Orthogonal Modes of Immunomodulatory Protein Release from Injectable Liposomal Nanocomposite Hydrogels

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Statement of Purpose: Lipid-based nanotechnologies have proven to be critically important for nanomedicine, but there has been limited research into how these technologies could be leveraged to fabricate multi-functional macroscopic biomaterials. Here, we report the synthesis of injectable, supramolecular hydrogels composed directly from liposomal nanoparticles and dodecyl-modified hydroxypropyl methylcellulose (HPMC-C_{12}). These liposomal hydrogels exhibited robust shear-thinning and self-healing behaviors enabling facile injectability for local drug delivery applications. By integrating modular lipid nanotechnology into this hydrogel platform, we introduced multiple mechanisms of protein release based on liposome surface chemistry. When injected into immuno-competent mice, these liposomal hydrogels exhibited formulation-dependent rates of dissolution and excellent biocompatibility. To fully validate the utility of this system for multi-protein delivery, we demonstrated the synchronized, sustained, and localized release of IgG antibody and IL-12 cytokine *in vivo*, despite the significant size differences between these two proteins.

Methods: Liposomes were prepared via thin-film hydration and extrusion methods, using DMPC, DMPG, and cholesterol, and NTA(Ni)-DGS. HPMC-C_{12} was prepared as described previously.[1] Hydrogels were formed by simple mixture of liposomes and HPMC-C_{12}. We confirmed that these materials afford multiple modes of release *in vitro* using GFP as a model drug. We then used whole animal IVIS imaging to validate that liposomal hydrogels are able to synchronize the sustained release of IgG antibody and IL-12 cytokine in C57Bl6 mice, despite the considerable size difference between these proteins.

Results: Liposomal hydrogels exhibit robust solid-like properties over physiologically-relevant frequencies, yet also possess shear-thinning and self-healing capabilities that make the material injectable (**Figure 1**). By engineering the surface chemistry of the liposomes, these hydrogels can be formulated to simultaneously exhibit three distinct modes of protein release: passive, electrostatic, and affinity governed release (**Figure 2**). Depending on the mode of release chosen, cargo release rates could be tuned for particular biomedical applications. We also demonstrated that these modes can be applied to individually tune the release rates of multiple cargo, allowing for synchronized and sustained local release of IgG and IL-12 cargo *in vivo* (**Figure 3**). Self-assembled liposomal hydrogels are a highly modular platform for designing multi-functional macroscopic biomaterials. They are injectable, biocompatible, and provide an opportunity to leverage the remarkable capabilities of lipid nanotechnologies toward localized therapeutics, with implications for both cellular and immuno-therapies.

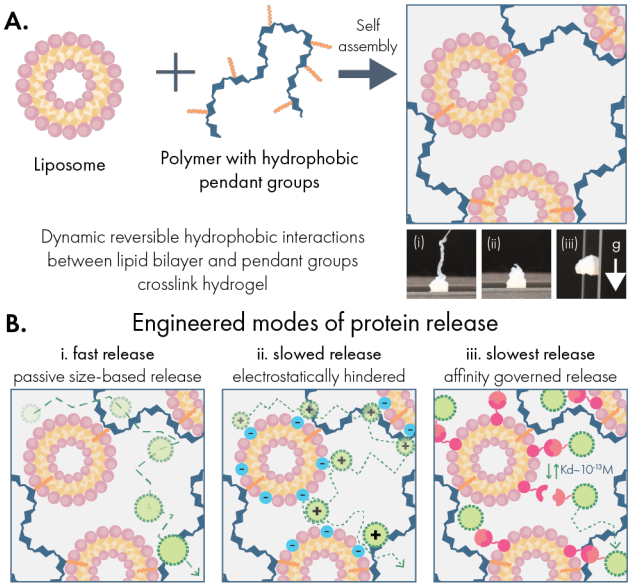

Fig. 1. (A) Injectable hydrogels are formed from dynamic interactions between liposomes and HPMC-C_{12} polymer. (B) Engineering the surface chemistry of liposomes to provide distinct modes of cargo release.

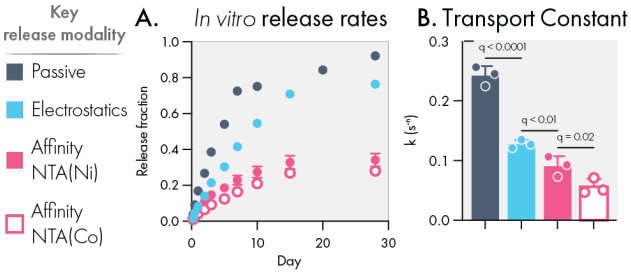

Fig. 2. Tunable release kinetics based on the mode of release chosen. Data acquired from in vitro release study of GFP model cargo.

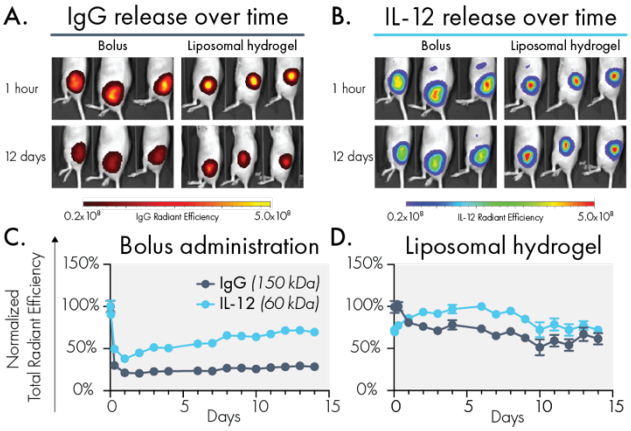

Fig. 3. Orthogonal modes of release from hydrogels. Using passive release for IgG and electrostatic release for IL-12, liposomal hydrogels allow for the synchronized and sustained release of cargo in mice for several weeks.

References: 1. Appel, E.A. et al. Nat Commun 6, 6295 (2015).