# Liposomal Delivery of FTY720 Modulates Inflammatory Response in Macrophages

Claire Segar[1], Edward Botchwey[1]
[1]Georgia Institute of Technology and Emory University

**Motivation:** The purpose of this research is to investigate the incorporation of the synthetic small molecule FTY720 into liposomes with the goal of targeted inflammatory modulation. Inflammation is an integral part of tissue regeneration that can be regulated to improve healing outcome. FTY720, a synthetic analog of the bioactive signaling lipid sphingosine-1-phosphate (S1P), has been shown to positively affect remodeling processes such as neovascularization and bone repair through modulation of inflammatory cell types[1,2]. FTY720 may exert its effects through both activity at its receptors and through interaction with intracellular components of the S1P metabolic pathway[3]. Intracellular phosphorylation of FTY720 is required for biological activity, suggesting that delivery to the cytoplasm via a nanoparticle vehicle may be beneficial. Additionally, nanoparticles passively accumulate in sites of leaky vasculature due to the enhanced permeability and retention effect, after which they are taken up by mononuclear phagocytes such as monocytes and macrophages. Consequently, a liposomal formulation of FTY720 may provide targeting to sites of inflammation *in vivo*, as well as improved drug activity and kinetics.

**Methods:** The small molecule FTY720 was incorporated into liposomes composed of the phospholipid DPPC and cholesterol and then extruded to 100 nm as confirmed by dynamic light scattering. High performance liquid chromatography and tandem mass spectrometry methods were developed to measure the drug incorporation efficiency, liposome stability, and effect on intracellular metabolites. Confocal microscopy was used to visualize the intracellular trafficking of liposomes, as the incorporation of FTY720 may cause changes in the zeta potential and subsequent endocytotic trafficking patterns. Multiplex cytokine profiling was performed on conditioned media of lipopolysaccharide (LPS)/ interferon-gamma (IFN-γ)-stimulated macrophages to test the hypothesis that liposomal FTY720 is a more potent modulator of inflammatory cytokine secretion. Proliferation assays were conducted to measure the mitogenic effects of liposomal FTY720 on multiple macrophage phenotypes, including naïve M0 macrophages, LPS/IFN-γ-stimulated M1-like macrophages, and IL-4-stimulated M2-like macrophages.

**Results:** Unloaded and FTY720 liposomes were 197 nm and 207 nm in diameter, respectively, with similar polydispersities. Confocal microscopy demonstrated that FTY720 loaded liposomes produced a more diffuse intracellular distribution within RAW264.7 macrophages, indicating that drug incorporation may change the trafficking pattern of liposomes and promote endosomal escape (**Figure 1**). Further studies are underway to directly track the trafficking and cytoplasmic release of FTY720 loaded liposomes using confocal live cell imaging. Luminex cytokine profiling demonstrated that liposomal FTY720 inhibited the secretion of inflammatory cytokines (such as TNF-α, IL-1, and MCP-1) compared to both unloaded liposomes and free FTY720 delivery (**Figure 2**). Proliferation assays indicated that liposomal FTY720 reduced the number of M1 macrophages in culture, while maintaining the number of M2 macrophages (data not shown), suggesting that FTY720 liposomes may be capable of shifting the ratio of M1:M2 macrophages, which has been correlated positively to wound healing outcome. Future studies will investigate the *in vivo* therapeutic effects of FTY720 liposomes in processes such as neovascularization and tissue regeneration.

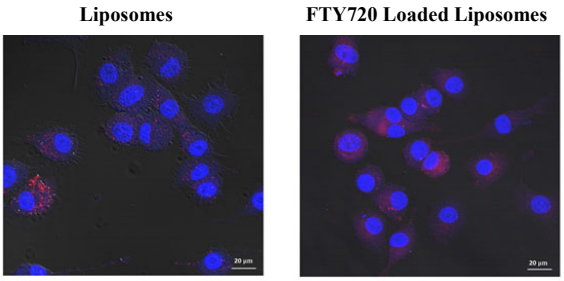


**Figure 1:** Representative confocal images of unloaded and FTY720 loaded liposomes 24 hours after delivery to RAW264.7 macrophages.

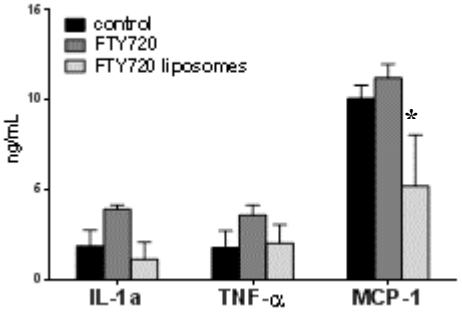


**Figure 2**: Inflammatory cytokine concentrations in condition media of LPS/IFN-γ-stimulated RAW264.7 macrophages treated with free or liposomal FTY720.

**Conclusions:** These results indicate that liposomal delivery of FTY720 is capable of modulating inflammatory responses *in vitro*. A reduction in the secretion of inflammatory cytokines and proliferation of M1 macrophages suggests that FTY720 liposomes may be therapeutic *in vivo*. Additionally, incorporation of FTY720 may change the endosomal trafficking of liposomes, promoting drug release into the cytoplasm for improved activity.

**References:** 1. Sefcik LS. Tiss Eng Part A. 2010:17; 617–629; 2. Huang C. Cell Tiss Res.2012:347; 553-662. Bandhuvula P. J Biol Chem. 2005:280; 33697–33700.