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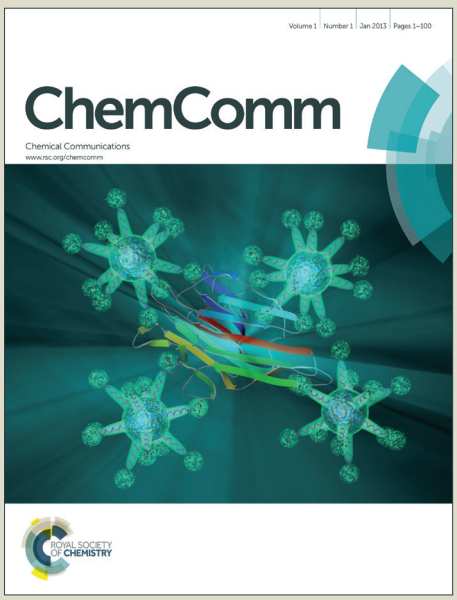

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A biodegradable adamantane polymer with ketal linkages in its backbone for gene therapy

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Abstract. In this report we present a polyketal, termed pADK, which has adamantane groups embedded in its backbone, and degrades into neutral excretable compounds. pADK was synthesized via click chemistry and had a MW of 49,472 and a PDI of 1.74. We demonstrate here that pADK can increase the transfection efficiency of CD1800 (PEI of 1800 modified β-cyclodextrin) by 60 fold, yet causes no increase in toxicity.

β-Cyclodextrin (β-CD) based cationic drug and gene delivery vehicles have found numerous applications in gene therapy because of their lower toxicity in comparison to other poly-cations.[1-12] Most β-CD based gene delivery vehicles are composed of either cationic β-CDs supramolecularly complexed with non-degradable guest polymers or directly conjugated with non-degradable polymers.[2-6, 8, 9] For example, Thompson and co-workers have developed poly-vinyl alcohol (PVA) based drug delivery vehicle that have numerous cholesterol or adamantane groups pendant on to the PVA, via an acid degradable acetal linkage,[13] which can efficiently deliver siRNA. The cholesterol or adamantane embedded PVA reversibly binds to cationic-β-CD and the resulting host-guest complex has a transient high charge density on the surface and therefore has minimal toxicity in-vitro.[14-17] The high surface charge density complex binds with siRNA with high affinity to form a polyplex, which is easily internalized by cells via endocytosis. After endocytosis, the polyplex is trafficked to the late endosome, where the acetal linkage hydrolyzes and the resultant complex degrades into monomeric cationic-β-CD, cholesterol and non-degradable PVA.

The non-degradable PVA can be problematic for translational studies due to its accumulation within cells in vivo and long term persistence.[18] To address this limitation, in this report we present a poly-ketal that has adamantane units on its backbone, termed pADK, which degrades into low molecular weight excretable compounds and can self-assemble with cationic-β-CD and deliver DNA efficiently to cells. The chemical structure of pADK is shown in Figure 1, is composed of a polyketal that has adamantane groups embedded in its backbone, flanked by triazole groups, and in the presence of acid degrades into low molecular weight diols and acetone, both of which are membrane permeable and potentially excretable. pADK is designed to complex cationic-β-CD, generating a multivalent high density polycation that can complex DNA and deliver it into cells. However, after endocytosis and trafficking into the late endosome, pADK hydrolyzes into small molecules, due to hydrolysis of the ketal linkage, and should cause endosomal disruption via the colloid osmotic mechanism.[19-26] Importantly, the cellular degradation products of pADK are an adamantane diol and acetone, both of which should cause minimal toxicity. For example

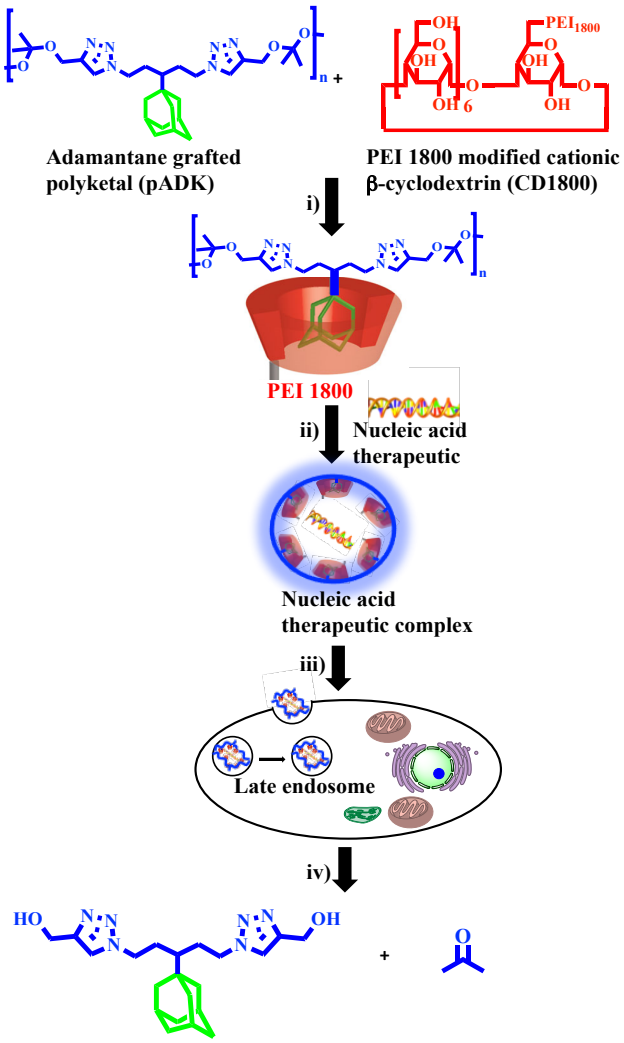

Figure 1: Polyadamantane-ketal (pADK), an adamantane grafted biodegradable polymer for nucleic acid delivery: i) pADK forms an inclusion complex with cationic-β-CD; ii) the host-guest complex electrostatically binds to nucleic acid therapeutics to form nanoparticles; iii) the pADK polyplex enters cells via endocytosis; iv) the acidic environment of the endosome degrades pADK into small molecules, causing the dissociation of nucleic acids and release into the cytoplasm, pADK degrades into membrane permeable small molecules that are excreted.

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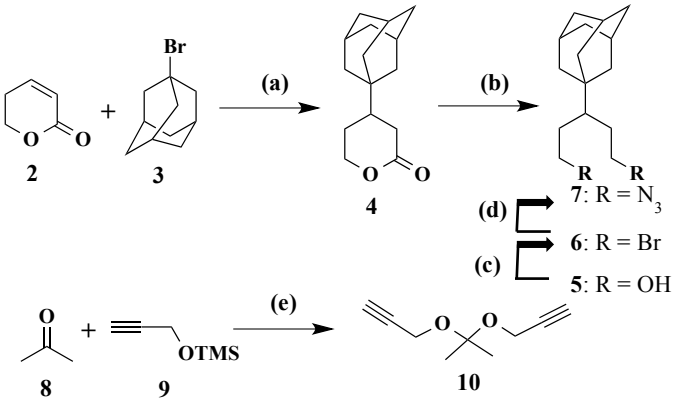
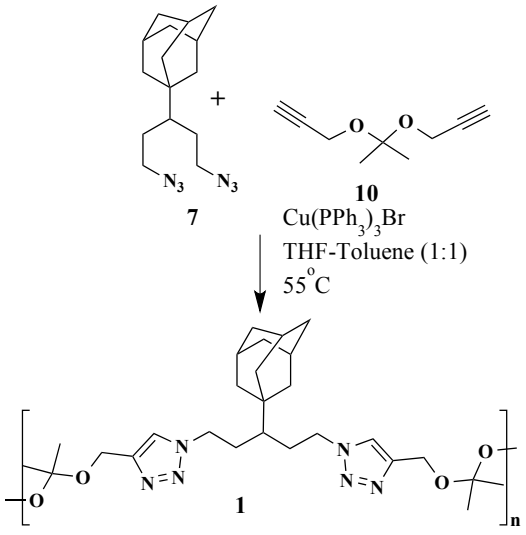


Scheme 1: Synthesis of pADK monomers: (a) AIBN, Bu_3SnH, Toluene, 100 °C, 61%; (b) $LiAlH_4$, THF, rt, 94%; (c) CBr_4, PPh_3, DCM, 0 °C to rt, 84%; (d) NaN_3, DMF-water (6:1), 50 °C, 97%; (e) TMS-OTf, DCM, -78 °C, 63%.

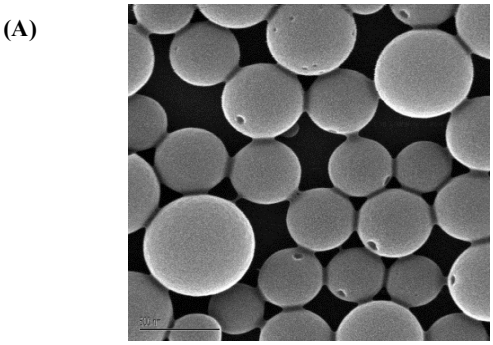
the released acetone should be metabolized to pyruvate and used for ATP production, and the 3-adamantaine-1,5-pentadiol should get excreted on the time scale of hours to days based on its small size and anticipated membrane permeability.[20,27,28]

The synthesis of pADK is shown in scheme 2 and was accomplished via a click polymerization between the diazide adamantane (**7**) and di-ketal alkyne (**10**). Diazide **7** was synthesized from 5,6-dihydro-2H-pyran-2-one **2**, via a 1,4-addition with the adamantane radical **3**, followed by reduction with lithium aluminium hydride, bromination and nucleophilic substitution with sodium azide. The monomer **10** was synthesized in one step via trimethylsilyl trifluoromethanesulphonate catalysed ketal formation of acetone (Scheme 1). pADK was synthesized via the click reaction between **7** and **10**. A key challenge in the synthesis of pADK was its solubility, which prevented successful polymerization in a wide variety of solvents, such as DMF, chloroform, tetrahydrofuran and dichloromethane. We identified a mixture of 1:1 tetrahydrofuran and toluene as a solvent, which has the proper balance between hydrophobicity and polarity to synthesize pADK. Using this solvent system a pADK (**1**) with Mn=49,472 (PDI=1.74) was obtained (polymer 1 in table 1 of the supporting information), which was used for further investigation.

pADK is designed to complex β-CD via supramolecular guest-



Scheme 2: Synthesis of pADK.



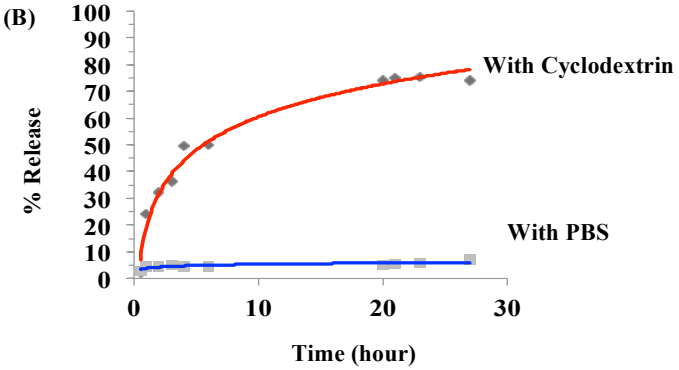


Figure 2: pADK microparticles respond to methyl-β-cyclodextrin (A) SEM image of rhodamine encapsulated in pADK microparticle (500 μM scalebar); (B) pADK microparticles respond to 5 mM methyl-β-cyclodextrin.

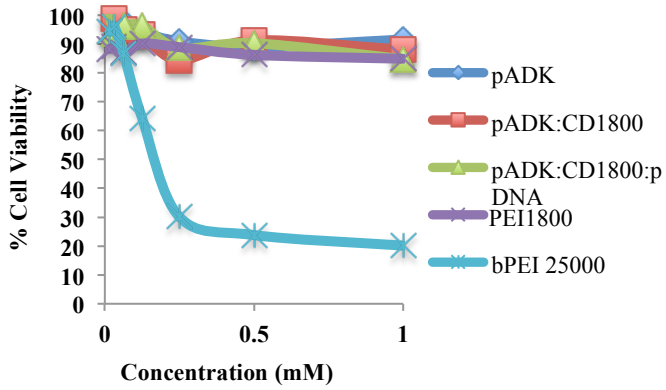


Figure 3: PEI1800, CD1800, pADK cationic complexes and pADK:CD1800:pDNA polyplexes have minimal toxicity

host interactions. To investigate if pADK can complex β-CD, we formulated microparticles from pADK (polymer 1 in table 1 of the supporting information), which encapsulated rhodhamine B (see Figure 2A), and investigated if β-CD could stimulate release from these microparticles. pADK microparticles were suspended into solutions that contained 5 mM methyl-β-CD or PBS, and the release of rhodamine-B was measured. Complexation of pADK microparticles with methyl-β-CD should increase the hydrophilicity of the particles, and catalyse their disassembly leading to release of the dye. This can be attributed to the dissolution of surface pADK polymers on the microparticles, and the creation of water channels in the microparticles. Figure 2 demonstrates that methyl-β-CD stimulates the release of rhodhamine-B from pADK microparticles.

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For example, in the presence of methyl-β-CD, pADK particles released their contents with a half-life of 15 hours, in contrast, in PBS, only 5% of the contents were released after 30 hours. Thus methyl-β-CD dramatically accelerates the release of compounds from pADK microparticles, demonstrating that pADK can complex β-CD.

A major application envisioned for pADK is for pendant polymer, cationic-β-CD mediated gene therapy. The toxicity of polycations is a major challenge with cationic drug/gene delivery vehicles, and we therefore investigated the in-vitro toxicity of pADK, pADK complexed with bPEI-1800 modified cyclodextrin

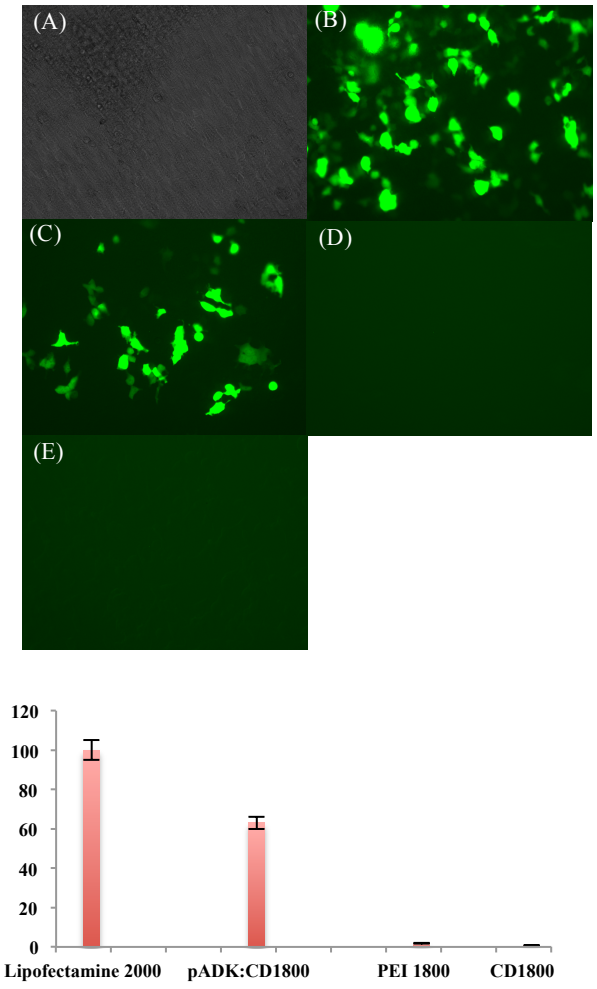

Figure 4: pADK improves the transfection efficiency of cationic cyclodextrins**:** (A) Bright field image of HeLa cells; (B) EGFP-pDNA transfection with lipofectamine 2000 (relative fluorescence intensity = 100) (C) EGFP-pDNA transfection with pADK:CD1800 complexes (relative fluorescence intensity to lipofectamine = 63%) (D) EGFP-pDNA transfection with CD1800 (relative fluorescence intensity to lipofectamine = 0.8%) (E) EGFP-pDNA transfection with PEI 1800 (relative fluorescence intensity to lipofectamine = 1.8%).

(pADK:CD1800), pADK:CD1800-pDNA polyplexes, PEI1800 by itself and CD1800, using the MTS assay. The cytotoxicity of bPEI (25000 MW) was used as a standard. CD1800 was chosen as the polycation for pADK complexation due to its previously demonstrated efficacy for pDNA delivery in pendant polymer systems.[13-16] Figure 3 demonstrates that pADK-cationic complexes have minimal toxicity. For example at a 1 mM concentration, none of the pADK formulations had any toxicity, whereas PEI 25,000 was cytotoxic at 100 μM (amine content). pADK:CD1800 should have the charge density of a high molecular weight polycation, yet its toxicity is 2 orders of magnitude lower than PEI of 25,000. The reduced cytotoxicity of pADK:CD1800 is presumably due to the biodegradability of the polymer and the transient nature of its cooperative cationic charges.

The ability of pADK:CD1800 complexes to deliver enhanced green fluorescent protein-pDNA (EGFP-pDNA) into HeLa cells was investigated and compared against CD1800. For these experiments an N/P ratio of 30, between the CD1800 and EGFP-pDNA was used, which was demonstrated to complex EGFP-pDNA as determined by a gel shift assay (see supporting information). pADK was complexed with CD1800 and then mixed with EGFP-pDNA and added to HeLa cells. Two negative controls were used for this experiment consisting of either EGFP-pDNA complexed with PEI1800 (N/P=30/1) or EGFP-pDNA complexed with CD1800 (N/P=30/1). Lipofectamine 2K was used as a positive control. After 36 hours the cells were analysed for green fluorescent protein (GFP) fluorescence via fluorescence microscopy. Figure 4 demonstrates that pADK can dramatically improve the transfection efficacy of CD1800. For example PEI1800 and CD1800 complexed with EGFP-pDNA had transfection efficacies that were approximately 1% of lipofectamine, whereas this increased to approximately 60% with the addition of pADK. pADK:CD1800's ability to increase the transfection ability of EGFP-pDNA and its low toxicity are presumably due to its high transient change density and ability to disrupt the endosome via either a proton sponge effect or acid catalyzed hydrolysis of pADK.[19-21]

Conclusions

In this report we present a new polymer, termed pADK, for gene delivery, which is a polyketal that has adamantane groups embedded on its backbone. pADK is capable of complexing with β-CD or β-CD derivatives and was able to improve the gene delivery efficacy of CD1800 by 60 fold. In addition, pADK complexed with CD1800 and pADK:CD1800-pDNA had minimal toxicity, even at a 1mM concentration (amine content). We anticipate numerous drug and gene delivery applications for pADK due to its ability to form host guest complexes with CD1800 and degrade into neutral excretable compounds.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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