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Diversely functionalised carbohydrate-centered oligomers and polymers. Thermoresponsivity, lectin binding and degradability

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ABSTRACT

Nature is capable of synthesizing perfectly defined, sequence-controlled oligomers and polymers, whereas synthetic polymerization methods inherently give rise to dispersity and limited reproducibility. This inherent dispersity provides a barrier to translation into biomedical applications and for probing material-biology interactions. Templating of polymers based upon biosynthesized cores offers a route to reproducible oligo/polymers if the template itself is readily available and highly tunable. Here oligosaccharides are employed as monodisperse scaffolds for the synthesis of highly functional biomaterials. The pendant hydroxyl units are converted to reactive methacrylates, which are themselves amenable for thiol-ene ('click') functionalization. Using this strategy, extremely well defined ($M_W/M_N < 1.05$) polymers are prepared bearing thermoresponsive or lectin-binding moieties. The templatation strategy ensures identical polymers are obtained from each synthesis. Their thermoresponsive behavior and multivalent interactions with a bacterial lectin are studied as a function of the discrete number of functional groups. Due to the ester linkage, these polymers are also shown to be inherently degradable.

1. Introduction

The development of biomaterials that are capable of interacting with and inducing a beneficial responsive from biological systems is providing new routes to treat human disease, infection and aging. For example, scaffolds for tissue or bone repair/re-growth [1], slow release formations [2], polymer–protein conjugation, nanoparticle delivery [3,4], gene delivery [5], sensing [6], diabetes treatment [7], cryopreservation [8] and nanomedicine [9].

The introduction of functionality that can enable 'smart' responses to biological cues [10], such as temperature, pH [11], biochemical gradients [12,13], light [14] or enzymes [15] is being used to enhance the complexity of materials. This enables dynamic interactions with biology to trigger cellular uptake [16,17], drug release, protein purification [18] or stem-cell differentiation [19]. Another approach focuses on incorporating biological binding motifs such as peptides [20], DNA [21], or carbohydrates [22] that trigger responses or highly specific binding events. In the development of all the above-mentioned materials, the key requirement is precise and reproducible synthesis; both to obtain structure–activity relationships, but also to meet regulatory requirements to ensure predictable pharmacokinetics, degradability and cellular trafficking.

The highly functional polymers described above are routinely accessible using controlled radical polymerization (RAFT, ATRP, SET and NMP) that allow the routine

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synthesis of highly functional polymers with excellent control over molecular weight and molecular weight distributions, and have been widely reviewed for biomedical applications [23,24]. The key challenge remains that these polymers are inherently non-biodegradable due to them being derived from vinyl monomers, with the rare exceptions of cyanoacrylates or copolymers made from radical-ring opening polymerizations [25]. Alternatively the use of ring-opening polymerization (ROP) of cyclic esters/amides [1,26] enables the introduction of a degradable backbone, but is limited in terms of functional group compatibility, requiring post-polymerization modification to access functional (e.g. hydroxyl) polymers [27]. (Bio)orthogonal (click) chemistries have revolutionized this synthetic approach, with particular attention paid to the [3+2] cycloadditions of azides/alkynes and either nucleophilic or radical additions of thiols onto alkenes. Despite these synthetic tools both ROP and radical polymerizations are limited by their statistical nature resulting in a distribution of molecular weights ($Đ < 1.3$ typically) even for the best single electron transfer (SET) processes [28]. This presents an additional challenge when it comes to the precision study of polymer–biological interactions where small differences in degree of polymerization (valency) and dispersity can have a significant influence on the observed biological outcome. This is a particular problem in the glycosciences where the binding affinity between multivalent scaffolds and their target proteins increases dramatically with valency. For example, Lee et al. observed a 10^5 fold reduction in the minimum inhibitory concentration upon changing from mono- to tetra-antennary lactosides against a hepatic lectin [29]. Many glycopolymer antagonists for pathogenic proteins with exceptionally high affinity/activity have been reported and the roles of polymer composition, architecture and length have been shown to be crucial [30,31]. Therefore there still exists huge chemical space to be explored to translate new anti-adhesive therapies as alternatives to traditional antibiotics [32,33], but this is somewhat constrained by the limitations of controlled radical polymerizations.

For these reasons dendrimers, perfectly branched macromolecules, have been extensively explored as biomaterials [34]. Due to their step-wise synthetic protocols, dendrimers or peptides (from solid phase synthesis) can be obtained with narrow dispersity and be constructed out of biodegradable units [35]. Detailed mass spectrometry and chromatographic analysis by Banaszak-Holl and co-workers has quantified the presence of generational defects in PAMAM dendrimers which increase their heterogeneity [36]. Perhaps the biggest constraint on the use of dendrimers is their high cost and relatively low availability compared to linear polymers. Nature is capable of synthesizing perfectly monodisperse macromolecules, in the form of proteins and DNA, and these have been studied extensively as templates for polymer synthesis [37,38]. Synthetic machines capable of assembling peptides have also been created [39]. (Pseudo) site-specific functionality can also be introduced at predetermined sites within the backbone of CRP derived polymers, based on differential reactivity of maleimides with other monomers [40].

Oligosaccharides are usually thought of as polymers of monosaccharides, but they could also be considered to be monodisperse multifunctional poly(ols) and are therefore ideal template molecules. STARFISH like multivalent glycoclusters [30], or PAMAM dendrimers around trehalose ('Octopus glycosides') are examples of these materials [41,42]. Gao et al. have made propargyl-functional galactose that was suitable for 'click' cycloaddition to make small (tetravalent) glycosylated clusters that displayed a 400-fold affinity enhancement relative to the carbohydrate alone [43] In particular, this functionalizable/clickable core is an appealing scaffold to generate libraries of monodisperse/polymers oligomers, and materials other than glycoconjugates.

Here we explore the synthesis and extend the application of carbohydrate-centered polymers *via* a thiol-ene modification strategy. This method is used to obtain stimuli responsive polymers, anti-adhesive pathogen inhibitors and also demonstrate their biodegradability.

2. Experimental

2.1. General procedures

Phosphate-buffered saline solutions were prepared using preformulated tablets (Sigma–Aldrich) in 200 mL of MilliQ water (>18 Ω mean resistivity) to give a buffered pH of 7.4. Cellobiose was purchased from Acros Organics. Benzylamine, Bovine serum albumen (BSA), FITC labeled Cholera Toxin B subunit, 2-(2-methoxyethoxy)ethanethiol, galactose, glucose, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), mannose, maltotriose, methacrylic anhydride, lactose, raffinose and tributyl phosphine were purchased from Sigma–Aldrich. Anhydrous pyridine was purchased from VWR. GM1 ganglioside, β-D-thiogalactose sodium salt and stachyose were purchased from Carbosynth (Berkshire, UK). Peanut Agglutinin-FITC was purchased from Vector Labs. Poly(diethylene glycol methacrylate) was prepared according to previous reported methods [47].

2.2. Physical and analytical methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers using deuterated solvents purchased from Sigma–Aldrich. Chemical shifts are reported relative to residual non-deuterated solvent. Infrared spectra was recorded on a Bruker Vector 22 GI003097. Mass spectrometry analyses were obtained using Bruker MicroTOF or Bruker MaXis electrospray instruments using positive or negative electrospray mode. Thermal transitions were measured using an optimelt MPA100 system and an Agilent Technologies Cary60 UV/Vis spectrometer equipped with a Quantum Northwest TC1 temperature controller. Size exclusion chromatography (SEC) was used to examine and differentiate between the molecular weights and polydispersities of the synthesized carbohydrates. The DMF GPC system comprised of a Varian 390-LC-Multi detector suite fitted with a differential refractive index (DRI) detector equipped with a guard