Carboxylated branched poly(β-amino ester) nanoparticles enable robust intracellular protein delivery and CRISPR/Cas9 gene editing

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Statement of Purpose: Direct intracellular delivery of protein drugs to target cells is safer than plasmid delivery as it eliminates risks of insertional mutagenesis. In the context of gene editing, there is the added benefit that delivery of CRISPR/Cas9 ribonucleoproteins (RNPs) reduces the probability of off-target editing by reducing RNP persistence time. However, intracellular protein delivery faces many challenges as the large size and hydrophilicity of proteins make them generally membrane impermeable. Poly(β-amino ester)s (PBAEs) are biodegradable, cationic polymers that self-assemble into nanoparticles with nucleic acids via electrostatic interactions and have been developed for effective nucleic acid delivery. In contrast to nucleic acids, different proteins carry different surface charges and nanoparticle encapsulation cannot rely solely on charge interactions. In this study, we modified branched PBAEs with amino acid-like carboxylate ligands and examined their ability to enable intracellular protein delivery. We hypothesized that the carboxylate end-caps facilitate protein encapsulation through hydrogen bonding and salt bridges while the PBAE polymer backbone can enable endosomal escape, resulting in a versatile protein delivery platform.

Methods: Carboxylate ligands were synthesized via acrylation of amino acid derivatives to yield a series of acrylated amino acids with varying numbers of carbons between the carboxyl and amide groups (ligands are referred to by the number of carbons, with C1 corresponding to glycine). Amine-terminated PBAEs were synthesized via a Michael addition reaction and end-capped with carboxylate ligands. Protein-encapsulated nanocomplexes were formed by mixing polymer and proteins in 25 mM sodium acetate solution (pH 5) for nanoparticle self-assembly.

Results: Carboxylated branched polymers complex with proteins to form nanoparticles between 100-600 nm in diameter assessed by DLS measurements. Using ferritin, an iron-rich protein that allows for direct visualization on TEM, we determined that each nanoparticle contained approximately 10 protein molecules (Figure 1). To determine intracellular protein delivery efficacy, we performed nanoparticle uptake experiments with FITC-labeled bovine serum albumin (Figure 2A) in CT2A murine brain cancer cells as well as human adipose-derived mesenchymal stem cells (MSCs). We found that protein uptake had a biphasic response relative to the number of carbons in the carboxylate end-cap, with C5 and C7 achieving the highest levels of uptake. Confocal laser scanning microscopy experiments revealed FITC-BSA fluorescence distributed throughout the cytosol 5 hours after delivery in HEK-293T cells (Figure 2B).

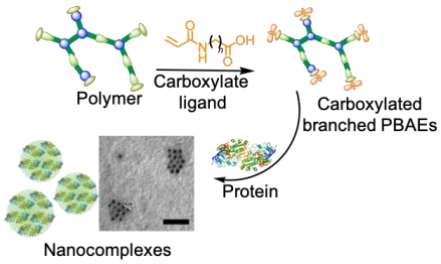

Figure 1. Assembly of carboxylated branched PBAEs with proteins. TEM of ferritin containing nanoparticles. Scale bar = 50 nm.

We then used polymer C5 to deliver the ribosome-inactivating protein saporin and demonstrated functional cell killing in several cell lines, with EC_{50} less than 2 nM; free saporin administered to cells without polymer failed to induce any cytotoxicity up to 15 nM (Figure 2C). Finally, we encapsulated CRISPR RNPs into nanoparticles and demonstrated high levels of knockout of a GFP reporter gene in HEK-293T as well as GL261 murine brain cancer cells (Figure 2D).

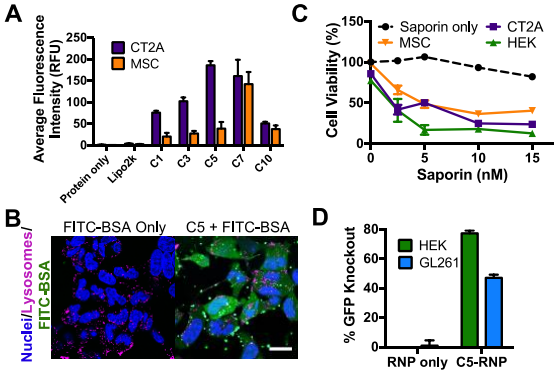

Figure 2. (A) Nanoparticle uptake using FITC-BSA reveals biphasic response. (B) Distributed cytosolic fluorescence visible 5 hours after nanoparticle delivery of FITC-BSA; scale bar = 100 μm. (C) Polymer C5 encapsulating saporin enables functional cytosolic delivery assessed by robust cell killing. (D) C5 polymer encapsulating CRISPR RNPs facilitates gene knockout.

Conclusions: We synthesized a series of carboxylated branched PBAEs and demonstrated that polymers with C5 end-groups enable high levels of cytosolic protein delivery in multiple cell types. Cytosolic delivery of saporin induced highly effective cell killing and delivery of Cas9 RNPs enabled CRISPR gene knockout up to 80%. Our results demonstrate that carboxylated branched PBAEs are a versatile protein delivery platform and a promising tool for gene editing applications.