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Glomerular disease augments kidney accumulation of synthetic anionic polymers

Gary W. Liu [a, 1], Alexander N. Prossnitz [a, 1], Diana G. Eng [b], Yilong Cheng [a, f], Nithya Subrahmanyam [c, e], Jeffrey W. Pippin [b], Robert J. Lamm [a], Chayanon Ngambenjawong [a], Hamidreza Ghandehari [c, d, e], Stuart J. Shankland [b], Suzie H. Pun [a, *]

[a] Department of Bioengineering and Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA, USA
[b] Department of Medicine, Division of Nephrology, University of Washington School of Medicine, Seattle, WA, USA
[c] Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT, USA
[d] Department of Bioengineering, University of Utah, Salt Lake City, UT, USA
[e] Utah Center for Nanomedicine, Nano Institute of Utah, University of Utah, Salt Lake City, UT, USA
[f] Department of Applied Chemistry, School of Science, Xi'an Jiaotong University, Xi'an, PR China

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ABSTRACT

Polymeric drug carriers can alter the pharmacokinetics of their drug cargoes, thereby improving drug therapeutic index and reducing side effects. Understanding and controlling polymer properties that drive tissue-specific accumulation is critical in engineering targeted drug delivery systems. For kidney disease applications, targeted drug delivery to renal cells that reside beyond the charge- and size-selective glomerular filtration barrier could have clinical potential. However, there are limited reports on polymer properties that might enhance kidney accumulation. Here, we studied the effects of molecular weight and charge on the *in vivo* kidney accumulation of polymers in health and disease. We synthesized a panel of well-defined polymers by atom transfer radical polymerization to answer several questions. First, the biodistribution of low molecular weight (23–27 kDa) polymers composed of various ratios of neutral:anionic monomers (1:0, 1:1, 1:4) in normal mice was determined. Then, highly anionic (1:4 monomer ratio) low molecular and high molecular weight (47 kDa) polymers were tested in both normal and experimental focal segmental glomerulosclerosis (FSGS) mice, a model that results in loss of glomerular filtration selectivity. Through these studies, we observed that kidney-specific polymer accumulation increases with anionic monomer content, but not molecular weight; experimental FSGS increases kidney accumulation of anionic polymers; and anionic polymers accumulate predominantly in proximal tubule cells, with some distribution in kidney glomeruli. These findings can be applied to the design of polymeric drug carriers to enhance or mitigate kidney accumulation.

1. Introduction

Polymeric carriers have been applied in drug delivery to improve circulation time, alter biodistribution, reduce metabolism, and facilitate cellular internalization of drug cargo [1–5]. The pharmacokinetics of polymeric carriers and their cargo depend on polymer properties including molecular weight, dispersity, charge, functionalization, and self-assembled size and shape [6–9]. Studies investigating polymer structure and resulting biodistribution have mainly focused on exploiting the enhanced permeability and retention effect for cancer applications [10–12]. However, polymeric carriers for kidney diseases remain relatively understudied despite the clinical potential of such technologies. For example, targeted drug delivery to glomerular podocytes could improve the standard of therapy for common glomerular diseases such as minimal change disease and focal segmental glomerulosclerosis (FSGS), and drug delivery to tubular epithelial cells may be strategic for acute kidney injury and polycystic kidney disease treatment [13,14]. The major challenge is that these cell populations reside

* Corresponding author.
E-mail addresses: spun@uw.edu, spun@u.washington.edu (S.H. Pun).
[1] Equally contributing authors.

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beyond the multi-layered glomerular filtration barrier, which comprises the innermost endothelial cells, a middle glomerular basement membrane, and the outer podocytes.

Given that the glomerular filtration barrier is both size- and charge-selective, these two parameters are likely critical when designing drug carriers to target cells past the barrier. Nanoparticle studies by the Davis group have revealed that gold nanoparticles of size ~75 nm target the kidney mesangium [15], and polycation-siRNA polymeric nanoparticles accumulate and disassemble in the anionic glomerular basement membrane [16]. However, the polymer physical properties required to cross this barrier for kidney targeting applications remain to be critically defined. Kamada et al. observed that hydrolyzed poly(vinylpyrrolidone-*co*-dimethyl maleic anhydride) copolymers of molecular weight approximately 10 kDa were anionic and distributed in kidneys up to 4 days after administration, with uptake primarily in proximal tubule cells [17]. Similarly, Borgman et al. reported that *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, functionalized with cyclo(RGDfK) targeting peptides and anionic penta-carboxylic acid residues, distributed preferentially in the kidneys compared to the designed target, tumors, and were retained up to 10 days after administration [18]. Recently, Bruni et al. reported on a panel of poly-ε-caprolactone and poly(ethylene glycol) methyl ether methacrylate star co-polymers (10–27 kDa), which exhibited evidence of kidney clearance *in vivo* [19]. While these reports have revealed in broad strokes that polymers with anionic charge and molecular weight less than 50 kDa accumulate in the kidneys, a rigorous evaluation of the individual and combined effects of polymer molecular weight and charge has yet to be reported.

Advances in controlled radical polymerization techniques have enabled polymer synthesis with precise control over molecular weight, dispersity, architecture, and chemical composition [20]. In this work, we used atom transfer radical polymerization (ATRP) to synthesize a panel of polymers to examine the effect of anionic charge density and molecular weight on kidney accumulation and distribution in mice. We first tested the effect of charge using a panel of low molecular weight (LMW) polymers, and then examined the effect of molecular weight in normal mice and mice with experimental FSGS, a model that results in loss of filtration size-selectivity and proteinuria [21]. Here, we report that highly anionic, LMW polymers preferentially accumulate in the kidneys and are internalized into proximal tubule cells. Conditions of experimental FSGS enhance accumulation of anionic LMW polymers.

2. Results

2.1. Polymer panel synthesis and characterization

We synthesized a panel of copolymers with varying ratios of anionic and neutral monomers by ATRP, with different degrees of polymerization (Table 1). Importantly, this approach yields well-defined polymers with tailored anion densities and molecular weights while keeping other properties constant. The hydrophilic, small molecular weight (~300 Da) monomer oligo(ethylene glycol) methyl ether methacrylate (OEGMA) was selected, as OEGMA-based polymers have been shown to exhibit favorable circulation times, low protein-binding properties, and reduced immunogenicity due to shorter ethylene glycol repeats [22–25]. The second monomer, *tert*-butyl methacrylate (tBuMA), yields methacrylic acids (MAA, anionic in charge) after deprotection. The monomer tBuMA was selected as an alternative to direct MAA polymerization, as MAA is insoluble in many organic solvents. Organic ATRP presents several advantages over aqueous ATRP, and results in polymerizations with less synthetic complexity and higher quality materials.

By varying the ratio of the two monomers and the polymerization time, p(OEGMA-*co*-MAA) copolymers with defined OEGMA:MAA ratios and molecular weights were synthesized (Table 1 and Fig. 1). Polymers with fixed molecular weight but varying anionic MAA content (0%, 50%, and 80%) were prepared to test the effect of charge on biodistribution. Two target molecular weight ranges were synthesized: low molecular weight (LMW) polymers of 20–25 kDa, and high molecular weight (HMW) polymers of 45–50 kDa. These two molecular weight regimes, which are either below or approximately at the renal filtration cutoff of ~50 kDa [26], respectively, were utilized to investigate the effect polymer molecular weight on kidney distribution. For biodistribution and tissue distribution analyses, polymers were fluorescently labeled with Cy3 fluorophore via a stable thioether bond by reduction of the disulfide bond of the pyridyl disulfide-terminated ATRP initiator and subsequent reaction with Cy3-maleimide (Fig. 1).

LMW polymers ranged in number average molecular weight (M_n) from 23 to 27 kDa, and HMW polymers had M_n of 47 kDa, as determined by gel permeation chromatography (GPC). All polymers exhibited dispersity ($Đ$) < 1.5. Within a molecular weight regime, the MAA monomer fraction during polymerization was varied at 0%, 50%, and 80%, resulting in polymer OEGMA:MAA ratios of 1:0 (homopolymer pOEGMA), 1:1, and 1:4, respectively. Monomer ratios within the copolymers, as determined by ^{1}H nuclear magnetic resonance spectroscopy (NMR), were in good agreement with the feed ratios and suggest similar reactivity of the two co-monomers under the polymerization conditions used (Table 1 and Fig. S1).

2.2. Biodistribution of LMW polymers in normal mice

The effect of polymer charge on kidney accumulation was first determined by evaluating the biodistribution of LMW 1:0, 1:1, and 1:4 copolymers 7 days post intravenous injection. This time point is significantly past the circulation half-life of similarly sized polymers (generally $t_{1/2} < 24$ h) [27,28] and was intentionally selected to measure organ accumulation. Fluorescence intensities of the three polymers prior to injection were comparable (Fig. S2). Polymer distribution to major organs (heart, lungs, liver, spleen, kidneys) was determined by whole organ fluorescence imaging after perfusion.

The LMW polymers exhibited a statistically significant increasing linear trend (p-value < 0.0001) in both kidney and liver

Table 1
Summary of p(OEGMA-*co*-MAA) copolymers. Number average molecular weight (M_n) and dispersity ($Đ$) values were determined by gel permeation chromatography. Polymer compositions were determined by ^{1}H NMR. PDS, pyridyl disulfide-functionalized ATRP initiator.

Polymer	Composition	OEGMA:tBuMA feed ratios	OEGMA:tBuMA measured ratios	M_n (Da)	$Đ$
LMW 1:0	p($OEGMA_{76}$)-PDS	100:0	N/A	23,000	1.125
LMW 1:1	p($OEGMA_{70}$-*co*-MAA_{70})-PDS	50:50	1:1.1	27,000	1.140
LMW 1:4	p($OEGMA_{39}$-*co*-MAA_{157})-PDS	20:80	1:3.5	25,000	1.370
HMW 1:4	p($OEGMA_{74}$-*co*-MAA_{296})-PDS	20:80	1:5.4	47,000	1.400

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