Molecular Beacon Technology for Tissue Engineering Applications
Jennifer C Alexander¹, Abhay Pandit¹, Gang Bao², Yury Rochev¹
¹Network of Excellence for Functional Biomaterials, National University of Ireland, Galway,
²Georgia Institute of Technology, USA
j.alexander1@nuigalway.ie

Introduction
Detection of RNA in cells is routinely performed by in vitro methods such as RT-PCR and fluorescent in situ hybridization (FISH). RT-PCR quantifies gene expression of a cell population. FISH requires fixation of cells, thus unable to give a true picture of mRNA localization. Molecular beacons (MBs) are antisense oligonucleotide stem-loop probes that fluoresce upon hybridization with target nucleic acids. The major advantage of MBs is the ability to detect, localize and monitor specific mRNA expression in single live cells in real-time. However, successful detection of target mRNA by MBs depends on a combination of factors such as target accessibility, efficiency of probe delivery, and the sensitivity of the imaging technique used. Here we report, for the first time, the live-cell imaging of specific mRNA in live cells in a 3D in vitro system using Streptolysin O (SLO) and TAT-peptide conjugation to deliver MBs targeting GAPDH mRNA.

Materials and Methods
Human fetal foreskin fibroblast (HFFF) or human dermal fibroblasts (HDF) were incorporated into 1 mg/ml collagen type I hydrogels before gel formation, and grown in complete medium for 24 h. Gel thickness was about 900 µM. Activated SLO (Sigma) delivered 1 µM GAPDH or ‘random’ MBs (MWG biotech) to the 3D scaffolding system. Cells were imaged by confocal microscopy 1 h after addition of complete medium. Amine-modified GAPDH MBs (10 µM) were cross-linked to N-succinimidyl 3-(2-pyridyldithio) propionate for 2 h, reduced with tris(2-carboxyethyl) phosphine (2 mM) for 30 min, and conjugated overnight to Cys-TAT-peptide (15 µM). Peptide-conjugated MBs (400 nM) were incubated with cells for 30 min before imaging.

Results
Positive signal from MBs targeting GAPDH mRNA (Fig. 1B & 1D), and non-specific signal from random-sequence MBs (Fig. 1A) were detected in the cytoplasm of cells in the 3D gel using confocal imaging. As a control for peptide-based delivery, amine-modified MBs without TAT-peptide showed no signal (Fig. 1C).

Discussion and Conclusions
Molecular beacons can be used in real-time visualization of mRNA expression in three dimensional type I collagen gel.

Reference

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Disclosures
Authors have nothing to disclose.