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Ultrasmall polymeric nanocarriers for drug delivery to podocytes in kidney glomerulus

Riccardo Bruni[a,b,c], Paolo Possenti[b], Carlotta Bordignon[c], Min Li[a,c], Stefania Ordanini[a,b,c], Piergiorgio Messa[c], Maria Pia Rastaldi[c], Francesco Cellesi[a,b,c,*]

[a] Fondazione CEN - European Centre for Nanomedicine, Piazza Leonardo da Vinci 32, 20133 Milan, Italy
[b] Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta", Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy
[c] Renal Research Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Pace 9, 20122 Milan, Italy

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ABSTRACT

We explored the use of new drug-loaded nanocarriers and their targeted delivery to the kidney glomerulus and in particular to podocytes, in order to overcome the failure of current therapeutic regimens in patients with proteinuric (i.e. abnormal amount of proteins in the urine) diseases. Podocytes are glomerular cells which are mainly responsible for glomerular filtration and are primarily or secondarily involved in chronic kidney diseases. Therefore, the possibility to utilise a podocyte-targeted drug delivery could represent a major breakthrough in kidney disease research, particularly in terms of dosage reduction and elimination of systemic side effects of current therapies. Four-arm star-shaped polymers, with/without a hydrophobic poly-ε-caprolactone core and a brush-like polyethylene glycol (PEG) hydrophilic shell, were synthesised by controlled/living polymerisation (ROP and ATRP) to allow the formation of stable ultrasmall colloidal nanomaterials of tuneable size (5–30 nm), which are able to cross the glomerular filtration barrier (GFB). The effects of these nanomaterials on glomerular cells were evaluated in vitro. Nanomaterial accumulation and permeability in the kidney glomerulus were also assessed in mice under physiological and pathological conditions. Drug (dexamethasone) encapsulation was performed in order to test loading capacity, release kinetics, and podocyte repairing effects. The marked efficacy of these drug-loaded nanocarriers in repairing damaged podocytes may pave the way for developing a cell-targeted administration of new and traditional drugs, increasing efficacy and limiting side effects.

1. Introduction

Chronic kidney diseases (CKD) are recognised as a major health threat worldwide. Over 10% of the global population is currently affected by CKD [1], and this number is expected to steadily increase in the next years [2]. Therapies which are able to treat or slow the progression of kidney damage are still limited [3,4]; steroids, immunosuppressive agents, and drugs interfering with the renin-angiotensin system cause a number of severe side effects, particularly when a systemic prolonged administration is required. When therapies fail, renal damage inevitably progresses to end stage renal failure, with the need of life-saving renal replacement therapies, which are charged with human and social high costs. Therefore, research is necessary to provide complete information about kidney cell physiology, to unveil disease mechanisms, and to design new and more specific therapies [5]. Technological progresses in nanomedicine already offer the possibility to plan a cell targeted administration of new and traditional drugs, increasing specific efficacy and limiting side effects [6]. Recent advances demonstrated how the majority of kidney diseases are characterised by damage of podocytes in the GFB [7]. Podocytes are highly specialised polarised cells composed by a cell body that bulges into the urinary space, and foot processes which interdigitate with neighbouring cells to completely enwrap the glomerular basement membrane [8]. Podocytes are known to be the primary glomerular target for different types of injuries, such as toxic, metabolic, hemodynamic, oxidant, and immune injury [9], therefore they are primarily or secondarily involved in all glomerular renal diseases. When podocytes work less efficiently due to stress or damage, proteinuria - the loss of proteins in the urine - and glomerular dysfunction inevitably take place. If not promptly treated, these conditions lead to progression of glomerular damage and renal failure [8]. Most interestingly, experimental results have also demonstrated that all drugs which are currently used to treat or slow progression of glomerular damage, including Angiotensin-converting enzyme (ACE) inhibitors [10], ster-

* Corresponding author.
E-mail address: francesco.cellesi@polimi.it (F. Cellesi).

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oids [11], cyclosporine A [9], and rituximab [12], have a direct action on podocytes [12–14]. Therefore, the possibility to utilise a podocyte specific delivery of these therapies could be beneficial in terms of dosage reduction and elimination of systemic side effects [15].

The most significant challenge in targeting podocytes is to design engineered nanocarriers which facilitate drug permeation through the GFB [16,17]. This biological barrier is a three-layer structure composed of fenestrated glomerular endothelial cells (with pores diameters in the range of 60–80 nm), podocytes (with interdigitating foot processes that form filtration slits of 32 nm) and glomerular basement membrane between the two cellular layers (rich in heparin sulfate and charged proteoglycans which provides size and charge selectivity) [18]. The glomerular filtration apparatus, taken in its entirety, possesses an effective size cut-off of 6–10 nm [19,20] (still highly debated in the context of renal function and glomerular pathologies). This size range is generally too small for the permeation of many nanocarriers, including several proteins, biomedical nanoparticles and liposomes. Taking into account these limitations, our approach focused on the design of ultrasmall colloidal polymeric nanomaterials able to permeate through the glomerular barrier (which may also increase its size cut-off under pathological conditions [21]), and reach podocytes.

A library of multiarm amphiphilic polymers were synthesised by Atom Transfer Radical Polymerisation (ATRP) of PEG-methacrylate, starting from either commercial 4-Arm ATRP initiators or custom-made multiarm poly(ε-caprolactone) macroinitiators. When dispersed in aqueous solutions, these macromolecules are able to form ultrasmall nanocarriers, stabilised by a dense hydrophilic corona of comb-like PEG (Scheme 1).

ATRP kinetics, reaction conditions, degree of polymerisation and final molecular weight distributions were optimised in order to obtain nearly monodisperse polymers of well-defined structure and molecular weight, and generate nanocarriers of uniform and tuneable particle size.

A subset of polymers with optimal physicochemical properties was identified and further used for biological tests. In particular, nanomaterials of target size < 30 nm (i.e. compatible with kidney filtration) were selected.

A hydrophobic drug, dexamethasone (DEX), was successfully encapsulated in the nanoparticle core and release profiles were analysed under sink condition.

In vitro tests on podocytes were carried out to exclude polymer cytotoxicity and possible damage to cell cytoskeleton.

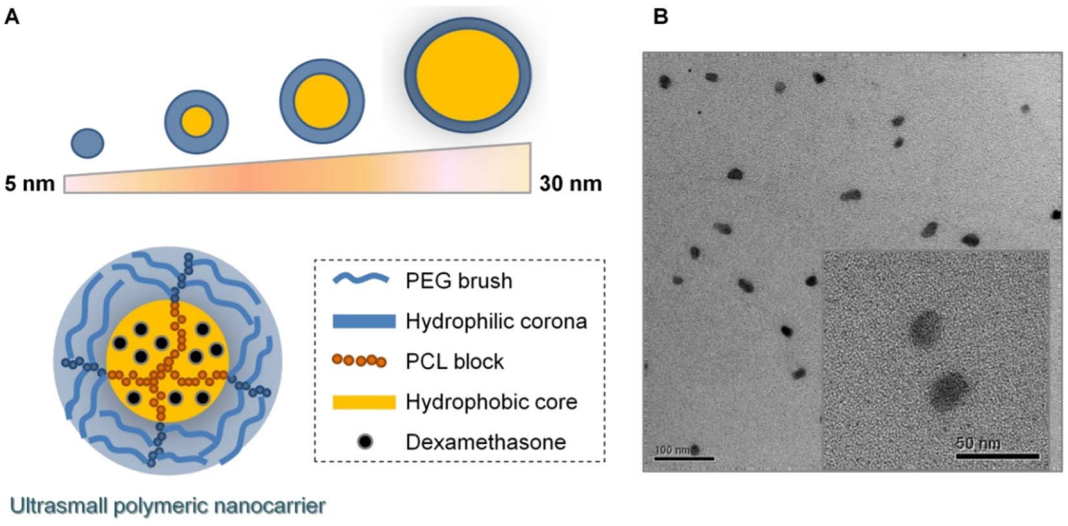
Podocyte repair by controlled nanodelivery of DEX was assessed. We took advantage of a recently developed 3D in vitro system, based on a co-culture of endothelial cells and podocytes [22–24], in mimicking the mechanisms of nanoparticle interaction with glomerular cells and the repair of the filtration barrier. Finally, in vivo tests on healthy and proteinuria-induced mice were carried out to investigate the biodistribution of these nanomaterials following intravenous administration, and their capacity to permeate through GFB and accumulate in urine.

2. Materials and methods

ε-Caprolactone 97%, Pentaerythritol > 99% (PTOL), Tin(II) 2-ethylhexanoate 92–100% ($Sn(Oct)_2$), α-Bromoisobutyryl bromide 98% (BiBB), Triethylamine > 99% (TEA), Sodium Sulfate > 99%, Poly (ethylene glycol) methyl ether methacrylate M_n 500 (PEGMA), Copper (I) Bromide 98% (CuBr), 1,1,4,7,10,10-Hexamethyltriethylenetetramine 97% (HMTETA), Pentaerythritol tetrakis(2-bromoisobutyrate) 97%, Dichlorometane > 99% (DCM), Isopropyl alcohol (IPA) > 99.8%, Methanol > 99.8%, Toluene > 99.7%, Tetrahydrofuran (THF) (HPLC grade, inhibitor free, 99.9%) Diethylether > 99.5%, Acetonitrile (ACN) > 99.5%, Ethylacetate > 99.5%, Acetone 99.5%, Dimethyl Sulfoxide 99.9%, 2-Hydroxyethyl methacrylate 97% (HEMA), Rhodamine B 95% (RhB), N,N′-Dicyclohexylcarbodiimide 99% (DCC), 4-(Dimethylamino)pyridine 98% (DMAP), Pyrene 99%, Sodium Chloride 99%, Potassium Chloride 99%, Sodium phosphate dibasic dihydrate 98.5%, Potassium phosphate monobasic 98%, Dexamethasone (DEX) 98%, Deuterochloroform 99.8%, Dimethyl sulfoxide-d6 100% were all purchased from Sigma (Italy) and used as received without further purification unless stated otherwise. MilliQ water was obtained by filtration of Helix 5 water using Easy Millipore System. Dialysis regenerated cellulose tubular membrane with 3500 MWCO were purchased by Orange Scientific (Belgium). 0.45 μm syringe nylon filter were purchased by Teknokroma (Italy).

2.1. Characterisation

The number-average molecular weight ($M_{n,GPC}$) values and dispersity ($Đ = M_w/M_n$) values of the polymers were evaluated using a Jasco LC-2000Plus gel permeation chromatograph (GPC) equipped with a refractive index detector (RI-2031Plus, Jasco) using 3 Agilent PL gel columns, 5 μM particle size, 300 × 7.5 mm (MW range: 5×10^2 to 17×10^5 g/mol). THF was chosen as eluent at a flow rate of 0.5 mL/min at 35 °C. The GPC samples (4 mg/mL in THF) were injected using a



Scheme 1. A) Four-arm star-shaped polymers, with/without a hydrophobic PCL core and a brush-like PEG hydrophilic shell, form stable ultrasmall colloidal nanomaterials which are able to encapsulate and release DEX and to cross the GFB. B) TEM image of a polymeric nanocarrier (A4CL10PEG20, scale bar 100 nm). Inset: details of the nanocarriers at higher magnification (scale bar 50 nm).