Drug-Loaded Nanoparticles Induce Gene Expression In Human Pluripotent Stem Cell Derivatives

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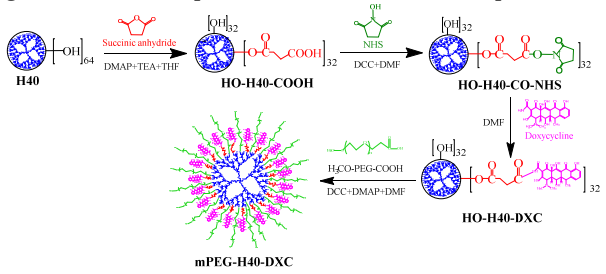
Statement of Purpose: Tissue engineering and advanced manufacturing of human stem cells requires a suite of tools to control gene expression spatiotemporally in culture. Inducible gene expression systems offer cell-extrinsic control, typically through addition of small molecules, but small molecule inducers typically contain few functional groups for further chemical modification. Doxycycline (DXC) is a potent small molecule inducer of tetracycline (Tet) transgene systems. To gain additional functional handles on drug release and thus gene expression, here we report the conjugation of DXC to a hyperbranched biodegradable and biocompatible polymer nanoparticle, i.e., Boltorn H40. The fourth-generation hyperbranched polymer nanoparticle offers a large number of peripheral functional groups which can be used to conjugate a large number of DXC molecules as well as polyethylene glycol (PEG) arms, thereby making the drug-polymer conjugate water soluble.

Methods: Methoxy-PEG-H40-DXC was synthesized via a two-step procedure as shown in **Scheme 1**. In the first step, H40-OH was partially converted to carboxylated H40 followed by conversion into NHS ester and then conjugated with DXC molecules. mPEG-COOH was subsequently conjugated with $_{32}$(OH)-H40-DXC to make the resulting PEG-H40-DXC water-soluble. In order to analyze the pH-sensitive drug release behaviors of the PEG-H40-DXC nanoparticles, *in vitro* drug release studies were carried out under simulated physiological conditions at pH 7.4 in PBS buffer and in intracellular acidic conditions at pH 5.0 in acetate buffer at 37ºC. To test the intracellular activity of the PEG-H40-DXC nanoparticles, human stem cell-derived secondary C1 fibroblasts [1] were cultured with DXC or PEG-H40-DXC. These fibroblasts contain a Tet-on DXC-inducible gene circuit for expression of Oct3/4 and Sox2 proteins.



Scheme 1. Synthesis scheme of PEG-H40-DXC.

Results: Every reaction step in Scheme 1 was followed by a purification step to remove un-reacted chemicals and by-products. To confirm conjugation, ^{1}H NMR spectra was collected. In drug release studies, at pH 5.0, 28.8±0.6% of DXC release was observed after 12 hrs, however, at pH 7.4, only 5.6±0.2% of DXC release was observed. Treatment of fibroblasts with DXC or PEG-H40-DXC at an equivalent DXC concentration of 5 µM for 24 hrs resulted in high levels of properly-localized, nuclear Oct3/4 and Sox2 proteins in a subset of cells, as determined by immunocytochemistry. Flow cytometry was used to quantify levels of protein produced from induced gene expression within single cells. The distribution of Oct3/4 and Sox2 proteins induced in non-treated, PEG-H40 treated, free DXC-treated, and PEG-H40-DXC treated cells (**Fig. 1**) was monitored. Similar percentages of cells contained induced gene protein products after 24 hrs of incubation with DXC or PEG-H40-DXC (equivalent DXC concentration 5 µM).

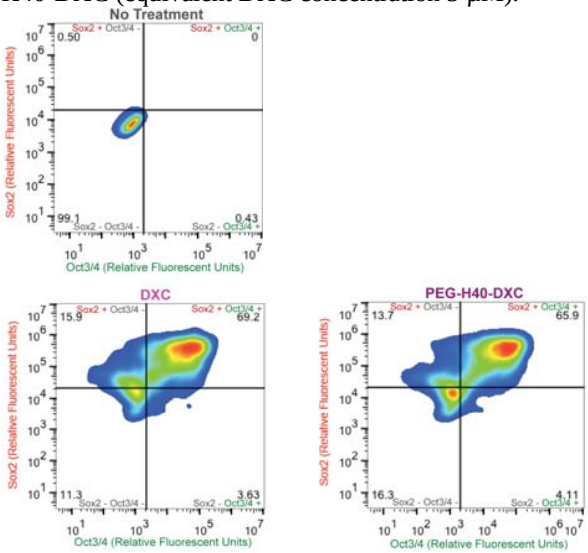


Figure 1. Quantification of DXC and PEG-H40-DXC nanoparticle-induced gene expression. Representative heat maps of induced protein expression in stem-cell derived human fibroblasts as measured by flow cytometry, which allows single-cell quantification of protein levels.

Conclusions: The PEG-H40-DXC nanoparticle serves as a versatile nanoplatform with the ability to control gene expression in human stem cell derivatives through the use of Tet-on transgene systems. Gene expression with the nanoparticle occurs as effectively as DXC, the currently utilized Tet inducer, while minimizing off-target effects that decrease cell health. Subsequent work with these nanoparticle systems could be used to intricately control Tet-on gene circuits developed by synthetic biologists [2] in stem cell-derived cells to model and treat diseases in innovative ways [3].

References: [1] Hockemeyer, D. *Cell Stem Cell*, 2008;3: 346–353. [2] Ruder, WC. *Science*, 2011;333:1248–1252. [3] Saha K., *Cell Stem Cell*, 2009;5:584–595.