist of degradable PEG-DTT and 2D nanosilicates. The mechanical properties, swelling kinetics, and degradation rate can be modulated via the amount of PEG-DA and nanosilicates. Using the cation exchange capacity of nanosilicates, growth factors were sequestered and released from the 3D printed structure. The activity of released protein therapeutics from 3D structure were verified *via* migration of cells. Overall, this study provides proof of principle to print therapeutics in 3D that can be used to control and direct cell functions.

Acknowledgement: AKG would like to acknowledge financial support from the National Science Foundation (CBET 1705852), and the National Institute of Health (DP2 EB026265).