Increased Cardiomyocyte Uptake of N-acetylglucosamine-Decorated Nanoparticles

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Statement of Purpose: An estimated 1.3 million Americans will suffer from myocardial infarction (MI) this year. MI leads to heart failure, for which the only successful treatment is organ transplantation. Currently, the scientific community lacks drug delivery systems and therapies to treat the damaged myocardium. To promote myocardial regeneration, we propose a polymeric nanoparticle drug delivery system decorated with N-acetylglucosamine (GlcNAc) to target cardiomyocytes (CM) (figure 1).

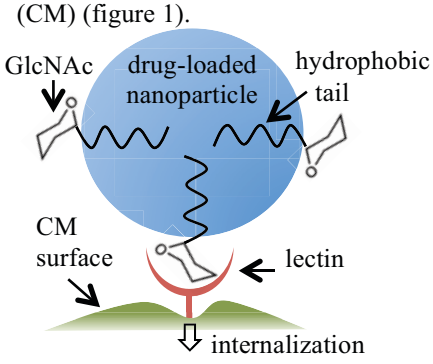

Figure 1. Targeting of NP to cardiomyocytes by GlcNAc.

Our rational lies in the recent discovery of a CM-expressed lectin that binds to GlcNAc[1] and can internalize GlcNAc-decorated liposomes[2]. We hypothesize that CMs will internalize acid-degradable polymeric nanoparticles (NPs) decorated with GlcNAc at a higher rate than undecorated NPs. We will then load CHIP, a recently discovered anti-apoptotic protein[3] to reduce CM death post-hypoxia and evaluate recovery of function *in vitro* and *in vivo*. Successful completion will lead to a novel drug delivery system for healing the post-myocardial infarcted heart.

Methods: We tethered GlcNAc to NPs by conjugating it to a hydrophobic tail (figure 2, molecule **I**) that embeds into a NP during formation due to the hydrophobic effect.

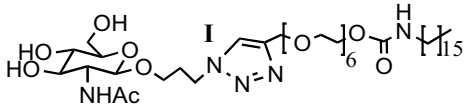

Figure 2. Chemical structure: GlcNAc tether.

To generate molecule **I**, we coupled molecules **II** and **III** (figures 3 and 4) by copper-catalyzed click chemistry. Selected steps of our synthetic scheme are as follows. We synthesized a GlcNAc derivative with a reactive azide handle (figure 3, molecule **II**) by reacting an oxazoline intermediate with azidopropanol (figure 3).

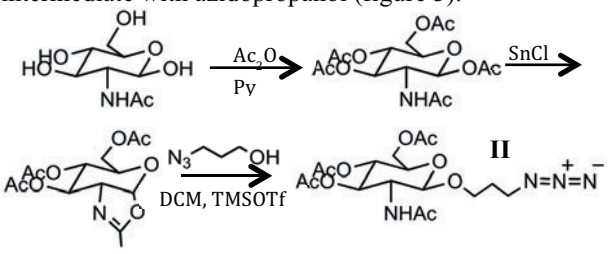

Figure 3. Synthetic scheme: N_3-functionalized GlcNAc.

Molecule **III** (figure 4) was synthesized by reacting one end of hexaethylene glycol with an alkyne and the other end with a 16-carbon alkyl chain.

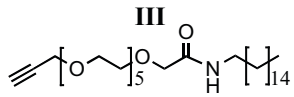

Figure 4. Chemical structure of synthetic molecule **III**.

Identity of each compound was verified by ^{1}H- and ^{14}C-NMR and mass spectrometry. Poly(cyclohexamine 1,4-diylacetone dimethylene ketal) (PCADK) NPs decorated with molecule **I** were formed by the solvent displacement method. NP images were obtained by scanning electron microscopy (SEM).

CMs were harvested from day-old Sprague-Dawley rat pups and treated within one week of plating. To examine the improved uptake of decorated NPs by CMs, we loaded the cell tracer dye 5-chloromethylfluorescein diacetate (CMFDA) into the NPs and evaluated internalization by fluorescence.

Results: The tethering of GlcNAc to NPs was verified by UV absorbance and mass spectrometry.

Sizing of NPs by SEM image analysis indicated NPs with an average diameter of 299 nm.

To evaluate increased internalization due to GlcNAc decoration, we treated CMs with CMFDA-loaded particles. We monitored the fluorescence of wells over 30 hours and calculated the fold increase of different treatment groups (figure 5). GlcNAc-decorated NPs ("dec/load") produced a 45% increase in fluorescence over undecorated NPs ("undec/load"). This indicated that GlcNAc decoration enhanced NP uptake, thereby confirming our hypothesis.

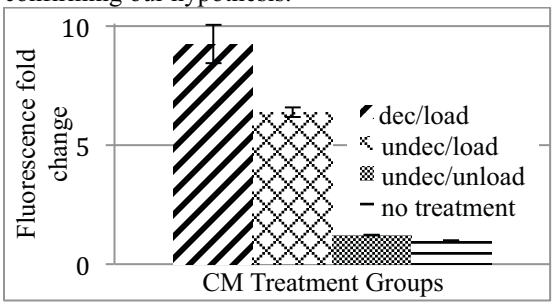

Figure 5. GlcNAc decoration increased uptake by CMs.

Conclusions: We demonstrate here that decorating NPs with GlcNAc enhances uptake by CMs. Future studies will include loading GlcNAc-decorated NPs with CHIP, an anti-apoptotic therapeutic. We will first treat hypoxic cells *in vitro* to reduce apoptosis and then deliver the NPs to the post-MI rat myocardium *in vivo* to evaluate improved ventricular remodeling. We anticipate the application of this drug delivery system utilizing GlcNAc internalization to various CM-targeting therapies.

References:

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(Aso S. J Cont Rel. 2007;122:189-198.)
(Naito AT. Circ Res. 2010;106:1692-1702.)

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